

THE INFLUENCE OF POLLEN QUALITY AND POLLEN-BASED
CUES ON THE NUTRITION AND FORAGING BEHAVIOUR
OF HONEY BEES, *Apis mellifera* L.

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Stephen Francis Pernal

In Partial Fulfilment of the

Requirements of the Degree

of

Doctor of Philosophy

Department of Entomology

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**The Influence of Pollen Quality and Pollen-Based Cues on the Nutrition and Foraging
Behaviour of Honey Bees, *Apis mellifera* L.**

BY

Stephen Francis Pernal

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree
of
Doctor of Philosophy**

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Dedicated to my wife,
Jeannie
and our children,
Katherine and Nicole
for their much tried patience.

Daddy is finally done his “peesis”.

And to my parents,
Nan Barton Robertson
and
Andrew Bolesław Pernal
for always having faith in my abilities.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF APPENDICES	xv
ABSTRACT	xvi
FOREWORD	xviii
CHAPTER I	
INTRODUCTION	1
CHAPTER II	
LITERATURE REVIEW	
Pollen Structure and Biology	
Wall Stratification and Surface Features	10
Contributions of the Tapetum	14
Dehiscence, Number and Size of Grains	16
Pollen Odours and Attractants	21
Pollen Foraging by Honey Bees	
Behaviour of Pollen Collectors	27
Attraction to Flowers and Learning	38
Influence of Colony State on Foraging	43
Pollen Utilization by Honey Bees	
Storage in the Hive	53
Digestion	55
Nutrition	56
Summary	60
Objectives	65

	Page
CHAPTER III	
Pollen Quality of Fresh and 1-Year-Old Single Pollen Diets for Worker Honey Bees (<i>Apis mellifera</i> L.)	68
Abstract	69
Introduction	69
Materials and Methods	
Pollen Collection	75
Experimental Design	77
Bioassay Cages	77
Diet Preparation	78
Bioassay Protocol	79
Hypopharyngeal Gland Protein	80
Protein Determination of Pollen Diets	82
Calculation of Diet Consumption	82
Statistical Analysis	83
Results	84
Discussion	88
Acknowledgements	95
CHAPTER IV	
The Influence of Pollen Quality on Foraging Behaviour in Honey Bee (<i>Apis mellifera</i> L.) Workers	118
Abstract	119
Introduction	120
Materials and Methods	
Honey Bees and Observation Hives	127
Treatment Structure	128
Experimental Trials	129
Experimental Protocol	130
Analysis of Pollen Loads	133
Preparation of Reference Slides	135
Scanning Electron Microscopy	136
Protein Determination	137
Analysis of Pollen Grain Size	139
Statistical Analysis	139

	Page
Results	
Pollen Species Collected, Size of Loads and Mixing	142
Quantitative Foraging Responses	144
Qualitative Foraging Responses	148
Combination of Quantitative and Qualitative Responses	151
Analysis of Species Assemblages Collected by Colonies	152
Discussion	156
Acknowledgements	171
 CHAPTER V	
Discrimination and Preferences for Pollen-Based Factors by Foraging Honey Bees (<i>Apis mellifera</i> L.)	237
Abstract	238
Introduction	239
Materials and Methods	
Pollen Collection	244
Pollen Analogues	245
Maintenance and Training of Bees	246
Common Bioassay Protocol	
Flight Room Conditions	248
Experimental Trials	249
Whole Pollen Odour Bioassay	250
Pollen Lipid Extract Bioassay	251
Particle Size Bioassay	252
Protein Content Bioassay	254
Handling Time Bioassay	254
Interaction of Multiple Factors Bioassay	255
Statistical Analysis	257
Results	
Whole Pollen Odour Bioassay	259
Pollen Lipid Extract Bioassay	260
Particle Size Bioassay	261
Protein Content Bioassay	263
Handling Time Bioassay	264
Interaction of Multiple Factors Bioassay	265
Discussion	270

	Page
Acknowledgements	287
CHAPTER VI	
An Improved Flight and Rearing Room Design for Honey Bees (<i>Apis mellifera</i> L.)	331
Abstract	332
Introduction	332
Materials and Methods	
Gross Room Features	336
Lighting	336
Heating, Cooling and Airflow	338
Humidity	339
Control Systems	340
Flight Cage and Feeding Station	340
Colony Maintenance	342
Results and Discussion	
Effect of Lighting	342
Airflow and Quality	347
Temperature and Humidity Control	348
Colony Dynamics and Foraging Behaviour	349
Future Directions	353
Acknowledgements	355
CHAPTER VII	
GENERAL DISCUSSION	374
Conclusions	381
Future Research	383
LITERATURE CITED	384

LIST OF TABLES

	Page
Table 1. Protein and water content of pollen diets	97
Table 2. Occurrence, protein content and water content of pollen species collected by honey bees, from analysis of corbicular pollen loads (N=5477)	173
Table 3. Wet weight (mean \pm SE) of corbicular pollen loads collected by honey bee foragers	176
Table 4. Linear relationships between number of pollen grains (y) and wet weight of pollen loads (x), used for the calculation of protein content of multifloral pollen loads containing species frequently collected by foragers	177
Table 5. Frequency of pollen loads comprised of different numbers of species, based on different thresholds for recognizing secondary species in mixed loads	178
Table 6. Frequency of co-occurring species in mixed pollen loads	179
Table 7. Main effects and significant interaction terms from log-linear analysis of pollen species assemblages collected by colonies	181
Table 8. Occurrences of species in pollen loads, as proportions (%) of occurrences within replicates	182
Table 9. Occurrences of species in pollen loads, as proportions (%) of occurrences within replicates and time periods	183
Table 10. Occurrences of species in pollen loads, as proportions (%) of occurrences within days of the experiment and time periods	185
Table 11. Occurrences of species in pollen loads, as proportions (%) of occurrences within time periods and levels of forager experience	186
Table 12. Univariate ANOVA statistics for behaviours analyzed by BIBD models for bioassays of whole pollen odour, pollen lipid extract, particle size, protein content and handling time	288

	Page
Table 13. Competing effects of pollen lipid extracts for numbers of bees approaching bioassay dishes (log-transformed LSMs)	290
Table 14. Competing effects of particle size classes for numbers of bees approaching bioassay dishes (log-transformed LSMs)	292
Table 15. Competing effects of particle size classes for total behaviours (log-transformed LSMs)	294
Table 16. Univariate ANOVA statistics for main treatment effects from BIBD models, by each behaviour scored for the interaction of multiple cues bioassay	296
Table 17. Competing effects of multiple cues for numbers of bees approaching bioassay dishes (log-transformed LSMs)	297
Table 18. Competing effects of multiple cues for total behaviours (log-transformed LSMs)	299
Table 19. Irradiance, illuminance and total light energy perceived in the bee flight and rearing room	356

LIST OF FIGURES

	Page
Figure 1. Structure of the pollen grain exine.	66
Figure 2. Bioassay cage used for experimental treatments, constructed from 1.3 cm thick spruce plywood and fibreglass screening.	98
Figure 3. Pollen consumption for worker bees fed freshly collected and 1-year-old pollen.	100
Figure 4. Pollen consumption during each experimental time interval, for worker bees fed freshly collected and 1-year-old pollen.	102
Figure 5. Protein consumption for worker bees fed freshly collected and 1-year-old pollen.	104
Figure 6. Protein consumption during each experimental time interval, for worker bees fed freshly collected and 1-year-old pollen.	106
Figure 7. Hypopharyngeal gland development for worker bees fed freshly collected and 1-year-old pollen.	108
Figure 8. Hypopharyngeal gland development during each experimental time interval, for worker bees fed freshly collected and 1-year-old pollen.	110
Figure 9. Ovary development for worker bees fed freshly collected and 1-year-old pollen.	112
Figure 10. Correlation between mean ovary score and hypopharyngeal gland protein for worker bees fed freshly collected and 1-year-old pollen.	114
Figure 11. Linear relationships between protein consumption per worker (from Fig. 4) and indices of worker development, for freshly-collected and 1-year-old pollen diets.	116
Figure 12. Observation hive with honey bees.	187
Figure 13. Dimensions of observation hive components.	189
Figure 14. Wet weight of pollen loads collected by experienced and inex- perienced foragers during morning and afternoon data collection periods.	191

Figure 15. Number of returning pollen foragers per min (pollen foraging rate) in experimental treatments during morning and afternoon sampling periods	193
Figure 16. Numbers of returning nectar and pollen foragers per min (total foraging rate) in experimental treatments during morning and afternoon sampling periods	195
Figure 17. Pollen intake rate for treatments by day of the experimental period.	197
Figure 18. Mean consumption of pollen supplements used in ‘low’ and ‘high’ quality treatments.	199
Figure 19. Consumption of pollen supplements (bars) and changes in the pollen foraging rate of colonies (line)	201
Figure 20. Temporal changes in protein content of pollen loads (% dry weight) for experienced and inexperienced foragers.	203
Figure 21. Mean cross-sectional area of pollen grains in loads collected by experienced and inexperienced foragers during morning and afternoon data collection periods.	205
Figure 22. Mean cross-sectional area of pollen grains in loads collected by experienced and inexperienced foragers for quantity and quality manipulated treatments.	207
Figure 23. Temporal changes in the number of pollen species collected by colonies receiving either low or high stimulus intensities.	209
Figure 24. Number of pollen species collected per colony during morning and afternoon data collection periods by inexperienced and experienced foragers.	211
Figure 25. Number of pollen species comprising individual loads of experienced and inexperienced foragers within levels of stimulus intensity and data collection period.	213
Figure 26. Protein intake rate (mg/min) in experimental treatments during afternoon sampling periods.	215
Figure 27. Temporal changes in protein intake rate (mg/min) averaged over all experimental colonies.	217

	Page
Figure 28. Occurrences of species (N=5615) in pollen loads, as proportions of occurrences from all pollen loads examined	219
Figure 29. Occurrences of species in pollen loads, as proportions of occurrences within morning (N=2247) and afternoon (N=3368) time periods	221
Figure 30. Occurrences of species in pollen loads, as proportions of occurrences within experienced (N=1947) and inexperienced (N=3668) foragers	223
Figure 31. Dish used for whole pollen odour bioassay, showing position of screens containing 9.5 and 3.2 mm openings.	301
Figure 32. Petri dish used for lipid extract bioassay, containing filter paper and a screen with 9.5 mm openings.	303
Figure 33. Bioassay dish used for particle size and protein bioassays.	305
Figure 34. Bioassay dish used for handling time bioassay	307
Figure 35. Bioassay dish used to test a combination of pollen-based cues, presented simultaneously to honey bees.	309
Figure 36. Behaviours of foragers scored to pollen and pollen analogues in whole pollen odour bioassay.	311
Figure 37. Behaviours of foragers scored to pollen and pollen analogues in pollen lipid extract bioassay.	313
Figure 38. Behaviours of foragers scored to different size ranges of soybean meal in particle size bioassay.	315
Figure 39. Weight of soybean meal collected per forager for size ranges used in particle size bioassay.	317
Figure 40. Behaviours of foragers scored to soy flours with different levels of crude protein (w/w), in protein content bioassay.	319
Figure 41. Behaviours of foragers scored to bioassay dishes having different levels of handling time.	321
Figure 42. Effect of simultaneous manipulation of three factors (presence of pollen lipid extract, size of particle and handling time), on worker foraging behaviour in the interaction of multiple factors bioassay.	323

Figure 43. Interactive effects of handling time and particle size on the number of bees approaching and landing on dishes during the interaction of multiple cues bioassay.	325
Figure 44. Interactive effects of pollen lipid extract and handling time on the number of bees crawling under the lower screen (screen #2) of dishes during the interaction of multiple cues bioassay.	327
Figure 45. Interactive effects of pollen lipid extract and handling time on the weight of cellulose collected per forager during the interaction of multiple cues bioassay.	329
Figure 46. Gross features of flight and rearing room, west-facing view (drawn as if east wall was transparent).	358
Figure 47. Gross features of flight and rearing room, top view (drawn without heating and cooling system).	360
Figure 48. Position of lighting in flight and rearing room, west-facing view.	362
Figure 49. Position of lighting in flight and rearing room, top view. Legend as in figure 3, except D, steel wall bracket.	364
Figure 50. Heating, cooling, airflow and humidification systems in flight and rearing room, west-facing view.	366
Figure 51. Heating, cooling, airflow and humidification systems in flight and rearing room, top view.	368
Figure 52. Flight cage and feeding station in flight and rearing room, west-facing view.	370
Figure 53. Flight cage and feeding station in flight and rearing room, top view.	372

LIST OF APPENDICES

	Page
Appendix 1. Untransformed cell means (\pm SE) for parametric analyses not containing forager experience as a model term	225
Appendix 2. Untransformed cell means (\pm SE) for parametric analyses containing forager experience as a model term	227
Appendix 3. Plate 1. Scanning electron micrographs of selected pollen species collected by foragers during this study: a-b, <i>A. lappa</i> (1400 \times); c, <i>A. lappa</i> (8600 \times); d, <i>Aster</i> spp. #1 (673 \times); e-f, <i>Aster</i> spp. #1 (2800 \times); g, <i>Aster</i> spp. #1 (9000 \times); h, <i>Aster</i> spp. #2 (673 \times); I-j, <i>Aster</i> spp. #2 (2800 \times); k, <i>Aster</i> spp. #2 (9000 \times); l, <i>C. arvensis</i> (1400 \times)	231
Plate 2. Scanning electron micrographs of selected pollen species collected by foragers during this study (continued): a, <i>C. arvensis</i> (1400 \times); b, <i>C. arvensis</i> (5820 \times); c-d, <i>V. angustifolium</i> (1800 \times); e-f, <i>M. alba</i> (2800 \times); g-h, <i>M. officinalis</i> (2800 \times); I-j, <i>T. hybridum</i> (2800 \times); k-l, <i>T. repens</i> L. (2800 \times)	233
Plate 3. Scanning electron micrographs of selected pollen species collected by foragers during this study (continued): a, <i>V. cracca</i> (2850 \times); b, <i>V. cracca</i> (1400 \times); c-d, <i>A. humilis</i> (2800 \times); e, <i>A. humilis</i> (14000 \times); f-g, <i>P. fruticosa</i> L. ssp. <i>floribunda</i> (2800 \times); h, <i>P. fruticosa</i> ssp. <i>floribunda</i> (11600 \times); I-j, <i>R. acicularis</i> (1800 \times); k-l, <i>R. idaeus</i> L. var. <i>strigosus</i> (2800 \times)	235

ABSTRACT

Pernal, Stephen Francis. Ph.D., University of Manitoba, July 2000. The influence of pollen quality and pollen-based cues on the nutrition and foraging behaviour of honey bees, *Apis mellifera* L. Major Professor: Robert W. Currie.

Changes in honey bee, *Apis mellifera* L., worker fitness were determined after feeding caged, newly-emerged bees one of eight pollen diets. Freshly-collected pollen was compared with pollen that had been stored for one year at -30° C in an oxygen-reduced atmosphere. Development of hypopharyngeal glands and ovaries was found to be correlated with the amount of protein consumed, and for some diets, protein appeared to be allocated between ovaries and glands differentially. Storing pollen for one year did not affect gland or ovary development.

A second experiment was conducted to determine whether honey bee colonies responded to changes in the nutritional quality of their stored pollen reserve. Colony pollen reserves were manipulated either quantitatively or qualitatively, at high and low levels. Foraging rates, and the weight and species composition of pollen loads were determined. Colonies responded to a decrease in the quantity or quality of their pollen reserve by increasing the proportion of pollen foragers, without altering the overall foraging rate. Inexperienced foragers collected heavier loads and more species of pollen per foraging trip, and specialized on larger, more proteinaceous pollen than experienced foragers. Colonies appear to respond to deficiencies in stored protein levels by increasing the gross amount of pollen returned to the colony, rather than by specializing on pollen

having a higher protein content. Colony-level responses to deficiencies in stored protein may be manifested by an increase in the ratio of naive to experienced foragers. Foragers receive colony-level feedback about pollen quality in order to match pollen intake with the protein need of the colony.

In a third study, pollen-based foraging cues were evaluated using two-choice bioassays in a flight and rearing room. The importance of pollen grain size, protein content, handling time and odour were assessed as foraging cues for worker bees. Pollen odour was the dominant cue foragers used to evaluate pollen, but bees also displayed preferences based upon the size of particles collected. Pollen-seeking behaviours decreased with increases in handling time and foragers did not discriminate on the basis of pollen protein content. Honey bees do not discriminate among food sources based on nutritional quality, but instead, evaluate cues that may affect their efficiency of pollen collection or recruitment to forage sources.

Technical details and advances in honey bee flight room design are also discussed.

FOREWORD

This thesis is written in paper style, with a review of pertinent literature necessary for understanding the overall body of research contained in Chapter II. Individual manuscripts (Chapters III - VI) are prepared as independent submissions to scientific journals. Chapter III is published in the journal *Apidologie* (Pernal and Currie 2000). Chapter IV has been submitted to the journal *Behavioral Ecology and Sociobiology*. Chapter V has been submitted to the journal *Animal Behaviour*, and Chapter VI has been submitted to the *Journal of Economic Entomology*. All publications are jointly authored by Pernal and Currie. Chapter VII contains an overall discussion of the research contained herein. The references cited in all manuscripts may be found in a combined Literature Cited section at the end of this thesis.

CHAPTER I

INTRODUCTION

Pollen is an essential nutritional requirement in the diet of the honey bee, *Apis mellifera* L., containing all of the protein, lipids, vitamins and minerals necessary for the development of larvae and adults. Although pollens vary widely in their nutritional composition among plant species, little work has been done to evaluate pollen quality using parameters directly associated with honey bee fitness. In addition, the influence of pollen quality and pollen-based cues on foraging decisions made by colonies or individual foragers is largely unexplored.

Hypopharyngeal gland and ovary development are good indicators of how the quality of pollen consumed by workers potentially affects colony-level fitness. Young bees that tend the brood are known as nurses; they are the principal consumers of pollen in the colony, converting it to a proteinaceous jelly which is secreted from their hypopharyngeal glands (Seeley 1982, Hrasnigg and Crailsheim 1998b). This secretion is fed to the developing larvae as their primary food source, but smaller amounts are also received by all adult members of the colony (Crailsheim 1992). The development of hypopharyngeal glands is influenced by the amount of pollen ingested by nurse bees (Maurizio 1954a; Haydak 1961; Hagedorn and Moeller 1968; Standifer 1967; Standifer et al. 1960, 1970; Knox et al. 1971; McCaughey et al. 1980; Hrasnigg and Crailsheim 1998b), and the size of glands is related to their total protein content and activity (Roşca et al. 1972, Brouwers 1982, Huang et al. 1989). The quantity and quality of brood food

produced by nurse bees have important ramifications for the fitness of the colony as a whole. The quality of food received by the brood, and especially the queen, has the potential to influence the overall rate of colony growth. In addition, the survival of workers is directly affected by the total amount of pollen protein consumed (Kleinschmidt and Kondos 1976, 1978; Schmidt et al. 1987). Therefore, diets that enhance hypopharyngeal gland development in nurses potentially influence colony population size, a variable that is related to such colony-level fitness components as increased colony survival and reproductive performance (Schmid-Hempel et al. 1993).

Pollen that is protein-rich also promotes ovary and egg development in workers caged without queens (Maurizio 1954a; Pain 1961, 1963; Jay and Jay 1993; Lin and Winston 1998), and a lack of pollen suppresses ovary development (Harris and Harbo 1990). Worker ovarian development provides a direct measure of the ability of bees to convert pollen proteins into vitellogenin (Bitondi and Simões 1996, Cremonese et al. 1998), a lipoprotein which is required for egg-laying. Greater, or more efficient, production of vitellogenin in queens could increase fecundity, colony population size, and therefore colony-level fitness.

Evaluating pollen quality using parameters that are related to a worker's ability to utilize pollen protein allows any inherent differences in the efficiency of pollen digestion and its relative assimilation into tissues of young workers to be addressed. The question of whether hypopharyngeal glands and ovaries utilize the nutritional components of pollen in a differential manner also remains poorly understood. Maurizio (1954a) indicated similar trends in hypopharyngeal gland and ovary development for bees fed different pollen diets, but Haydak (1961) showed that hypopharyngeal glands are more

sensitive to reductions in pollen quality. If unequal partitioning of protein between developing hypopharyngeal glands and ovaries exists, it would affect our judgement of how these indicators should be used in making assessments of pollen quality.

Determinations of pollen quality are also affected by the length of time and conditions under which pollen is stored. The protein content, vitamin content, and brood rearing capacity of pollen are known to diminish with age (Svoboda 1940, Haydak 1963, Hagedorn and Burger 1968; Hagedorn and Moeller 1968, Dietz and Stevenson 1980). Much ambiguity remains over the value of stored pollen for honey bees because many studies are confounded by the use of diets with mixed, and often unidentified, pollen species and provide inadequate descriptions of the storage techniques employed. Thus, it is difficult to assess the effects of specific storage parameters on pollen quality, and whether such effects are consistent among species. Freezing pollen, in combination with storage in an oxygen-reduced environment, might prevent degradation of pollen protein while preventing the oxidation of other non-protein constituents. A nutritional comparison between fresh and 1-year-old stored pollen, using several identified pollen species, would provide useful information for beekeepers, who typically use the previous year's pollen to feed colonies, and for researchers interested in aspects of pollen nutrition and consumption.

Honey bees store approximately 1 kg of pollen in their colonies during summer conditions as a reserve, a small amount compared with the 50 kg of honey that a typical temperate colony accumulates (Seeley 1995). The constant demand for protein to rear brood and feed adult bees can rapidly deplete this small reserve, thereby making colonies quite susceptible to sudden fluctuations in the supply of pollen in their environment.

Therefore, it is important that colonies tightly regulate their collection of pollen to meet the requirements for protein, particularly for the developing brood. Within the colony the demand for protein is reflected by the rate and quantity of pollen consumed by nurse bees. Variation in the amount of brood within colonies results in corresponding adjustments to pollen foraging activity (Filmer 1932; Cale 1968; Barker 1971; Todd and Reed 1970; Al-Tikrity et al. 1972; Free 1967, 1979; Calderone 1993; Eckert et al. 1994; Pankiw et al. 1998b; Dreller et al. 1999), and changes in the quantity of the pollen reserve also result in differences in the numbers of foragers and their rate of pollen collection (Barker 1971, Free and Williams 1971, Moeller 1972, Fewell and Winston 1992, Camazine 1993, Dogterom and Winston 1999; Dreller et al. 1999; Fewell and Bertram 1999).

Two competing hypothesis have been proposed to explain the mechanisms by which colonies regulate the quantity of pollen that they collect. The direct assessment hypothesis suggests that workers perceive physical or chemical cues produced by the brood and stored pollen as direct stimulatory feedback (Free 1967, Pankiw et al. 1998b, Dreller et al. 1999), while the indirect assessment hypothesis suggests that the trophallactic exchange between nurses and foragers transmits an inhibitory signal that regulates pollen collection (Camazine 1993, Camazine et al. 1998). According to the latter model, high levels of protein from the hypopharyngeal gland secretions of nurses act to inhibit pollen collection, while low levels stimulate pollen collection. However, it is not known whether colonies regulate pollen collection in a manner that is sensitive to the amount of protein within the hive independent of its quantity, and whether foragers receive feedback about the quality of pollen that they collect. Although foragers have

been suggested to change the quality of pollen that they collect in relation to the size of their colony's pollen reserve (Fewell and Winston 1992) and pollen quality may be coded in dance information (Waddington et al. 1998), other evidence indicates that the choice of pollen by foraging bees is not directly motivated by its nutritional content (Levin and Bohart 1955, Schmidt 1982, Shaw 1990). Therefore, it remains to be determined whether foragers can assess the nutritional status of the colony's pollen reserve, and whether colonies modify their foraging behaviour strictly in response to levels of stored protein.

To examine whether honey bees adjust their foraging behaviour in response to changes in pollen quality, responses must be examined both at the level of the colony and at the level of the individual forager. The total amount of protein being returned to a colony can be adjusted by changes in the quantity or quality of pollen being collected by foragers, or both. Changes in the quantity of pollen returned to a colony can be effected by adjustments to the rate of foraging, the proportion of pollen-collecting specialists, or the effort of individual pollen collectors. Qualitative changes may result from colonies or individuals specializing on higher quality sources of pollen. Shifts in colony foraging strategy in response to pollen quality can be detected by evaluating the protein content of pollen loads, and the sampling activities of returning foragers. Any change in the breadth of flora sampled by a colony is reflected in the total number of species being returned to a colony by its scouts, and by the number of species sampled by individual foragers. The qualitative and quantitative contributions of forager choice on colony protein intake can ultimately be estimated from the average amount of protein collected per forager, or the amount being returned to colonies per unit time.

The behaviour of naive foragers compared with those of known pollen foraging

experience is also of interest. Examination of these two cohorts of foragers is biologically meaningful, as previous learning (Dukas and Visscher 1994) and restrictions to the lifetime energy budget (Neukirch 1982) of more experienced foragers could be significant factors in altering their foraging strategies. If differences between these two foraging cohorts exist, the relative proportion of each within the foraging population could serve as a mechanism by which bees respond to changes in colony state.

The behaviour of foragers on flowers has shown that bees collect pollen from a wide variety of floral sources and have distinct preferences for some pollen types over others, as demonstrated in natural settings (Linsley and McSwain 1947, Bohart 1957, Nye and Mackensen 1965, Olsen et al. 1979, Jay and Jay 1984, Free 1993) or by controlled choice experiments (Levin and Bohart 1955, Doull 1966, Wahl 1966, Boch 1982, Schmidt 1982, Boelter and Wilson 1984). Although it is clear that bees possess the ability to discriminate among pollen types, the cues used to accomplish this remain poorly understood.

Honey bees rely primarily on visual and olfactory stimuli to locate flowers and their rewards (Butler 1951, von Frisch 1967, Menzel et al. 1997, Backhaus 1993). Although colour is the main stimulus used by bees to locate flowers at a distance, odour is also used in flower selection (DeGrandi-Hoffman 1987; Beker et al. 1989; Masson et al. 1993, Kirchner and Grasser 1998; Pham-Delègue et al. 1989, 1993, 1997; Blight et al. 1997; Le Métayer et al. 1997). Floral odour allows bees to discriminate between floral species, decide whether or not to land, and may influence their expression of discrete foraging behaviours (McNaughton and Harper 1960; Dobson 1991, 1994; Dobson et al. 1996). Overall flower odour is the result of compounds produced from several floral structures (Dobson et al. 1990), however in many insect-pollinated plants, the odour of pollen is

qualitatively distinct from the remaining floral odour (von Frisch 1923; von Aufsess 1960; Porsch 1954, 1956; Buchmann 1983; Dobson et al. 1987, 1990, 1996; Bergström et al. 1995). Within pollen, odour-producing compounds are associated with the oily pollenkitt layer surrounding each grain (Dobson 1988). Honey bees possess the ability to discriminate between the odour of pollen and that of other floral volatiles, and can be trained to collect pollen based its odour alone, even in the absence of supplementary dance information (von Frisch 1923, von Aufsess 1960). Furthermore, the selection by bees of food substances that have little or no nutritional value may be stimulated by the addition of pollen lipid odour components (Taber 1963, Hohmann 1970, Starrat and Boch 1971). Therefore, pollen odour functions as a distinct and important cue for honey bee foragers.

The nutritional value of pollen, as measured by its protein content, is another potential cue that honey bee foragers may assess. The question of whether individual foragers can perceive the nutritional value of the pollen they are collecting has never been adequately tested. The inability of foragers to assess the quantity of pollen protein is supported by many documented accounts of bees collecting pollen or pollen-like substances with little or no nutritive value (Shaw 1990), as well as choice tests in which foragers were allowed to select among pollens having varying protein contents (Levin and Bohart 1955, Wahl 1966, Schmidt 1982). However, other evidence suggests that bees possess an independent ability to discriminate the protein content of pollen (Rasheed and Harder 1997a, Waddington et al. 1998) and may select pollen species in a way that is related to colony need (Fewell and Winston 1992). Hence, the perception of pollen quality by honey bee foragers is in need of clarification.

The influence of other pollen-based cues on the foraging decisions made by *A. mellifera* are even less well known. For example, the size of pollen grains could function as a cue for foragers to elicit pollen-collecting or packing behaviours because grain size is related to the efficiency of pollen collection, and its nutritional content (Baker and Baker 1979, Simpson and Neff 1983). The time necessary for a forager to work a flower and extract pollen (“handling time”), may also influence its decision-making process. Foragers incur higher costs as floral architecture becomes more complex (Heinrich 1979; Lavery 1980, 1994a), and the perception of this complexity via the handling time incurred, could be used as a cue for bees to exhibit continued pollen-seeking or collection behaviours.

The dearth of information regarding the importance of pollen-based cues on the pollen collection behaviours exhibited by foragers clearly indicates the need for further study. The ability of foragers to perceive pollen odour and protein content and the influence that these factors have on individual foraging decisions merits careful investigation, because of their known importance to colony-level pollen collection. Proper evaluation of such cues requires their controlled and discrete manipulation in the absence of other competing stimuli. By presenting foragers with simultaneous combinations of attractive cues, the hierarchy of stimuli used in their decision-making may also be deduced. Such studies are best conducted within the precisely controlled environment of an indoor flight and rearing room, where conditions are consistent and free of confounding influences.

Finally, technical improvements for honey bee flight and rearing rooms must be explored and championed. Successful establishment and long-term maintenance of a

colony for indoor foraging studies requires an ambient light environment that is similar to the natural energy spectra, produced in a manner which satisfies the visual requirements of bees. Furthermore, the rate of air exchange and general air quality must meet the demands of evacuating odours within the room in a rapid, draft-free manner, and prevent the distribution of airborne allergens that have seriously affected the health of previous investigators. Controls for such regulated environmental chambers must also be capable of keeping the room within tight tolerances of humidity and temperature for a wide range of extremes that represent the diurnal conditions in a temperate climate.

CHAPTER II

LITERATURE REVIEW

Pollen Structure and Biology

Wall Stratification and Surface Features

Angiosperm pollen grains resemble other types of plant cells, having their cytoplasm enclosed by a plasmalemma and an outer wall of cellulose. In pollen grains, the cellulose wall is known as the intine and is surrounded by an outer wall called the exine (Iwanami et al. 1988). The exine is composed of sporopollenin, a complex polymer of carotenoids and carotenoid esters with oxygen, together with small quantities of polysaccharides, known as the glycocalyx. (Brooks and Shaw 1968, Rowley et al. 1981). Sporopollenin is relatively inert, being resistant to chemicals such as hydrogen fluoride and concentrated sulfuric acid, but may be affected by severe oxidizing reactions (Moore et al. 1991).

The exine typically consists of two layers: an inner, basal layer called the nexine (or "non-sculptured part"), and an outer layer called the sexine (or "sculptured part") (Moore et al. 1991) (Fig. 1). The sexine is composed of a set of radially directed rods supporting a roof which may be complete, partial or absent. Based on the classification of Reitsma (1970), the roof, containing the exterior sculptured elements, is called a tectum. Any rod supporting the tectum is termed a columella, and a rod not supporting anything is called a baculum. In Fig. 1, the system by which pollen wall layers are named in (a) differs from that of (b). System (a) is derived from the appearance of pollen wall layers after staining with fuchsin, rather than the morphological differences between them (Fægri 1956, Fægri

et al. 1989). Differences in staining characteristics may result from the way in which the sporopollenin of the exine is deposited (Moore et al. 1991). In the ektexine the sporopollenin is laminated radially, with this pattern disappearing as it consolidates. However in the endexine, sporopollenin is deposited tangentially and retains its characteristic lamination pattern even when the grain is mature.

Although a chemical and developmental division of the exine into ektexine and endexine seems logical, it is difficult to distinguish these layers with a light microscope (Kapp 1969). Moore et al. suggest that a foot layer (nexine 1) is not always present and is often difficult to distinguish from the endexine (nexine 2). Therefore, morphological divisions of the exine are more commonly employed as terminology when describing pollen grains. This system was first proposed by Erdtman (1966a) and was modified by Reitsma (1970). A morphologically-based system of naming exine layers is of use to palynologists and pollination biologists, whose main concern is describing the characteristics of the pollen grain exine in order to identify or compare it to others.

The fine structure of the exine of angiosperm pollen may have several adaptive functions. First, features of exine sculpture in entomophilous flowers may play a role in electrostatic charge sharing (Erickson and Buchmann 1983, Chaloner 1986). It is suggested that a prominent exine ornament can physically separate charged pollen from the surface to which it is attracted (the bee or stigma). This delays charge sharing and prolongs the adherence of pollen to such a charged surface. A second reason for exine ornamentation has to do with its capacity to carry sporophytically or gametophytically derived materials (Heslop-Harrison 1976). In certain plant families, the intra- or interspecific compatibility control system between pollen and stigma is mediated through

sporophytic proteins conveyed in the cavities of the exine. In addition, intine-borne enzymes, carried on the exine, function in the penetration of the stigma and early pollen tube growth. (Heslop-Harrison 1976). The fine structure of the exine also has some relation to the "stickiness" of a given pollen (Hesse 1981). For pollen grains without a tectum, sticky pollen results from the deposition of a great amount of homogeneous, electron-dense pollenkitt (Knoll 1930, Pankow 1957) between the columellae. For grains with a tectum, sticky pollen results when the same type of pollenkitt is deposited on the exine surface as well as in the exine cavities. In contrast, powdery pollen in angiosperms results when heterogeneous, electron-transparent pollenkitt is deposited in tiny amounts between the columellae or as a thin film on the surface of the tectum. Finally, the surface ornamentation of pollen grains may also affect their collection by honey bees (*Apis mellifera* L.). For example, the length of spines on the surface of upland cotton pollen grains, *Gossypium hirsutum* L., physically interferes with the pollen aggregating process used by bees thereby precluding foragers from collecting it in appreciable quantities (Vaissière and Vinson 1994).

Other surface features of pollen grains, as described by Iwanami et al. (1988), serve to increase the probability of successful dispersal and pollination. Pollen produced by members of the Onagraceae and *Rhododendron* spp. possess cord-shaped structures called viscin threads, which serve to attach grains to insect legs. Several viscin threads occur per grain, each thread having one end attached the exine surface and the other free. The threads often form twisted, branching strands which arise in clumps and fuse along their length. Viscin threads hold large numbers grains together from dehisced anthers, thereby facilitating increased pollen transfer to an insect vector. Other thread-like

structures, known as exinal connections, are found on the pollen grains of some Leguminosae. Only one or two exinal connections occur on each grain, with the structures being absent on others. Ends of the exinal connection are attached to adjacent grains, thereby holding small numbers of them together for more efficient pollen transfer. In contrast, pollen grains produced by many gymnosperms, such as members of the *Pinaceae*, possess a pair of sacs (sacci) to enable their dispersal by wind. While dispersing, these grains shrink because of dehydration, but upon contact with a stigmatic surface they become fully expanded after resorbing moisture. The shapes of the sacci vary among types of pollen, and by comparing the relative distances between the grain body and the sacs, species can be identified.

Another important feature associated with pollen grains are their characteristic apertures, also known as pores or furrows. An aperture is a site in the pollen wall where sporopollenin is deposited in thin sheets or granules, thereby exposing the underlying intine (Stanley and Linskens 1974). Two basic types of pollen grains can be distinguished based on their apertures. Porate pollen grains have round apertures in their exines, and colpate (sulcate) pollen grains have long furrows called colpi (sulcae); those with colpi and pori combined in the same aperture are termed colpporate (Iwanami et al. 1988). Some species have the central areas of the aperture membrane surrounded by granules. These structures are known as opercula (aperture caps), and are often lost as a pollen grain expands with moisture. Apertures are the sites through which pollen tubes germinate, and also permit the grain to expand after moisture uptake (Wodehouse 1935). At a later stage in the pollination process, apertures also provide a pathway for the mobile gametophytic fractions of the intine domain (Heslop-Harrison 1976). For honey bees,

apertures further provide the site at which pollen grain digestion is initiated (Peng et al. 1985).

Contributions of the Tapetum

Early in the development of pollen grains, sporogenous tissue inside the anther is enclosed by a nutritive cell layer known as the tapetum. Two types of tapeta exist: secretory and amoeboid, the former being more common in angiosperms (Pacini and Franchi 1991). Secretory tapeta is characterized as having cells that maintain a continuous position along the inner lining of the locule (cavity of the anther) which eventually become disorganized, undergoing autolysis (Moore et al. 1991). In amoeboid tapeta, cell walls break down at a very early stage, forming a plasmodium associated with the microsporocytes (pollen mother cells). A distinguishing feature of secretory tapeta is the production, on the inner locular surface, of spheroidal particles known as orbicules or Übisch bodies (Brooks and Shaw 1971). The orbicules are released into the locular fluid as the walls of the tapetum break down, and gain a covering of sporopollenin with ornamentation similar to that of the mature pollen grains (Rowley 1963, Echlin and Godwin 1968). As the locule wall shrinks at dehiscence, the orbicules approach each other to form a non-wettable surface on the inside of the cavity, so that pollen grains can more easily be dispersed (Heslop-Harrison 1968a, Heslop-Harrison and Dickinson 1969, Keijzer 1987b).

The tapetum is responsible for producing many substances, including locular fluid, exine precursors, callase, sporophytic proteins and enzymes, viscin threads and the orbicules (Pacini et al. 1985, Pacini 1990). However, the tapetum serves its primary

function only after the microspores exhaust their individual food reserves. At this time, the tapetal plasmalemma breaks down and the cytoplasmic contents are emptied into the locule (Shivanna and Johri 1989). These contents serve as food precursors for the young microspores. Just before anther dehiscence, many substances are coated on the exine of the mature pollen grain from the disintegrating tapetum. These substances are collectively known as pollenkitt or tryphine, and comprise a highly hydrophobic layer surrounding the exine of mature grains (Stanley and Linskens 1974). Tryphine, in the more recent literature, has been distinguished as a separate substance from pollenkitt. Tryphine is a complex mixture of hydrophilic and hydrophobic substances, often containing cytoplasmic elements (degenerated organelles). It is formed by the *extra situm* degeneration of tapetal cell protoplasts (Pacini et al. 1985, Pacini and Franchi 1991). Pollenkitt, however, is entirely hydrophobic, comprised of lipids and species-specific carotenoids (Dickinson 1973). It is formed by the *in situ* degeneration of products in the tapetal cytoplasm (Pacini and Franchi 1991). The endoplasmic reticulum and plastids of tapetal cells are known to participate in pollenkitt formation. Both of these organelles deposit precursors within vesicles, which fuse to form pollenkitt as the protoplasts degenerate (Weber 1992). In addition to carotenoids, other major classes of neutral lipids found in pollenkitt include hydrocarbons, terpenoids, fatty acids, sterol esters and glycerides; polar lipids are restricted to the internal domain of pollen grains (Dobson 1988). Proteins (Abadie and Hideux 1979) and polysaccharides (Klugness and Peng 1984) are also present in the pollenkitt. Some of the proteins produced by the tapetum are those responsible for pollen-stigma recognition (Heslop-Harrison 1987).

Pollenkitt is omnipresent in angiosperms, and is lacking in gymnosperms (Hesse

1984). Pollenkitt lipids are readily held in the cavities of the exine, and act as adhesives if sufficient amounts are present to fill in the interbacular spaces and flow between the pollen grains (Heslop-Harrison 1968b). It has been suggested that the dissolved pigments in pollenkitt, which make pollen conspicuous, serve to guide pollinating insects and protect the generative nucleus against mutagenic effects of ultraviolet rays (Stanley and Linskens 1974, Willemsse 1985). In addition, the material serves to adhere pollen grains to the bodies of insect pollinators (Pacini and Franchi 1991). Pollenkitt components also provide olfactory cues for pollinating insects (Hügel 1962, Lepage and Boch 1968, Hopkins et al. 1969, Dobson 1987), which will be reviewed in detail, later.

Dehiscence, Number and Size of Grains

In angiosperms, pollen is shed through openings in the anther-sac walls. The most common form of shedding is through longitudinal dehiscence, in which pollen is released through a tear in the stomium (the site of anther opening) along the length of the thecae (anther halves or lobes) (Buchmann 1983). In porose dehiscence, pollen is discharged through apical slits, valves or pores, often after a cap has opened at the distal end of the anther sac; other forms of dehiscence have structures intermediate between pores and longitudinal slits (Schmid 1976). Some anthers are non-dehiscent, relying on insects to transfer pollen or entire anthers to the stigmatic surface, and in a small number of tropical flowers, insects liberate pollen by squeezing the anthers or manipulating the anther filament (Fægri and van der Pijl 1979).

The process of longitudinal dehiscence is well described by Keijzer (1987a) and Keijzer et al. (1996). For dehiscence to occur, a mechanically-active tissue layer known

as the endothecium must differentiate thickened secondary cell walls. The endothecium completely surrounds the loculi, except at the stomium where the cells are thin-walled. Dehiscence begins when all or part of the anther tissues dehydrate, under the influence of environmental temperature and relative humidity (Yates and Sparks 1993, Bianchini and Pacini 1996). The cells of the wall separating the anther sacs (the septum) then dissociate by enzymatic lysis, and the tapetum becomes mechanically ruptured by the expansion of the pollen mass. The stomium is opened by the inward bending of the two adjacent locule walls, caused by water uptake in the epidermis and endothelial cells. The locule walls then bend outward due to evaporation in the same layers, resulting in an even distribution of pollen over the surface of the desiccated anther.

Pollen production per anther varies greatly with species as well as plant age and environmental conditions. Plant height and anther size are also known to be correlated with the amount of pollen shed, and differences also exist between varieties, years and climates (Stanley and Linskens 1974). In general, anemophilous (wind pollinated) flowers produce much more pollen than entomophilous (insect pollinated) flowers, with hydrophilous (water pollinated) species producing the least (Shivanna and Johri 1989). For example, a single pine floret can produce about 1.5 million grains of pollen, for a total production per tree exceeding 4.5 billion grains. The number of grains produced per anther in most entomophilous flowers, however, ranges from several hundred to a few thousand, but may be as great as 10,000 in species such as *Camelia japonica* L. (Iwanami et al. 1988). In contrast, the hydrophilous species *Vallisneria spiralis* L. produces as little as 72 to 144 grains per plant (Stanley and Linskens 1974). Among species of pollen flowers (those producing no nectar) greater quantities of pollen are produced than those

species offering both nectar and pollen as a reward (Baker and Baker 1979). A more biologically relevant index of pollen production is the quantity of grains produced per viable ovule. Based on this measure, the ratios for wind and insect pollinated species are relatively similar (Fægri and van der Pijl 1979).

Pollen grain size varies considerably among species, but appears to be dependent on its transport mechanism and its role in post-pollination processes. The relationship between pollen size and post-pollination processes is well supported by interspecific correlations between the size of grains and such pistil characteristics as style length, stigma depth and papillae size (Heslop-Harrison 1981, Cruden and Lyon 1985, Plitmann and Levin 1983, Williams and Rouse 1990, Kirk 1993). Abiotic transport mechanisms also appear to have influenced pollen-size evolution. For example, species that are pollinated within water currents (hyphydrophily) have long, linear pollen to enhance deposition in a relatively viscous medium (Ackerman 1995). One of the largest and most unusual types of pollen belongs to a hyphydrophilous plant called eelgrass (*Zostera marina* L.) (Fægri and van der Pijl 1979). Its thread-like pollen grains are 2-3 mm in length and have the ability to twist rapidly around any narrow object in their path (such as the stigma). In contrast, most anemophilous plants produce pollen within the range of 17 - 58 μm in diameter, while zoophilous (animal pollinated) species range between 5 - 200 μm (Wodehouse 1935). Anemophilous species have a smaller range of pollen sizes because they must “compromise” between the benefits of small pollen for removal and transport, and the deposition benefits of large pollen (Niklas 1985). The greater range of sizes for zoophilous species reflects the lack of such constraint, and presumably, the wide array of zoophilous transport mechanisms and floral morphologies. In contrast to the

identified functions of pollen size in abiotic pollination, however, the effect of pollen size in zoophilous pollination systems is largely unknown (Harder 1998).

If one examines the size of pollen specifically collected by bees, 97 % of grains have dimensions between 10 and 100 μm , with an overall mean of 34 μm (Roberts & Vallespir 1978). Examples of bee-collected species with very large grains ($> 100 \mu\text{m}$) include morning glory (*Impomoea purpurea* (L.) Roth), pumpkin (*Cucurbita pepo* L.) and flower-of-an-hour (*Hibiscus trionum* L.), while intermediate-sized grains ($50 < x < 100 \mu\text{m}$) are produced by yellow evening-primrose (*Oenothera biennie* L.), corn (*Zea mays* L.) and fireweed (*Epilobium angustifolium* L.) (Crompton and Wojtas 1993). Examples of small ($10 < x < 20 \mu\text{m}$) pollen grains are those produced by bird's-foot trefoil (*Lotus corniculatus* L.), blueweed (*Echium vulgare* L.) and golden-cress (*Alyssum saxatile* L.), while very small grains ($< 10 \mu\text{m}$) include forget-me-not (*Myosotis alpestris* L.) and bluebell (*Mertensia asiatica* (Takeda) Macbr.) (Iwanami et al. 1988).

Variation in the size of mature pollen grains among plant species may also be affected by the process by which pollen grains differentiate. For most species, four microspores from a pollen mother cell separate from each other to develop into individual pollen grains. In others, the tetrads of developing pollen grains do not separate but remain attached to each other, even when they are fully developed, to form a large mass of four to sixteen grains (Shivanna and Johri 1989). Such masses are common in the Lauraceae and Ericaceae and are typical of such species as azalea (*Loiseleuria procumbens* (L.) Desv.), rhododendron (*Rhododendron canadense* (L.) B.S.P.) and *Vaccinium* spp. (Crompton and Wojtas 1993).

Several investigations have addressed the question of whether pollen grain size is an

adaptive trait, selected for by the actions of bees. Harder (1998) examined whether the grooming behaviour of bees influenced the evolution of pollen size. He suggested that the pollen collection by bees was fundamentally different than pollen transport by many other pollinators, because bees actively groom pollen from their bodies for subsequent consumption, rather than incidentally transporting it. Harder postulated that selection in bee-pollinated plants should favour smaller pollen, because plants would achieve higher reproductive success if grains were more difficult to groom off a bee's body. However, after examining pollen grain size in several bird and bee-pollinated syndromes, Harder (1998) concluded that interspecific-pollen size variation in angiosperms was more indicative of the differences in conditions for pollen germination, pollen-tube growth and ovule fertilization than the transport conditions provided by the pollinator. In another study, Roberts and Vallespir (1978) examined the specialized pollen collection structures from several species of Apoidea. Their results indicated that the size of angiosperm pollen had influenced the morphology of pollen-collecting structures in bees, but there was no evidence to conclude that the evolution of bees had in any way influenced the size of pollen grains. In an additional study, Baker and Baker (1979) examined 990 species of angiosperm pollen and provided indirect evidence supporting the adaptive significance of grain size for bee-pollinated plants. For those plants offering pollen as part or all of a reward, Baker and Baker concluded that bees and flies selected for oil-containing (starchless) pollen, and furthermore, that such oil-rich grains were smaller than those containing starch. Baker and Baker (1979) also determined that the tendency toward starchlessness and small pollen grain diameter was most extreme in those species requiring vibratile ("buzz") pollination, in which pollen is liberated through small pores

in the anther.

Inherent variation in pollen size may be related to a number of internal and external influences. The average size of pollen grains can be positively correlated with chromosome number, which is sometimes used as a direct measure of ploidy (Kapadia and Gould 1964). Stanley and Linskens (1974) reviewed several other factors that affect the size and variability of pollen grains produced. For example, changes in pollen size may occur among individual flowers on the same plant, between wild versus cultivated species, and at different times during the flowering season. Various sizes of pollen may also be associated with specific height classes of anthers within a species. In addition, external conditions such as high temperatures tend to increase pollen size, and good mineral and water allocation are necessary to prevent plants from developing abnormally small pollen grains.

Pollen Odours and Attractants

Foraging honey bees show preferences for the type of pollen that they collect in natural settings (Linsley & McSwain 1947, Bohart 1957, Nye & Mackensen 1965, Olsen et al. 1979, Jay & Jay 1984, Free 1993) and controlled choice experiments (Levin & Bohart 1955, Doull 1966, Wahl 1966, Boch 1982, Schmidt 1982, Boelter & Wilson 1984). Karl von Frisch (1923) also recognized the pollen-collecting preferences of *A. mellifera*, and focused his attention on the odour produced by pollen in several floral species. Von Frisch (1923) noted that to a human observer, pollen odours are distinct from, and generally more intense than, the odour produced by the same plant's petals. Similar investigations by von Aufsess (1960) showed that among 17 species of flowers

visited by insects, 13 had pollen odours stronger and quantitatively different from the rest of the flower, two had pollen odours less intense but of a different “quality”, and two species had pollen odour similar to their floral odour, but stronger. Honey bees were also shown to perceive qualitative differences between pollen and flower odours produced by *Polyantha*-Rose hybr. ht. (Wildfire) and *Oenothera fruticosa* L. (von Aufsess 1960). In addition, distinct pollen odours used by pollinating insects have been described from species of *Dodecatheon* (Primulaceae) and *Solanum* (Solanaceae) (Buchmann et al. 1977, Buchmann 1983). Pollen odour also plays a major role allowing the pollen beetle *Meligethes aeneus* F. to find its cruciferous hosts (Charpentier 1985), and halictine bees are capable of locating their host, pollen-flowers (Dilleniaceae), using pollen odour alone (Bernhardt 1986). Finally, olfactory cues for oviposition by the sunflower moth, *Homoeosoma electellum* (Hulst), can be provided by sunflower (*Helianthus annuus* (L.)) pollen, or an ethanol extract thereof (Delisle et al. 1989).

The characteristic odour that many pollens possess is capable of guiding honey bees to flowers (von Frisch 1923). Honey bees foraging on plants which possess strong pollen odours also use these fragrances for communicating in the hive. Pollen foragers in the hive often turn towards a homecoming worker from distances of 2 cm, even before her dance begins, and excitedly examine the pollen in her corbiculae with their antennae (von Frisch 1967). The relative importance of pollen odour in relation to the fragrance produced by other floral structures was shown by von Frisch (1923) in an experiment with roses (*Rosa moschata* hybr.) and Canterbury bells (*Campanula medium* L.). From a single honey bee colony, a group of numbered bees collected pollen from roses, while 8 m away a second group collected pollen from Canterbury bells. Pollen foraging was then

interrupted and all of the flowers were removed. Next, roses, whose stamens had been removed and replaced with fresh *Campanula* pistils (with copious adherent pollen), were put at the rose feeding station. A scout bee then collected *Campanula* pollen from the modified roses, flew back to the hive, and by dancing, dispatched six of seven bees that had previously worked *Campanula* flowers. These workers flew to the *Campanula* station, where now, there was nothing to be obtained. However, none of the bees that had previously worked the roses were recruited, and they remained in the hive. The reciprocal experiment produced exactly the opposite effects. The results of this and other of von Frisch's experiments demonstrate that honey bees can differentiate between the odour of pollen and that of the flower, and can be conditioned on each separately.

Several studies have documented the importance of pollen-based compounds for the selection and consumption of pollen by honey bees. Levin and Bohart (1955), using an outdoor array containing six different pollen sources, concluded that the only parameter associated with the selection of pollen by foragers was the intensity of its odour. Subsequent investigations identified the first pollen-based attractants as phytosterols or steroids, extracted using benzene or diethyl ether (Louveaux 1959, Hügel 1962). Taber (1963) showed that hexane or diethyl ether extracts of pollen contained substances that are very attractive to foraging honey bees. The addition of these extracts to cellulose stimulates foragers to pack this non-nutritive substance into their corbiculae. When such attractants are removed from pollen, bees do not collect it, even when it contains over 97% of the total dry weight and most nutritive substances. Robinson and Nation (1968) compared the consumption of an artificial diet, inside the hive, to which extracts of pollen had been added. They found that the addition of the acetone soluble lipid fraction

increases the amount of food taken. Similarly, Hohmann (1970) determined that extracts of alder and hazel pollen could elicit “vivid” dancing and intensified foraging among honey bees collecting powdered cellulose as a pollen surrogate. Doull and Standifer (1970) reported that some of the attractant properties of clover pollens could be isolated in chloroform-methanol extracts. Doull (1974) found that when a benzene extract of almond pollen was added to a pollen supplement within the hive, it was preferentially visited by 90% of bees over one that was untreated. Schmidt (1985) examined several species of pollen and found that consumption was influenced by the cumulative effects of phagostimulants present in extracts prepared with a range of solvent polarities. Singh et al. (1999) examined the flabellogustatory responses of *A. mellifera* and *A. dorsata* to pollen lipids from several bee-preferred and non-preferred pollen sources. They showed that preference for certain pollen species may be related to stimulatory or inhibitory effects of pollen lipids on bee flabellar receptors. The presence of phagostimulants or phagodeterrents in pollen lipids is further supported by studies documenting pollen consumption preferences of bees (Synge 1947, Purdie and Doull 1964, Campana & Moeller 1977, Boch 1982, Schmidt 1984, Schmidt and Johnson 1984, Schmidt et al. 1995), particularly where such preferences have no clear nutritional basis.

It is likely that the odour-producing components of pollen are associated with the oily pollenkitt that surrounds grains (Fægri and van der Pijl 1979, Buchmann 1983, Bernhardt 1986). Most plant volatiles are lipid soluble, and in some species pollenkitt has been known to undergo a change in consistency and volatilization with time (Gori 1983). The presence of odour-producing compounds in the pollenkitt would allow them to be easily dispersed in the air and detected by pollinators (Dobson 1988). Early

attempts to examine the attraction of honey bees to pollenkitt extracts characterized compounds only into broad organic classes (Louveau 1959, Hügel 1962, Taber 1963, Hohmann 1970). The only researchers to identify a discrete honey bee foraging attractant from pollenkitt were Lepage and Boch (1968), who isolated a novel fatty acid from a mixture of clover pollens (*Melilotus*, *Trifolium*, *Lotus spp.*). The compound, (*E*)-2-(*Z,Z*)-9,12-octadecatrienoic acid, is unique because of the conformation of the double bond in the Δ^2 position (Hopkins et al. 1969, Starrat and Boch 1971). This C_{18} fatty acid is also structurally similar to the major components of honey bee queen mandibular pheromone: 9-keto-(*E*)-2-decenoic acid (9ODA), (*R*)-(-)-9-hydroxy-(*E*)-2-decenoic acid (-9HDA) and (*S*)-(+)-9-hydroxy-(*E*)-2-decenoic acid (+9HDA) (Winston and Slessor 1992).

Discerning the relative importance of a flower's pollen odour in relation to the whole floral fragrance is crucial to understanding host-plant recognition in pollinating insects. For the nectarless *Rosa rugosa* Thunb. (Rosaceae), the whole floral fragrance is dominated by compounds emitted from the petals (monoterpene and aromatic alcohols), while the pollen shows an overall different and complex assemblage of volatiles (principally eugenol and methyl eugenol) (Dobson et al. 1990). Further analysis of this plant shows that the major pollen volatiles can be extracted from the pollenkitt alone (Dobson et al. 1987). Observations of bumble bees (*Bombus terrestris* L.) foraging on *Rosa rugosa*, indicate that at long range the absence of pollen does not decrease the flower's attractiveness, but at close range, only the presence of pollen or a synthetic pollen odour initiates landing and foraging responses (Dobson 1991). Among several species of the nectariferous Compositae, pollen volatile profiles are predominantly comprised of monoterpenes, in proportions similar to those of the whole flowers (Dobson

1991). Solitary bees, *Colletes fulgidus longiplumosus* Stephen, distinguish their Compositae host on the basis of odours from whole flowers, pollen or pollenkitt (Dobson 1987). However, other nectariferous flowers such as *Ranunculus acris* L. (Ranunculaceae), have dissimilar odour profiles (Bergström et al. 1995). As a result, the solitary bee *Chelostoma florissomme* L. (Megachilidae), a specialist on *Ranunculus* flowers, can recognize its host plant if offered a choice of pollen odours, but not if offered flower odours (without pollen). This indicates that pollen is providing the insect with a key recognition stimulus, within the full floral odour bouquet. Therefore, pollen odour is likely used by many pollen-foraging insects to discriminate among plant species, and to assess reward availability within individual flowers (Dobson et al. 1996).

Aside from the work of Dobson, the relationship between pollen and flower odours has not been well investigated. Many studies have identified floral volatiles, but few have attempted to separate out the contribution of pollen from the flower as a whole. In such studies, any insect attractant found among floral volatile compounds could be partially, or exclusively, pollen-based. For example, honey bee foraging attractants and repellents have been identified (linalool, 3-octanone, methyl salicylate) from whole-flower volatiles of alfalfa (*Medicago sativum* L.). Such compounds may have important uses for plant breeding purposes, as they enhance the attraction of cultivars to foraging honey bees (Buttery et al. 1982; Henning and Teuber 1992a, b; Henning et al. 1992). In sunflowers (*Helianthus annuus* L.), differences in the volatile profiles from whole-head extracts are suggested to account for the ability of honey bees to discriminate among different genotypes and flowering stages (Pham-Delègue et al. 1989, 1990). In oilseed rape (*Brassica napus* L.), honey bees (Pham-Delègue et al. 1993, 1997; Blight et al.

1997; Le Métayer et al. 1997) and cabbage seed weevils (*Ceuthorrhynchus assimilis* Payk.) (Blight et al. 1995) are also capable of discriminating volatile plant metabolites. Fractionation of headspace volatiles from *Cucurbita maxima* Duchesne (Cucurbitaceae) led Anderson and Metcalf (1986) to identify an attractant for *Diabrotica* and *Acalymma* spp. of beetles, and similar techniques allowed Lewis et al. (1988) to identify compounds attractive to several species of fruit fly (*Dacus* spp.) from the fruit-fly lily (*Spathiphyllum cannaefolium* L.). In addition, Bergström et al. (1991) identified fatty acid esters from the floral volatiles of *Eupomatia* spp. (Eupomatiaceae), which are suspected to serve as attractants for the plant's highly specific and mutualistic pollinating weevils (*Elleschodes* spp.). As the efficiency of analytical techniques improves, the number of insect foraging attractants identified, pollen-based and otherwise, is likely to proliferate.

Pollen Foraging by Honey Bees

Behaviour of Pollen Collectors

Pollen is an essential resource for honey bee colonies and many foragers specialize in its collection. In healthy, queen-right colonies, foraging is typically performed by older workers. Initial foraging flights occur on about day 23 of a worker's life (Winston 1987), and most foragers die after collecting food for 7 - 8 d (Neukirch 1982, Visscher and Dukas 1997). The life of foragers may be related to the accumulated distance that they have flown, as nectar foragers die after flying approximately 800 km, irrespective of the time needed to fly the distance. The mechanism causing this process may be related to the reduced ability of older foragers to synthesize glycogen (Neukirch 1982).

However, more recent evidence indicates that bees outside the colony experience a

constant probability of death per unit time, implying that age-independent factors such as predation play a strong role in the survivorship of foragers (Visscher and Dukas 1997). To compensate for the death of older foragers, approximately 10% of the foraging population is replaced by novice foragers each day (Sakagami and Fukuda 1968, Dukas and Visscher 1994, Seeley 1995).

In a typical honey bee colony containing 20,000 - 40,000 bees, 25 % of the adult workers are foragers, of which 80 % are actively employed in the collection of food during daylight hours (Seeley 1995). Not all honey bee foragers collect pollen: in fact, most specialize in the collection of nectar. Studies that have examined the division of labour in colonies show that about 58% of foragers collect only nectar, 25% collect only pollen and 17% collect both nectar and pollen (Parker 1926, Free 1960). This specialization of tasks is not arbitrary, but is influenced by genetic composition of individual workers, and the behaviour of nestmates through their effects on the shared colony environment (Robinson and Page 1989; Oldroyd et al. 1991; Page and Robinson 1991; Robinson 1992; Calderone and Page 1992, 1996; Hunt et al. 1995; Page and Fondrk 1995; Page et al. 1995, 1998; Dreller 1998; Fewell and Bertram 1999; Pankiw and Page 1999). During a single foraging trip, foragers visit anywhere between 1-500 flowers to gather sufficient quantities of pollen and make an average of 10 to 15 trips per day (Winston 1987).

While in flight, foragers groom pollen from their bodies and form it into pellets which are transported back to the colony in their corbiculae (Hodges 1984). The two pellets gathered by a forager are collectively referred to as a pollen load. Foragers make 10 to 15 foraging trips per day, spending an average of 10 minutes (but sometimes up to

187 min.) to collect a pollen load, which weighs between 10-30 mg (Park 1922, Parker 1926, Maurizio 1953). Approximately ten pollen loads are necessary to provide enough protein to rear one bee, and 2 million pollen loads (20 kg of pollen per year) are needed to rear all the brood of a strong colony (Todd and Bishop 1941, Crailsheim et al. 1992, Seeley 1995). Other studies have estimated that colonies require between 15-30 kg of pollen a year, although workers in some colonies may collect as much as 55 kg (Eckert 1942, Schaefer and Farrar 1946, Hirschfelder 1951, Louveaux 1958, Seeley 1985a).

The great diversity of flowers in nature leads to several different ways in which pollen may be presented to foragers. Depending on the way in which honey bees work to collect pollen, flowers are classified into five different groups: open flowers, in which a worker bites the anthers with her mandibles and uses her forelegs to grasp them and move them toward her; closed flowers, where a worker forces the petals apart with her forelegs and gathers pollen on her mouthparts and forelegs; tubular flowers, in which a bee pushes her proboscis into the corolla to search for nectar, with pollen transferring to her mouthparts and legs; spike or catkin flowers, where a worker runs along the flower shaking off pollen onto her body; and presentation flowers, where a bee presses her abdomen against the inflorescence causing a pollen mass to be ejected (Parker 1926).

Pollen-collecting workers deliberately scabble over the anthers of a flower to transfer as much pollen as possible to their bodies. Most pollen is groomed off, but some typically remains on the back of the head, the central dorsal part of the first thoracic segment and the first and second abdominal segments (Lukoschus 1957). Generally, twice as much pollen accumulates on a bee's thorax than its abdomen, with lesser amounts on the head (Free 1993). Pollen foragers have greater amounts of pollen on their

bodies than nectar foragers, and for this reason, they are more successful at pollinating flowers even in those situations where their foraging behaviour is not more conducive to pollination (Free and Williams 1972, Free 1993). Pollen foragers are as likely as nectar foragers to pollinate flowers when their heads make contact with the stigmas, but pollen foragers are more likely pollinate than nectar foragers when the remaining portion of their bodies do so (Free and Williams 1972). Although many nectar foragers collect the pollen that has incidentally transferred to their body by combing and pressing it into their corbiculae, others scrape it from their bodies and discard it. Discarding behaviour has been recorded from plant species that produce abundant pollen, such as *Helianthus annuus* L., *Taraxacum officinale* Weber, *Rubus idaeus* L. and *Brassica napus* L., where pollen rejection is easily observed (Synge 1947; Free 1968a, b; Free and Nuttall 1968); similar, but less obvious behaviour may occur on species where pollen is less plentiful (Percival 1955). Pollen rejection is suggested to occur when nectar-gathering bees have attained a full load of pollen before their honeystomachs are completely filled with nectar (Free 1993). Discarding a heavy load of pollen would allow nectar collection to continue to capacity.

Honey bees exhibit considerable fidelity to a crop or floral patch during successive foraging flights. If pollen rewards are offered continuously over several days, a worker will often visit the same group of flowers at the same time of day, for many days. This effect was demonstrated by Free (1963) in a study using marked pollen foragers, in an area containing a diversity of floral species. Free showed that on the second day of observations, 70-90% of foragers collected the same type of pollen they had during the first day, with this proportion declining to 40-60% after one week. Bees collecting the

most common type of pollen were also the most constant, and no bees were ever observed to collect different pollens at different times of day. If the pollen they were accustomed to collecting was unavailable for a day, most of the pollen foragers did not forage or switched to nectar foraging. Free further determined that switching between species only occurred when the previously collected pollen became scarce or unattractive over long periods of time. Other studies have shown that an individual worker may restrict foraging to a site only a few metres in diameter and continue to visit it all of her foraging life (Free 1966, Levin 1966). Even if the same patch of flowers is not revisited, bees will still tend to forage on the same height of plants on successive visits (Levin and Kerster 1973, Faulkner 1976). Bees also exhibit considerable constancy to a single floral species within individual foraging trips. On average, pollen loads containing more than one species are found in less than 3% of foragers returning to the hive, although multi-species visits as high as 13% have been recorded (Betts 1920, 1935; Brittain and Newton 1933; Percival 1947; Maurizio and Kollman 1949; Maurizio 1953; Free 1963; Stimec et al. 1997). Most mixed loads contain only two species of pollen, but as many as five species have been recorded (Betts 1920, Brittain and Newton 1933, Free 1963).

Honey bees are known to collect pollen from different plants at different times of day (Parker 1926). The peak period of pollen presentation differs widely for different species, and correlations between the time of day when pollen is most abundant and its collection by honey bees have been established (Synge 1947; Percival 1950, 1955).

When anthers of a flower dehisce in the bud, the time the flowers first open will determine the availability of pollen to the bees (Free 1993). Flowers may open for a single day, for many days or may open on successive days, closing each night. The time

of flowering may also vary with the age of the flower (Synge 1947). When time of opening of the flower is not a limiting factor, the time at which the anther dehisces tends to dictate pollen collection (Free 1993). All of a flower's anthers may dehisce simultaneously, or anthers may dehisce individually over a period of several days. Although the amount of pollen produced among species differs greatly, there is no connection between the amount of pollen produced per flower and the tendency for bees to collect it (Free 1993).

While foraging, honey bees receive feedback about the resource they are collecting including such information as the distance flown to a forage site, the distribution of flowers, the relative abundance of food, the duration of intrafloral trips, the handling costs associated with floral morphologies, learning costs, and the weight of food collected. In addition, nectar foragers measure the concentration of sugars in the nectar they imbibe as a measure of quality, and using knowledge of their current floral patch, assess profitability (Seeley 1995). This information enables nectar foragers to behave in a manner that optimizes their collection efficiency (Schmid-Hempel et al. 1985; Kacelnik et al. 1986; Schmid-Hempel 1986, 1987, 1991, 1993; Seeley 1986, 1994, 1995; Wolf and Schmid-Hempel 1990, Dyer and Seeley 1991; Waddington 1980, 1985; Núñez 1982; Varjú and Núñez 1991, 1993). Honey bee foragers do not appear to maximize the rate at which they collect nectar (Stephens and Krebs 1986), but instead maximize their energetic efficiency (Schmid-Hempel et al. 1985; Kacelnik et al. 1986; Seeley 1986, 1994; Wolf and Schmid-Hempel 1990; Dyer and Seeley 1991; Schmid-Hempel 1986, 1993), or rate of information exchange with the colony (Núñez 1982; Varjú and Núñez 1991, 1993). By measuring the net energetic gain associated with a nectar reward per

unit energy expended by a forager, optimal foraging models can be used to predict the movement of foragers between flowers and floral patches, as well as the size of the nectar load gathered. For example, these models predict that bees move short distances and turn frequently within profitable forage patches, but when flowers are less rewarding, change direction less frequently and fly longer distances (Pyke 1978, Waddington 1983a, b). Foragers also change direction between flowers less frequently as handling time increases (Schmid-Hempel 1984), and abandon some non-depleted food sources with a partially filled crop so as to maximize the ratio of nectar load to flight distance (Schmid-Hempel et al. 1985). Although some of the general principles of these models may apply to the floral visitation patterns of pollen foragers, they remain inappropriate for characterizing pollen foraging because of their insensitivity to a proteinaceous currency.

In contrast to nectar foragers, most evidence indicates that pollen foragers are incapable of directly assessing the quality of food they collect. Honey bees have been documented to collect large quantities of pollen or pollen-like substances having little or no nutritional benefit including: sawdust, earth from swamps, coal dust, and animal feed (Root and Root 1935); rotted wood (Haydak and Tanquary 1943); road dust (Morse 1975); coal dust and flakes of paint (Fægri and van der Pijl 1979); epidermal hairs from plants, tar, and tree gum (Johannsmeier 1981); and fungal spores (Shaw 1990). Data from experiments in which foragers were allowed to choose from a controlled array of pollen species also demonstrate that the choice of pollen by foragers is not directly related to its protein content (Levin and Bohart 1955, Schmidt 1982). The discrimination of pollen quality by foragers has, however, been suggested by Waddington et al. (1998), who characterized the dance rate and the probability of dancing by foragers that had

collected either a mixture of several pollen species or the same mixture diluted with cellulose powder. Unfortunately, the conclusions of this study are highly confounded because of the relative differences in pollen odour created by the treatments, as well as other factors affecting the efficiency of pollen collection. Recent findings further suggest that bumble bees forage for pollen in a manner consistent with maximizing the site-specific efficiency of protein collection (Rasheed and Harder 1997a, b). Hence, the perception of pollen quality by honey bee foragers is a matter in need of clarification. In addition, the quantitative and qualitative mechanisms of protein feedback employed by foragers must be elucidated in order that the process of pollen foraging can be modelled successfully.

Based on the energy received from the consumption of pollen, a honey bee is rewarded with 8 calories for every one it spends in foraging flight (Seeley 1995). For nectar foraging the return is even greater (10:1), yet pollen foragers will travel much further to obtain a full pollen load (Seeley 1995). This behaviour may be related to the fact that pollen loads are lighter than nectar loads, and take less time per flower to collect (von Frisch 1967). In addition, colonies do not have large pollen reserves compared to that of honey which may cause workers to travel further for pollen in order to satisfy the constant demand for nutrients imposed by brood rearing (Gary et al. 1972). In agricultural areas, the median foraging radius for pollen collection is only a few hundred metres (Ribbands 1953, Michener 1974), although significant forager populations have been found as far as 3700 m from apiaries (Gary et al. 1972). The method by which bees of adjacent colonies apportion foraging sites in different sections of fields is not well understood (Levin and Glowska-Konopacka 1963). Recruitment from feeding stations

can occur at up to 10 km if no other food sources are made available (Knaffl 1953). In forested areas, the median foraging distance for nectar and pollen is 1.7 km with 95% of foraging sites being located within a radius of 6 km from the colonies (Visscher and Seeley 1982). However, in a suburban environment with dense resources and larger floral patches, most nectar and pollen foraging occurs within 2 km of colonies (Waddington et al. 1994).

Environmental factors affect the pollen collection abilities of individual workers. The major factors affecting honey bee flight initiation are temperature and solar radiation (Burrill and Dietz 1981). Although a worker can fly in winter temperatures only slightly above 0°C, and pollen collection has been observed at temperatures as low as 5°C, foraging activities for both nectar and pollen typically do not occur until about 12-14°C (Szabo 1980, Burrill and Dietz 1981). Even if temperatures are suitable, flight will not occur without suitable light. Honey bees will fly on cloudy days, but stay close to the hive (Phillips 1930). In the morning and afternoon, flight activity is positively related to solar radiation, but when the sun is at its zenith, a negative relationship exists (von Frisch 1967, Burrill and Dietz 1981). Difficulty in communicating the location of food sources when the sun is directly overhead may explain the general decline in mid-day foraging activity, but it is unclear whether a such a decline is also related to the decreased production of nectar by flowers during this period (Winston 1987). Precipitation, and wind speeds greater than the average speed of honey bee flight ($6.3 \text{ m} \cdot \text{sec}^{-1}$), diminish or preclude flight activity (Williams and Sims 1977). Observations of honey bees foraging on apple blossoms show that foraging activity starts to decline above wind speeds of $3.1 \text{ m} \cdot \text{sec}^{-1}$ (Brittain 1933). Environmental conditions directly affect the amount of pollen

collected by foragers by constraining their flight activities, and indirectly affect pollen collection by influencing the amount of pollen made available by plants. For example, a rise in temperature from 10° to 30°C results in greater pollen collection because of a steady increase in the number of stamens ripening and presenting pollen (Free 1993). Light intensity, precipitation and relative humidity also influence pollen production, although their individual effects are not easily separated (Synge 1947, Ribbands 1953, Percival 1955). Pollen collection is also more intense when a favourable day follows an unfavourable one. This response is governed both by tendency of bees to respond to improved conditions as well as an increased need for pollen in the colony (Free 1993).

Foraging behaviour in honey bees is also influenced by the genetic composition of individual workers. Free and Williams (1973) showed that the progeny of different queens that were reared and lived under identical conditions, had different floral preferences. A honey bee colony composed of genetically different subfamilies may have foragers differing not only in their host plant preferences, but also in their temporal patterns of foraging and preferred foraging distances (Oldroyd et al. 1992, 1993). Mackensen and Nye (1966, 1969) and Nye and Mackensen (1968, 1970), showed a genetic basis for the collection and storage of pollen by demonstrating variability in these traits between colonies, and then selecting for high and low phenotypes. A two-way selection of colonies over six generations led to colonies with high and low preferences for alfalfa pollen. In the high category, 86% of the workers visited alfalfa, but in the low category, only 8% of the workers did. These traits were also shown to be polygenic and to have high heritability. Pollen hoarding is another characteristic that can be influenced through selection. After four generations of selective breeding, Hellmich et al. (1985)

obtained colonies that stored anywhere from 2 to 13 times as much pollen as the low hoarding strain colonies. Differences in pollen hoarding between the two strains were only found in the presence of larvae or capped brood (Hellmich and Rothenbuhler 1986). Continued research with high and low pollen hoarding strains of bees reveals that “high strain” workers specialize in pollen collection, while “low strain” workers specialize in nectar collection (Page et al. 1995). “High strain” colonies have a higher proportion of foragers returning with loads of pollen than nectar, however the total number of foragers in “high” and “low strain” colonies is similar (Page and Fondrk 1995). Using the proboscis extension reflex to test the threshold response of bees to varying concentrations of sucrose, Page et al. (1998) and Pankiw and Page (1999) showed that colony-level selection for stored pollen could also alter a forager’s response to sucrose and its corresponding foraging behaviours. Nectar foragers from high hoarding strains respond to lower concentrations of sucrose and are more likely to collect water or dilute nectar than foragers from low hoarding strains. Low hoarding strain foragers have a higher threshold response to sucrose and collect nectar containing high concentrations of sucrose, or none at all. These studies demonstrate how a genotypically varied sensory-physiological process is directly associated with foraging behaviour. Selection for pollen hoarding, however, does not change preferences for the sources of pollen collected by bees, and high strain colonies are successful at improving the pollination of alfalfa and almonds (Gordon et. al 1995). The probability that foragers will independently scout for food is also genetically-based, along with their preference to collect either pollen or nectar (Dreller 1998). Hence, honey bee foragers may be genetically predisposed to gather either pollen or nectar, and collect food from specific host plants.

Attraction to Flowers and Learning

Individual foraging behaviour is guided by the perception and learning of floral cues. Honey bees rely primarily on olfactory and visual stimuli to locate flowers and their rewards (Butler 1951, von Frisch 1967, DeGrandi-Hoffman 1987, Beker et al. 1989, Backhaus 1993, Menzel et al. 1997, Kirchner and Grasser 1998). During orientation experiments, von Frisch (1967) showed that honey bees can detect the odour produced by small groups of flowers within several meters, and Free (1970) discovered that odour is the most important distinguishing feature that bees evaluate when training to flower models. Newly-recruited foragers seeking a food source for the first time rely upon odour, and are unable to locate a food source that is not scented (Wenner et al. 1969, Wenner 1974, Wells and Wenner 1971). In addition, if the target that workers are recruited to lacks significant odour (weakly scented flowers or water), honey bees will mark the site with scent from their Nasanov gland (Free 1968c, Free and Williams 1970). Plants coordinate the production of floral odours with times and temperatures during which their pollinators are most active. For example, the emanation of floral volatiles in alfalfa flowers is photoperiodically induced, and is released in discrete cycles throughout the day when pollinators are most active (Loper and Lapioli 1971). These compounds attract honey bees and allow them to differentiate between different alfalfa clones (Kauffeld and Sorensen 1971, Loper et al. 1974). For many species of bees, the use of odour is most important during close-range orientation, when bees inspect flowers both before and after alighting (von Frisch 1950, Butler 1951, Manning 1957, Galen & Kevan 1980, Zimmerman 1982, Dobson 1991, Lunau 1991). This inspection allows bees to discriminate between floral species, decide whether or not to land, and may influence

their expression of discrete foraging behaviours (McNaughton & Harper 1960; Dobson 1991, 1994; Dobson et al. 1996).

A honey bee's visual orientation toward a flower is guided by achromatic and colour vision (Lehrer 1997). Achromatic signals are mediated using the green receptors of the honey bee ommatidia, and are detected from further distances than colour signals (Giurfa et al. 1997, Giurfa and Vorobyev 1997). The far-distance green (achromatic) signal and close-up colour signal are both used in sequence during the approach to a particular flower (Menzel et al. 1997). Chromatic and achromatic vision are tuned to different angular sizes of objects. Objects subtending a visual angle of less than 15° are detected using green contrast against the background colour, but objects subtending a visual angle greater than 15° are detected using chromatic contrast; flowers subtending an angle of less than 5° are not detected (Giurfa et al. 1996b, Giurfa and Vorobyev 1997). Based on these relationships and assumptions of suprathreshold contrast, a flower having a 45 mm corolla would be detected from its background by a bee at a distance of 52 cm using achromatic vision, and at 18 cm using colour vision (Menzel et al. 1997). The threshold distance at which honey bees detect flowers from their background foliage can be increased by strategies such as combining flowers in dense inflorescences, enlarging the signalling parts of flowers or plants, and by the temporal synchronization of flowering.

Flower colour is determined by the quantity and proportion of floral pigments present in the inflorescence. Principal classes of floral pigments consist of the flavonoids and carotenoids, while minor classes include the chlorophylls, quinones and beta alkaloids (Harborne 1967, 1976, 1982). Honey bees are attracted to flowers that appear blue or yellow to the human eye, and are also able to perceive differences in the

absorption of ultraviolet light by flowers (von Frisch 1950). Ultraviolet absorbing compounds such as flavones and flavonols are present in almost all white flowers and are also found in varying proportions in many others flower colours (Kevan and Baker 1983). Honey bees, though insensitive to red, will forage on red flowers that intensely absorb or reflect ultraviolet light (Harborne 1982). The innate abilities of foragers for discriminating and learning specific colours (zu Oettingen-Speilberg 1949, von Frisch 1967, Menzel 1990) have been extensively utilized to study foraging decisions made by bees (Jones 1978; Waddington & Holden 1979; Wells & Wells 1984, 1986; Giurfa & Núñez 1989; Wells et al. 1992; Banschbach 1994; Giurfa et al. 1994).

In addition to colour, the shape, outline or three dimensional form of a flower can also influence honey bee foraging preferences (Free 1970; Anderson 1977a, c; Wehner 1981; Kevan and Baker 1983; Gould 1985; Lamb & Wells 1995). Honey bees also respond to differences in the pigmentation patterns on flower petals (Anderson 1977b, Wehner 1981, Gould 1986a, Lehrer 1991, Petrikin & Wells 1995) and are sensitive to symmetry of floral structures (Free 1970, Møller & Eriksson 1995, Giurfa et al. 1996a). The size of flowers, inflorescences and floral masses coupled with their movement also provide cues for pollen-foraging bees (Fægri and van der Pijl 1979).

Once bees land on flowers they may receive additional "signposts" about food rewards. Such signals include marks or odours on the flower petals which serve as nectar guides, or prominently displayed, colour-contrasting stamens which advertise the source of pollen (Manning 1956, Free 1970, Jones and Buchmann 1974, Barth 1985, Lunau 1991). Another interpretation of the function of guide marks is that they serve as pollen models, or imitations of an original pollen signal (Vogel 1978, Osche 1979, 1983). Such

a hypothesis is borne out of the evolutionary history of angiosperms, in which the earliest flowering plants had only pollen as an insect food source (Barth 1985). Some of these plants employed infertile pollen or false anthers to attract pollinating insects, while their real anthers remained hidden. Pollinating insects were attracted by these pollen imitations, and while feeding, incidentally transferred the fertile pollen to their bodies.

A final method by which insects may be directed toward a food source after landing on a flower is by tactile cues. The identity of a floral source, or the direction of a food reward, may be discerned by detecting microsculptural features on the petals of flowers (Kevan and Lane 1985). These tactile cues are species specific and may provide a means by which pollinators can discriminate between species of similar morphology. Because these features differ from one end of the petal to another, they may also serve as nectar guides.

As previously discussed, foragers tend to collect only one species of pollen per trip, and will continue to make foraging flights to the same group of flowers as long as the source of pollen remains available (Free 1963). The previous experience of the forager reduces the time spent searching for flowers on successive trips, and eliminates the costs associated with learning different floral handling techniques (von Frisch 1953). Workers quickly learn to orient to the shape, colour and odour of the flowers that they are working (Menzel et al. 1973). Very few visits are necessary to learn such cues; a single experience of an odour with a reward is all that is necessary to induce constancy in 90% of workers (Koltermann 1969), but the same association with colour requires five exposures to achieve a similar level of fidelity (Menzel & Erber 1978). Bees also learn colours that are more likely to be associated with food signals (violet, blue and bee-

purple) at a faster rate than others (Menzel et al. 1973). If visual cues at a flower are different from those encountered previously, foragers do not depart immediately but turn around in flight and look at the feeding place (Lehrer 1991). Bees learn the new conditions during this look-back behaviour, and use this information in their choice of flowers for subsequent visits. During departure from a newly-discovered food source, bees also perform circular flights of ever-increasing size to learn distant signals such as landmarks, celestial cues, and the direction and distance between the food source and colony (Menzel 1993). Once a forager establishes a foraging pattern, the memory of location and visual cues such as landmarks become more predominant (Levin 1966, Wells and Wells 1985). Couvillon et al. (1991) determined that foragers learn about landmarks near a feeding place upon arrival and departure from it, as opposed to learning about certain characteristics only upon arrival (Opfinger 1931), or only upon departure (Gould 1988). Long-distance orientation requires learning of signal sequences and the retrieval of context-specific memories (Menzel 1993). Evidence for this type of learning is provided by studies showing that bees can be trained to a sequence of different visual cues within a single foraging trip (Collett and Kelber 1988). Honey bees apply a hierarchy of best-matching rules to different kinds of learned orientation marks (Wehner and Menzel 1990, Wehner et al. 1990, Kirchner and Braun 1994). Bees orient using celestial cues first (sun compass, polarized light), but if orientation flights produce a mismatch with the direction, sequence or number of long distance landmarks, then the latter are used according to the best match between the learned and currently experienced flight pattern (Menzel et al. 1990, Chittka and Geiger 1995a, b; Chittka et al. 1995). When foragers approach flowers, they home in by a successive search for the best match

with local landmarks (Menzel 1993). Bees may also possess the ability to construct a cognitive map from landmarks within the foraging radius around their hive (Gould 1986b, Gould and Towne 1987, Menzel et al. 1996). This would allow bees to calculate the shortest flight path between two objects, even if this flight path has never previously been experienced. Foragers are capable of retaining the memory of orientation cues by workers for weeks, and in overwintered bees, trained workers can return to the same locale to search for food after 175 days of confinement in the winter (Menzel et al. 1973).

Influence of Colony State on Foraging

Honey bee foragers are uniquely adapted to perceive and satisfy a colony's need for food. Pollen foraging behaviours increase with increases in the amount of brood in colonies (Filmer 1932; Cale 1968; Todd and Reed 1970; Al-Tikrity et al. 1972; Free 1967, 1979; Calderone 1993; Eckert et al. 1994; Pankiw et al. 1998b; Dreller et al. 1999), and within normal increases of unsealed brood, the longevity and average age of initiation of foraging by workers remains unchanged (Winston and Fergusson 1986). Hence, colonies respond to increased amounts of eggs and larvae by increasing the amount of pollen collected by existing foragers, rather than lowering the age at which workers start foraging. Although more extreme changes in colony state may accelerate changes in caste ontogeny, the age of individual workers does not influence their foraging distance or tendency to collect pollen (Gary et al. 1981). Increased rates of pollen intake (Filmer 1932, Dreller et al. 1999) appear to be mediated through a switch in task specialization from nectar to pollen foraging (Free 1967, Eckert et al. 1994), and the collection of larger pollen loads by individual foragers (Eckert et al. 1994).

The relative quantity of pollen stored within honey bee colonies is also perceived by workers, and variations in its level cause changes in the dynamics of foraging. For example, adding pollen or artificial pollen supplements to colonies decreases the amount of pollen collected by workers, until the excess is consumed by nurses (Barker 1971, Free and Williams 1971, Moeller 1972, Fewell and Winston 1992, Camazine 1993, Dreller et al. 1999) Conversely, large decreases in the amount of pollen resulting from the use of pollen traps (Lindauer 1952, van Laere and Martens 1971) or by direct manipulations of pollen stores (Barker 1971, Fewell and Winston 1992; Camazine 1993, Dogterom and Winston 1999) cause increases in foraging until preexisting levels are restored. Food shortages within colonies also result in the acceleration of behavioural development, whereby greater numbers of bees become precocious foragers at an earlier age than in non-starved colonies (Schulz et al. 1998). Fewell and Winston (1992) documented changes in the foraging activities of workers in response to pollen deprivation. In such colonies, pollen intake is maximized by the increased effort of individual foragers, and to a lesser extent, by an increase in the proportion of pollen foragers. Pollen foragers work harder by increasing their rate of foraging, shortening the duration of foraging trips, and collecting heavier loads. Foragers from pollen starved colonies also appear to be less “choosy” than those from supplemented colonies, as indicated by an average decrease in the nitrogen (protein) content of pollen returned to the colony. Food stressed colonies return their pollen stores to original levels within 8-16 days, suggesting that colonies regulate pollen stores around a homeostatic set point (Fewell and Winston 1992, Dogterom and Winston 1999). Camazine (1993) also examined individual and colony-level responses to manipulations of pollen stores and determined that pollen starved

colonies can detect the supplementation of pollen overnight, and respond by restricting their pollen foraging. This is accomplished by having pollen foragers switch tasks to nectar foraging, or not forage at all. If pollen stores are manipulated in a graded manner, by successively increasing or decreasing the amount of accessible pollen within hives every 48 h, colonies do not respond by incrementally adjusting their foraging activity (Fewell and Bertram 1999). Instead, colonies show a pronounced step-wise change in pollen foraging activity as pollen storage levels move above or below a specific set point of quantity. Under these conditions, changes in colony performance take place as a result of the recruitment of new foragers into pollen collection (rather than by task switching), without change in the effort of individual foragers.

The proportion of workers collecting pollen also increases with the egg-laying rate of the queen (Cale 1968). The stimulatory effect that the queen and brood have upon pollen collection is likely under pheromonal control (Jaycox 1970a, b; Todd and Reed 1970; Barker 1971; Free 1967, 1979; Free et al. 1984, 1985; Kolmes and Sam 1990; Pankiw et al. 1998b). Applications of synthetic queen mandibular pheromone increase the number of foragers collecting pollen and the average size of their loads, but not the total foraging rate (Higo et al. 1992). In addition, applications of this pheromone enhance the pollination of several fruit and berry crops by increasing the recruitment of foragers and the duration of their floral visits (Currie 1992a, b; Naumann et al. 1994, Higo et al. 1995). Queen mandibular gland pheromone also plays an important role in regulating the age-related division of labour within colonies. Queen-right colonies supplemented with large amounts of the pheromone suffer reduced rates of foraging because the pheromone suppresses juvenile hormone which in turn delays worker ontogeny (Pankiw et al. 1998a).

A pheromone produced by honey bee brood also influences pollen foraging behaviour. Free (1967) performed a series of experiments using single and double screens within a colony to separate brood and nurse bees from foragers, and allow them different levels of physical contact. He found that foragers separated from the brood could achieve slight increases in pollen collection over broodless colonies, but that contact with nurse bees was necessary to further increase activity, purportedly because of better pheromone transmission. Moreover, Free determined that foragers required unencumbered access to the brood in order to elicit maximum rates of pollen foraging. For normal beekeeping practices, positioning the brood near the entrance of a colony enhances pheromone transmission to foragers and stimulates greater pollen collection than the placement of brood in a more distant position (Free 1979). Extracts of larvae also stimulate pollen foraging behaviour (Jaycox 1970b). In fact, broodless colonies can be stimulated into collecting pollen to the same extent as brood-right colonies by the use of a hexane brood extract, with these effects lasting several hours; a similar extract added to a brood-right colony can boost pollen foraging almost three-fold (Pankiw et al. 1998b). Honey bee brood pheromone has also been chemically isolated and synthesized as a blend of 10 fatty aliphatic esters (Le Conte et al. 1990). These compounds have been shown to affect the chemical communication between larvae and workers (Le Conte et al. 1994), the feeding behaviour of workers tending larvae (Le Conte et al. 1995), and several physiological processes including inhibition of worker ovary development (Le Conte 1990, Arnold et al. 1994) and the development of hypopharyngeal glands (Le Conte 1990).

Pollen collection also increases when colonies are fed with carbohydrate solutions (Free and Spencer-Booth 1961; Free 1965, 1967; Barker 1971; Goodwin 1986; Goodwin

and Houten 1991). Free (1965) found a two-fold increase in the collection of sweet cherry pollen, a three-fold increase for field beans and a five-fold increase in red clover pollen collection after feeding syrup. Similarly, Goodwin and Houten (1991) found that feeding a 2 M sucrose solution increases the collection of kiwifruit pollen to a maximum of eight times that of unfed colonies. When a colony is fed a carbohydrate solution, many of the house bees that usually wait at the hive entrance to collect nectar from foragers, simply collect syrup themselves. As a consequence, nectar foragers have difficulty in finding food handlers to accept their nectar load and switch to pollen collection (Free 1965).

The discovery and collection of pollen is critical to the survival of the colony. In order to maximize the efficiency of pollen collection, honey bees use a system in which a small group of foragers specializes in finding pollen and then recruits other foragers from a larger population within the hive (Seeley 1995). Scout bees locate pollen sources independently and typically account for 10% of the general foraging population. However, their abundance ranges from as low as 5% when good forage is plentiful, to as high as 36% in times of dearth (Seeley 1983, 1995). Scout bees are not of any specific age (zu Oettingen-Spielberg 1949, Dreller 1998), and both novices (Lindauer 1952) and experienced foragers (Seeley 1983, Seeley and Visscher 1988) engage in scouting activities. The choice of collecting either pollen or nectar by scouts is not opportunistic, but appears to be related to the genetic background of the bee (Dreller 1998).

Seeley and coworkers developed the concept of the “information centre” strategy of foraging (Visscher and Seeley 1982; Seeley 1985a, b; Seeley et al. 1991). They found that the foraging pattern of bees is characterized by daily changes in the number of

workers visiting particular floral patches, with individual workers using only a few patches on any one day. In an environment of small, widely-scattered patches of food, colonies employ scouts to monitor a radius of 4-6 km around the hive, equivalent to an area of approximately 100 km². Reconnaissance by scouts results in recruitment only to those flower patches that scouts have visited and deemed to be profitable. Thus colony foraging activity is adjusted on a daily basis as food patches are depleted and new patches of food are found. The colony is used as an exchange centre for the information brought back by scouts and their recruits, so that workers can concentrate their efforts on profitable, high quality resources.

The information centre model assumes that task recruitment is based primarily on worker communication of the task need or opportunity (Seeley et al. 1991, Gordon 1996). This information is provided through the social interactions between workers engaged in the task (nectar or pollen foraging) and workers available to perform it. In contrast, the “foraging-for-work” model suggests that workers evaluate colony task stimuli (e.g. levels of stored food or young brood) directly from the environment of the hive, independent of social interactions (Tofts and Franks 1992, Franks and Tofts 1994). This model states that a worker’s probability of performing a task is directly related to the probability of encountering task stimuli, independent of social interactions. Recent evidence, however, has shown that the foraging flexibility of a colony can be explained by an integrated model incorporating the influence of worker genotype on task choice, and the ability of the colony to amplify its response via social interactions (Fewell and Bertram 1999). Because little evidence exists to support the foraging-for-work model, descriptions of honey bee foraging will be presented in the context of workers receiving task information

through interactions with nestmates.

Seeley (1995) characterized the interactions of honey bee foragers with nestmates and modelled the decision-making processes for allocating labour among foraging sites. He showed that foragers returning to the colony “report” on the profitability of their food source through the strength of their dance. This is accomplished by altering the number of waggle runs (duration) of each dance, and to a lesser extent, by changing the vigour of each waggle run. Foragers do not perform recruitment dances about a food source unless its profitability exceeds a dance threshold. Dance thresholds are adaptively tuned in relation to the colony’s influx of food and the external environmental conditions for its collection. This adaptation allows colonies to exploit a wider range of food sources when food is sparse, and only the most profitable ones when resources are abundant. The criterion that individual foragers use to measure the profitability of a nectar source is the energetic efficiency of its collection (Seeley 1986, 1994). It is not known whether honey bees use a similar currency to assess the profitability of pollen collection, however bumble bees are known to collect pollen in a manner that maximizes their site-specific efficiency of protein collection (Rasheed and Harder 1997a, b). Honey bee foragers independently assess the profitability of a floral patch by integrating information about the energetics of foraging in the patch with variables affecting the threshold for dancing. They then make a decision about whether, and how much, to dance. Potential recruits assess the profitability of a floral source by observing a forager’s dance and may receive additional orientation cues by licking or antennating the odour of pollen on its body. Rather than following the dances of many foragers and selectively responding to the one advertising the best source of food, recruits follow one, randomly-chosen dance before

leaving the hive (Seeley and Towne 1992). In contrast to information being obtained from a well-informed cohort of “supervisors” within the hive, the allocation of bees among floral patches results from the differential persistence and recruitment of foragers to their own food sites, in the absence of knowledge about the profitability of competing food sources (Seeley et al. 1991).

The regulation of the quantity of pollen collected by honey bee colonies has recently been explained using two competing hypothesis (Camazine 1993, Camazine et al. 1998, Pankiw et al. 1998b, Dreller et al. 1999). In the “direct assessment” class of hypotheses, each pollen forager assesses the size of a colony’s pollen supply. Such direct assessment might be accomplished by a forager counting or inspecting the number of cells with pollen, or by evaluating the intensity of pollen odour within the colony. The need for pollen could also be directly assessed by detection of the quantity or production of volatiles from young brood. In an alternate class of hypotheses known as “indirect assessment”, a different group of bees in the colony assimilate information about the need for pollen and then transmit that information to the pollen foragers.

Camazine (1993) and Camazine et al. (1998) proposed a mechanism for regulating the quantity of pollen collected by honey bee colonies based on an indirect assessment hypothesis. They suggested that pollen foragers obtain information about the need for pollen in the form of an inhibitory cue provided within the normal trophallactic exchanges between workers and nurse bees. This cue is the amount of protein fed to foragers, secreted as brood food from the hypopharyngeal glands of the nurses. When pollen stores are high, nurse bees distribute large amounts of protein-rich hypopharyngeal gland secretion to foragers, inhibiting their foraging. Under conditions of dearth, nurses

have little protein to distribute to foragers, thereby stimulating pollen foraging. Hence, pollen collection of foragers is suggested to be driven by a physiological requirement for protein in adult bees (Crailsheim 1986, 1990a). Distribution and concentrations of protein among nestmates, measured using radioactive phenylalanine, support Camazine's hypothesis (Crailsheim 1990a, b; 1991; Crailsheim 1992; Camazine et al. 1998).

Trophallactic interactions between colony members potentially provide them with information about the quantity and quality of food in the hive, and are considered by some to be a form of communication as important as dance language or pheromones (Farina 1996, Camazine et al. 1998, Crailsheim 1998).

Pollen foraging may be also regulated by direct assessment of the quantity of brood and pollen found within a colony. Early studies showed that larval volatiles are important for stimulating pollen foraging, but that foragers require direct physical contact with the brood to further increase foraging rates (Jaycox 1970b; Free 1967, 1979). Recently, Pankiw et al. (1998b) proved that a hexane extract of 2- to 4-day-old larvae (brood pheromone) provides a direct stimulatory effect on pollen collection. Because colonies used by Pankiw et al. (1998b) contained equal amounts of stored pollen, the stimulatory effect seen after applying brood pheromone in the absence of brood contradicts the indirect, inhibitor hypothesis of Camazine (1993). According to the latter hypothesis, the level of protein inhibitor in all broodless colonies should be high because of the absence of larvae to consume it. Hence, foragers should have received sufficient quantities of protein to satiate their protein hunger, thereby suppressing foraging. In a second experiment, Pankiw et al. (1998b) added brood pheromone to queen-right colonies that contained young brood, and left similar colonies untreated. In treated colonies, foraging

rates increased 2.5 times over untreated colonies. This also contradicts the indirect, inhibitor hypothesis which predicts that the foraging rates of both groups of colonies should have remained the same, because they contained the equal amounts of brood and stored pollen.

Dreller et al. (1999) proposed a direct, multifactor hypothesis to explain the regulation of pollen foraging. They showed that several cues, including the amount of young brood, stored pollen and empty comb space in colonies provide important physical stimuli affecting pollen foraging behaviour. Unlike Camazine (1993), Dreller et al. (1999) did not detect an inhibitory signal that could be passed by trophallaxis through a single screen separating nurse bees and foragers in a colony. When foragers were physically separated from the brood but still had trophallactic contact with nurse bees, the addition of eggs and larvae did not increase pollen collection. Increases in pollen foraging were only observed when foragers had direct contact with the brood. Pollen foraging was inhibited when foragers were given access to supplemental pollen and had trophallactic exchange with nurses, but remained separated from the brood. Furthermore, empty comb stimulated pollen foraging, but only when it was placed next to the brood nest. Therefore, the need for foragers to directly physically assess these cues is clearly at odds with the indirect, inhibitory hypothesis for the regulation of pollen foraging, and also weakens support for a pheromonally-mediated system of regulation. Alternatively, a hierarchy of redundant cues may be utilized by foragers.

There is little doubt that the regulation of pollen foraging by honey bees is a topic needing further clarification. Foremost, we do not understand what currency foragers assess while collecting pollen. It is also unknown whether honey bees assess the

nutritional value of the pollen they collect, and if they do, whether such feedback occurs at the individual or colony level. In addition, we have conflicting information about what cues the colony may be using to regulate the collection of pollen to meet colony demand. Further investigation is required to elucidate the mechanisms behind the system of feedback between the colony and its foragers. Only quantitative testing and experimental manipulation of the mechanisms associated with negative inhibition, pheromone stimulation or direct forager assessment will truly answer these questions.

Pollen Utilization by Honey Bees

Storage in the Hive

Pollen foragers returning to the hive place pollen pellets from their corbiculae into cells in the comb. Nest workers moisten the pellets by regurgitating honey and saliva, and pack them into the bottom of cells with their mandibles (Parker 1926). The pollen is then covered by a thin layer of honey, which preserves it for several months, even though the cells remain uncapped. After pollen has been completely processed and packed into cells it is known as "bee bread".

Pollen obtained from the corbiculae of foraging honey bees has good germination potential but stored pollen becomes non-viable in 1 to 8 days, depending on the species (Singh and Boynton 1949, Stanley and Linskens 1974). The lack of viability of stored pollen is related to the activity of several different compounds. In addition to honey, nest workers add a phytocidal acid to stored pollen thereby preventing its germination within the hive (Maurizio 1959). A specific germination inhibitor can also be isolated from the hypopharyngeal glands or alcohol extracts of workers, larval food, royal jelly and honey

(Chauvin and Lavie 1956, Lavie 1960, Pain and Maugenet 1966). This substance is thermostable, bactericidal and phytocidal, but has not been chemically characterized. An additional germination-inhibiting factor is known to originate from the mandibular glands of workers and can be extracted in water, ethanol or diethyl ether from pollen pellets or bee bread (Keularts and Linskens 1968). The inhibitory action of this substance is also effective on germinating pollen, and comparative chromatographic investigation suggests that its activity is linked to 10-hydroxy-(*E*)-2-decenoic acid, which strongly inhibits pollen respiration. However, specific assays for 10-hydroxy-(*E*)-2-decenoic acid in corbicular loads or bee bread of almond pollen (*Prunus dulcis* (Mill.) D. A. Webb) indicate that this fatty acid is absent, thereby casting doubt on the participation of the mandibular glands during pollen collection or storage (Standifer et al. 1980).

Pollen stored in the moist, warm environment of the colony undergoes a number of biochemical changes. Published accounts of the relative composition and nutritive value of pollen before and after it is stored are, however, contradictory. Preliminary digestion of pollen is known to occur as a result of enzymes added by workers and by the action of beneficial bacteria. The addition of invertase by workers results in bee bread having a greater proportion of reduced sugars than fresh pollen (Loper et al. 1980). When pollen is stored in the hive, its acidity and water-soluble protein increase because of lactic acid fermentation by bacteria present in the pollen (Hitchcock 1956, Stanley and Linskens 1974). However, acidity is not always increased as Standifer et al. (1980) found no appreciable change in pH between the corbicular loads and bee bread of almond pollen. Molds that utilize much of the lactic acid in bee bread subsequently develop so that the pollen becomes somewhat predigested, and possibly more palatable for bees (Pain and

Maugenet 1966). High levels of vitamin E, vitamin K and histamine are also found in stored pollen, and are associated with bacterial metabolism (Vivino and Palmer 1944, Haydak and Vivino 1950). Protein, vitamins (ascorbic acid and pyridoxine) and unsaturated fatty acids decrease in concentration when pollen is converted into bee bread, while saturated fatty acids and sterol esters increase (Loper et al. 1980, Standifer et al. 1980, van der Vorst et al. 1982). The enzyme-rich honey added to the stored pollen prevents anaerobic metabolism and fermentation, which contributes to the longevity of stored pollen in cells (Winston 1987).

Digestion

The digestion of pollen by adult and larval honey bees is difficult because of the indigestible components of the pollen exine including cellulose, pectic acids and sporopollenin (Klugness and Peng 1984). After pollen is eaten by the bee, it quickly passes through the honey stomach into the midgut, where digestion takes place. Little mechanical breakdown of pollen is caused by the mouthparts or proventricular valve (Parker 1926, Whitcomb and Wilson 1929). In the midgut, the enzyme-secreting peritrophic membrane tightens around the pollen bolus, contracting mostly at the ends to create a "sausage-like" package. The peritrophic membrane also functions to protect the midgut from the abrasive texture of pollen grains (Barker and Lehner 1972). Actual crushing of the pollen grain is not necessary, as digestion of its contents can occur through germination pores or by rupturing the grain through osmotic shock (Kroon et al. 1974, Stanley and Linskens 1974, Klugness and Peng 1984, Peng et al. 1985). Proteases and lipases of the midgut digest the cell contents causing most grains, now devoid of

material, to collapse. Estimates of the apparent efficiency of pollen digestion by adult workers range from 89% by gravimetric analysis, to 77% using a Cr_2O_3 internal food marker placed in the diet (Schmidt and Buchmann 1985). One to three hours is necessary for the passage of the pollen mass through the midgut, and pollen residue is stored in the hindgut until it is discharged in flight (Stanley and Linskens 1974). Individual worker larvae consume between 125-145 mg of pollen, containing about 30 mg of protein (Rosov 1944).

Nutrition

Numerous studies have evaluated the quality of bee-collected pollen by direct measurement of nutritional factors (Svoboda 1940, Todd and Bretherick 1942, Vivino and Palmer 1944, Auclair and Jamieson 1948, Sarkar et al. 1949, Weaver and Kuiken 1951, Levin and Bohart 1955, Lubliner-Mianowska 1956, Hagedorn and Burger 1968, McLellan 1977, Herbert and Shimanuki 1978, Youssef et al. 1978, Gilliam et al. 1980, Kauffeld 1980, Loper et al. 1980, McCaughey et al. 1980, Solberg and Remedios 1980, Standifer et al. 1980, Rabie et al. 1983, Schmidt and Schmidt 1984, Rayner and Langridge 1985, Herbert et al. 1987, Loper and Cohen 1987, Shower et al. 1987, Schmidt et al. 1989, Day et al. 1990, Stace 1992). Pollen nutrition has also been assessed by quantifying brood production and evaluating the growth, longevity, survival or protein content of workers (de Groot 1951, 1952, 1953; Haydak 1961, 1963; Wahl 1963; Hagedorn and Moeller 1968; Townsend and Smith 1969; Standifer et al. 1960, 1970; Herbert et al. 1970; Knox et al. 1971; Campana and Moeller 1977; Kleinschmidt and Kondos 1976, 1978; Loper and Berdel 1980a, b; McCaughey et al. 1980; Lehner 1983;

Dietz and Stevenson 1980; Shower 1987; Schmidt et al. 1987, 1989, 1995).

Pollens contain anywhere from 6-28% (w/w, dry weight) protein and are the only natural source of protein available to honey bees. The nutritive value of pollen from different plants is highly variable, because of different amounts of protein present in each. Differences in the amino acid composition from the same plant species may even occur from different geographical populations (Loper and Cohen 1987). Pollens contain between 1-30% lipid (with most below 5%), and less than 0.5% sterols. In addition, sugars, starches, vitamins and minerals, all of which are required for bee nutrition, are minor components of pollen. Pollens are rich in water-soluble vitamins such as the B-complex (thiamine, riboflavin, pyridoxin, pantothenic acid, niacin, folic acid and biotin), inositol and ascorbic acid, but are poor in fat-soluble vitamins (Nielsen 1955, Augustin and Nixon 1957, Dadd 1973). The age of stored pollen is also important. Protein content, vitamin content, and the ability of colonies to use pollen for brood rearing diminishes with age (Svoboda 1940, Haydak 1963, Hagedorn and Burger 1968; Hagedorn and Moeller 1968, Dietz and Stevenson 1980). The relative importance of lipids, vitamins and minerals in the diet of the honey bee is not well understood (Parker 1926, Vivino and Palmer 1944, Maurizio 1954b, Stanley and Linskens 1974). For a more thorough review of these components, the reader is directed to Herbert (1992).

Worker larvae are fed brood food produced by the hypopharyngeal and mandibular glands of the nurse bees. Secretions from the hypopharyngeal glands are clear, containing mostly protein, while secretions from the mandibular glands are white, containing mainly lipids. Worker larvae are fed secretions consisting of 20-40% white component for the first two days of life; on the third day the white component ceases and

only the clear component with small amounts of pollen and honey are fed. Heaviest pollen feeding occurs on the fifth day of development (Michener 1974). The exact food requirements of larvae have never precisely been determined, and no artificial diet can completely replace pollen and honey (Chalmers 1980). However, some nutritional information has been obtained from experimental manipulation of diets. Developmental failure of a colony's brood is high and dwarf adults are produced if less than 65% of the normal amount of food is fed to larvae (Jay 1964a). Brood can be reared by adult bees fed a pure carbohydrate diet, but they must break down their own body tissues to produce larval food, and the resulting offspring have lower nitrogen content in their abdomens than in colonies with access to pollen (Haydak 1935). The vitamins pyridoxine and inositol are important for larval development, and if added to a vitamin-free diet, allow normal brood rearing for four generations (Haydak and Dietz 1965, 1972; Anderson and Dietz 1976). Ascorbic acid fed to adult workers also results in higher brood survival rates than in those colonies deprived of it (Herbert et al. 1985). Larvae fed lipid-deprived diets have shorter developmental times, but have increased mortality at the prepupal stage and cannot spin silk (Haydak 1970). An imperative nutritional component for larval diets is the fatty acid 10-hydroxy-(*E*)-2-decenoic acid. It is produced by the mandibular glands of nurse bees and makes up about 4% of the brood food (Callow et al. 1959). Sterols (mostly 24-methylene cholesterol) are important for the production of cholesterol and comprise about 0.25% of the brood food (Barbier and Bogdanovsky 1961). The addition of these components to a lipid-free diet prevents the aforementioned effects (Kinoshita and Shuel 1975).

For adult workers, pollen is necessary for proper post-emergent gland development

and growth of internal structures during the first 8-10 days of life; after this time it is not essential unless older workers begin to produce brood food and feed larvae or develop ovaries. Lack of protein during the first 8-10 days of adult worker life results in reduced longevity (Haydak 1937a, b; Maurizio 1950; de Groot 1951, Weiss 1984). The extent of hypopharyngeal gland development during this period is also influenced by the quantity and quality of protein consumed (Maurizio 1950, 1954a; Haydak 1961; Hagedorn and Moeller 1968; Standifer 1967; Standifer et al. 1960, 1970; Knox et al. 1971; McCaughey et al. 1980; Crailsheim et al. 1992; Hrassnigg and Crailsheim 1998b). Pollen that is protein-rich also promotes ovary and egg development in caged workers without a queen (Maurizio 1954a; Pain 1961, 1963; Jay and Jay 1993; Lin and Winston 1998), and a lack of pollen protein can retard or prevent ovary development (Harris and Harbo 1990). Pollen protein also promotes growth of the fat body (Maurizio 1950, 1954a) and haemolymph vitellogenin titre has been linked to the level of pollen in worker diets (Bitondi and Simões 1996, Cremonez et al. 1998). The consumption and utilization of pollen by workers is correlated with their age, function, hypopharyngeal gland development and content of proteolytic enzymes (Crailsheim et al. 1992, Hrassnigg and Crailsheim 1998a).

The type of pollen utilized by workers is of great importance. Pollens have been classified into groups based on their nutritive value (Maurizio 1960). For proper growth, only ten of the amino acids are considered to be necessary: arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine (de Groot 1953). Deficiencies of these amino acids, in species such as dandelion (*Taraxacum officinale* Weber ex Wigg.), may cause reduced longevity in workers and preclude brood

rearing (Herbert et al. 1970, Knox et al. 1971, Loper and Berdel 1980b), Little is known of the requirements of lipids, vitamins and minerals for adult workers. Standifer and Mills (1977) found that vitamins were necessary for hypopharyngeal gland development, but Maurizio (1954b) determined that gland and fat bodies were normal during a vitamin-free study. Adult workers appear to have similar lipid requirements as larvae, although in smaller quantities, but whether they require more lipid in their diet than what has been consumed as larvae is not known. Older workers show increased mineral content, which may be related to the tasks they perform (Dietz 1971).

Little information exists about the nutritional requirements of drones. Drones receive more food than workers because of their size, and the range of proteins found in their diet is somewhat greater (Haydak 1970). In addition, the food of older drone larvae contains more carbohydrates, riboflavin, and folic acid than younger larvae and less thiamin, biotin, pantothenic acid, choline, pyridoxine, protein, fat, ash, and niacin (Haydak 1970). These changes reflect increased quantity of pollen and honey in the diet of drone larvae as well as variations in the composition of brood food (Haydak 1957a). The production of spermatozoa in young drones is not dependent on the amount of protein fed, but brood food and pollen feeding may affect longevity and mating ability (Free 1957, Haydak 1970).

Summary

The nutrition of a honey bee colony is dependent on the quantity and quality of pollen collected by foragers and its consumption and physiological utilization by nurse bees. Evaluation of dietary quality must address how well different pollens or pollen

substitutes promote the development of organs and glands that are associated with individual and colony-level fitness parameters. The development of hypopharyngeal glands and ovaries serves as a useful indicator of how the quality of pollen consumed by workers affects such processes. In nurse bees, the size and activity of hypopharyngeal glands are directly influenced by the amount and type of pollen diet consumed (Maurizio 1954a; Haydak 1961; Hagedorn and Moeller 1968; Standifer 1967; Standifer et al. 1960, 1970; Knox et al. 1971; Roşca et al. 1972; McCaughey et al. 1980; Brouwers 1982; Huang et al. 1989; Hrassnigg and Crailsheim 1998b). Similarly, ovary development is promoted by pollens that are rich in protein (Maurizio 1954a; Pain 1961, 1963; Jay and Jay 1993; Lin and Winston 1998), providing a direct measure of the ability of bees to convert pollen into vitellogenin (Bitondi and Simões 1996, Cremonez et al. 1998). Evaluating pollen quality using parameters that are related to a worker's ability to utilize nutrients from their diet also allows any inherent differences in the efficiency of pollen digestion and its relative assimilation into tissues of young workers to be addressed.

Depending on the species of pollen that workers are fed, the assimilation of dietary protein by developing hypopharyngeal glands and ovaries could occur in different ways (Maurizio 1954a, Haydak 1961). Coincident evaluation of glands and ovaries within cohorts of workers would allow us to judge how these indicators should be used in making assessments of pollen quality. Determinations of pollen quality are also affected by the length of time and conditions under which pollen is stored because the protein content, vitamin content, and brood rearing capacity of pollen are known to diminish with age (Svoboda 1940, Haydak 1963, Hagedorn and Burger 1968; Hagedorn and Moeller 1968, Dietz and Stevenson 1980). Comparing carefully collected and identified sources

of pollen under standardized storage regimens would provide definitive information in an area where much ambiguity exists.

Pollen quality may influence the choices made by honey bee foragers, and serve as a criterion by which colonies allocate labour among forage sites and optimize their collection of food. However, this aspect of foraging theory has never been directly examined and it is unknown whether individual foragers or colonies qualitatively adjust their pollen-collection behaviours in relation to protein demand within the colony. Shifts in foraging strategy in response to changes in the quality of stored pollen could be detected by intensively sampling colonies that have undergone pronounced changes in their need for pollen protein. By examining the protein content of pollen loads and the sampling activities of returning foragers, shifts in the pollen selection strategies of foragers could be detected. In addition, changes in the breadth of flora sampled by a colony could be verified by examining the total number of species being returned to a colony by its scouts, and by the number of species sampled by individual foragers. The qualitative and quantitative contributions of forager choice on colony protein intake can be ultimately estimated from the average amount of protein collected per forager, or the amount being returned to colonies per unit time. The behaviour of naive foragers compared with those of known pollen foraging experience is also of great interest in relation to shifts in colony protein demand. Examination of these two cohorts of foragers would be biologically meaningful, as previous learning (Dukas and Visscher 1994) and restrictions to the lifetime energy budget (Neukirch 1982) of more experienced foragers could be significant factors in altering their foraging strategies. If differences between these two cohorts exist, adjusting their relative proportion within the foraging population

could serve as an elegant mechanism by which bees respond to changes in colony state.

Honey bees collect pollen from a wide variety of floral sources and have distinct preferences for some pollen types over others (Linsley and McSwain 1947, Levin and Bohart 1955, Bohart 1957, Nye and Mackensen 1965, Doull 1966, Wahl 1966, Olsen et al. 1979, Boch 1982, Schmidt 1982, Boelter and Wilson 1984, Jay and Jay 1984, Free 1993). Foragers primarily rely on visual and olfactory stimuli to locate flowers and their rewards (Butler 1951, von Frisch 1967, Menzel et al. 1997, Backhaus 1993), with floral odour allowing bees to discriminate between species, determine the availability of a reward and elicit discrete foraging behaviours (McNaughton and Harper 1960; Dobson 1991, 1994; Dobson et al. 1996). The importance of pollen odour as a component of the overall floral bouquet is demonstrated by the ability of honey bees to discriminate and be trained to collect pollen based on its odour alone, even in the absence of supplementary dance information (von Frisch 1923, von Aufsess 1960). Moreover, bees can be stimulated to collect food having little or no nutritional value by the addition of pollen odour components (Taber 1963, Hohmann 1970, Starrat and Boch 1971).

The importance of other floral cues that honey bees use to discriminate and select among different pollens is poorly understood. For example, the nutritional value of pollen, as measured by its protein content, could be a primary cue that foragers assess. The question of whether individual foragers can perceive or modify their foraging behaviours based on the nutritional value of the pollen they are collecting has never been experimentally tested without coincident modification of other pollen-based cues. The inability of foragers to assess the quantity of pollen protein is supported by many documented accounts of bees collecting pollen or pollen-like substances with little or no

nutritive value (Shaw 1990), as well as choice tests in which foragers were allowed to select among pollens having varying protein contents (Levin and Bohart 1955, Wahl 1966, Schmidt 1982). However, other evidence suggests that bees possess an independent ability to discriminate the protein content of pollen (Rasheed and Harder 1997a, Waddington et al. 1998) and may select pollen species in a way that is related to colony need (Fewell and Winston 1992).

Whereas the perception of pollen quality by honey bee foragers is in need of clarification, the role of additional pollen-based cues on the foraging decisions made by *A. mellifera* are virtually unknown. For example, the size of pollen grains could function as a cue for foragers to elicit pollen-collecting or packing behaviours because grain size is related to the efficiency of pollen collection, and its nutritional content (Baker and Baker 1979, Simpson and Neff 1983). The time necessary for a forager to work a flower and extract pollen (“handling time”), may also influence its decision-making process. Foragers incur higher costs as floral architecture becomes more complex (Heinrich 1979; Lavery 1980, 1994a) and the perception of this complexity, as measured by the handling time incurred, could be used as a cue for bees to exhibit continued pollen-seeking or collection behaviours.

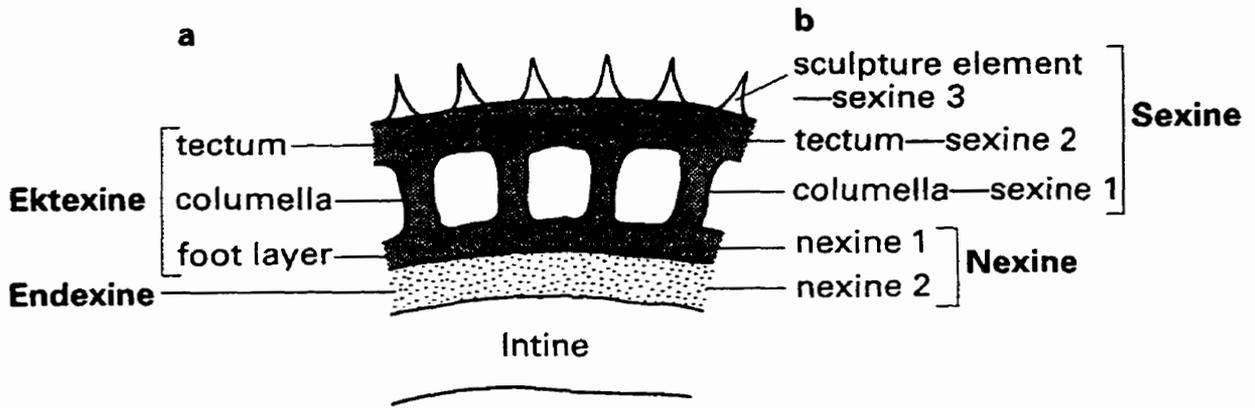
The ability of foragers to perceive pollen odour and protein content and the influence that these and other potential cues have on individual foraging decisions is worthy of experimental investigation, because of their known importance to colony-level pollen collection. Proper evaluation of such cues requires their controlled and discrete manipulation in the absence of other competing stimuli. Such studies are best conducted within the precisely controlled environment of an indoor flight and rearing room, where

conditions are consistent and free of confounding influences.

Objectives

1. To evaluate the quality of single pollen diets for honey bees, by measuring the development of hypopharyngeal glands and ovaries in newly-emerged workers.
 - a. To determine if hypopharyngeal glands or ovaries utilize protein derived from pollen in a similar manner, and are equally sensitive indicators of pollen quality.
 - b. To examine the relationship between the quality of a pollen as a food source for bees, and its protein content.
 - c. To evaluate whether freezing pollen and storing it for one year in an oxygen-reduced environment cause a change in its quality.
2. To determine whether honey bees can respond to changes in the quality of pollen stored in their hive.
 - a. To examine how manipulations of the quantity or quality of a colony's pollen reserve change components of colony-level and individual-level foraging behaviour.
 - b. To compare the behaviour of inexperienced and experienced pollen foragers, in response to manipulations of a colony's pollen stores.
3. To evaluate the importance of pollen odour, pollen lipid odour, particle size, protein content and handling time on the collection of pollen by individual foragers.
 - a. To determine whether interspecific differences in pollen odour affect the behaviour of foragers.
 - b. To determine the hierarchy of stimuli used in the decision-making processes of foragers while collecting pollen.
4. To improve lighting, airflow and control systems used in a honey bee flight and rearing room.

Fig. 1. Structure of the pollen grain exine. (a) As defined by Fægri (1956), Fægri et al. (1989). (b) From Reitsma (1970). The structures named by Fægri are based on the staining characteristics of the ectexine and the endexine. In contrast, Reitsma's division [based on that of Erdtman (1966a)] is a formal morphological description, dividing the exine into descriptive subunits. (From Moore et al. 1991).



CHAPTER III

**Pollen Quality of Fresh and 1-Year-Old Single Pollen Diets for Worker Honey
Bees (*Apis mellifera* L.)**

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Bees (*Apis mellifera* L.)**

Abstract

Newly-emerged honey bees were placed in cages and fed sucrose syrup and one of the following single-pollen diets: *Malus domestica* Borkh., *Brassica rapa* L., *Phacelia tanacetifolia* L., *Melilotus officinalis* (L.) Pall., *Helianthus annuus* L., *Pinus banksiana* (Lamb.), artificial supplement (Bee-Pro[®]) or nothing. Freshly collected pollen was compared with pollen that had been stored for one year at -30° C in an oxygen-reduced atmosphere. Hypopharyngeal gland protein was determined on day 0, the beginning of the experiment, and at 3, 8 and 14 days after diets were supplied; ovary development was visually scored on day 14. The development of hypopharyngeal glands and ovaries varied with diet and, collectively, proved to be sensitive measures of protein utilization and pollen quality. For workers fed 1-year-old *Phacelia* pollen, protein was utilized in a differential fashion, promoting the development of ovaries over that of hypopharyngeal glands. Development of glands and ovaries was strongly correlated with the total amount of protein workers consumed from pollen diets, and to a lesser extent, the percent crude protein content of pollen diets. Storing pollen for one year did not affect gland or ovary development.

Introduction

Pollen is the only source of protein in the diet of honey bees, *Apis mellifera* L., and also contains all of the lipids, vitamins and minerals necessary for normal growth and

development of the colony. Numerous studies have evaluated the quality of bee-collected pollen by direct measurement of nutritional factors (Svoboda 1940; Todd and Bretherick 1942; Vivino and Palmer 1944; Auclair and Jamieson 1948; Sarkar et al. 1949; Weaver and Kuiken 1951; Levin and Boihart 1955; Lubliner-Mianowska 1956; Hagedorn and Burger 1968; McLellan 1977; Herbert and Shimanuki 1978; Youssef et al. 1978; Gilliam et al. 1980; Kauffeld 1980; Loper et al. 1980; McCaughey et al. 1980; Solberg and Remedios 1980; Standifer et al. 1980; Rabie et al. 1983; Schmidt and Schmidt 1984; Rayner and Langridge 1985; Herbert et al. 1987; Loper and Cohen 1987; Shower et al. 1987; Schmidt et al. 1989; Day et al. 1990; Stace 1992), or by such parameters as: brood production; the growth, longevity, survival or protein content of workers; or the development of hypopharyngeal glands and ovaries (de Groot 1952, 1953; Maurizio 1954a; Pain 1961; Haydak 1961, 1963; Wahl 1963; Standifer 1967; Hagedorn and Moeller 1968; Townsend and Smith 1969; Standifer et al. 1960, 1970; Herbert et al. 1970; Knox et al. 1971; Campana and Moeller 1977; Kleinschmidt and Kondos 1976, 1978; Loper and Berdel 1980b; McCaughey et al. 1980; Lehner 1983; Dietz and Stevenson 1980; Shower 1987; Jay and Jay 1993; Schmidt et al. 1987, 1989, 1995). The direct measurement of nutritional factors alone may be misleading, as the importance of the non-protein components of pollen are not well understood (Vivino and Palmer 1944, Maurizio 1954b, Stanley and Linskens 1974).

Evaluating pollen quality by measuring colony growth and development would provide the most pertinent information about its potential impact on honey bee fitness. However, the collection of large quantities of pollen for use in studies on full-size colonies is not feasible, at present. Measuring factors that are related to the worker's

ability to utilize pollen, such as hypopharyngeal gland and ovary development, could address any inherent differences in the efficiency of pollen digestion and its relative assimilation into tissues of young workers or brood. Because young worker bees are responsible for feeding all castes and age cohorts within a colony (Crailsheim 1991, 1992, 1998; Crailsheim et al. 1992), measurements of hypopharyngeal gland development could also provide information about the amount of protein potentially disseminated to the rest of the colony, relative to a particular pollen source. Furthermore, by feeding similar groups of caged workers single pollen diets, relative consumption can be measured and the palatability of pollen diets may be discerned.

Hypopharyngeal gland development is influenced by the quantity and quality of protein ingested by workers (Maurizio 1954a; Haydak 1961; Hagedorn and Moeller 1968; Standifer 1967; Standifer et al. 1960, 1970; Knox et al. 1971; McCaughey et al. 1980; Hrassnigg and Crailsheim 1998b). The size of hypopharyngeal glands, as measured by acinal diameter, is related to their total protein content (Brouwers 1982). In nurse bees, the protein content of hypopharyngeal glands can also be used as an indication of gland activity (Roşca et al. 1972, Huang et al. 1989). Examination of hypopharyngeal glands from caged newly-emerged workers is a reliable measure of dietary protein assimilation because these glands will develop in the absence of brood and their total protein content is unaffected by brood quantity (Huang et al. 1989, Crailsheim and Stolberg 1989).

Hypopharyngeal gland development is also a good indicator of how pollen diets potentially affect colony-level fitness. The development of these glands in nurse bees is positively correlated with pollen consumption (Hrassnigg and Crailsheim 1998b) and

protein synthesis from these glands utilizes protein derived from pollen (Crailsheim 1992). In a colony, nurse bees actively consume and digest the largest quantities of stored pollen (Crailsheim et al. 1992) and secrete it as brood food from their hypopharyngeal and mandibular glands (Seeley 1982). The quantity and quality of brood food produced by nurse bees have important ramifications for the fitness of the colony as a whole. Most brood food is fed to the developing larvae within the colony, however a significant proportion is also fed to the adult members of each caste (Crailsheim 1998). The quality of food received by brood and the queen, especially, has the potential to influence the overall rate of colony growth. In addition, the survival of workers is directly affected by the total amount of pollen protein consumed (Kleinschmidt and Kondos 1976, 1978; Schmidt et al. 1987). Therefore, diets that enhance hypopharyngeal gland development in nurses potentially influence colony population size, a variable that is related to such colony-level fitness components as increased colony survival and reproductive performance (Schmid-Hempel et al. 1993).

A second physiological parameter used to evaluate the quality of a pollen diet is the extent of ovary development in newly-emerged workers. Pollen that is protein-rich usually promotes ovary and egg development in workers that are caged without queens (Maurizio 1954a; Pain 1961, 1963; Jay and Jay 1993; Lin and Winston 1998) and a lack of pollen protein can retard or prevent ovary development (Harris and Harbo 1990). Pollen protein promotes growth of the fat body (Maurizio 1954a) and haemolymph vitellogenin titre has been linked to the level of pollen in the diet of workers (Bitondi and Simões 1996, Cremonez et al. 1998), thereby establishing the importance of pollen protein for egg development. Worker ovarian development provides a direct measure of

the ability of bees to convert pollen proteins into vitellogenin (Bitondi and Simões 1996, Cremonez et al. 1998), a lipoprotein which is required for egg-laying. Greater, or more efficient, production of vitellogenin in queens could increase fecundity, colony population size, and therefore colony-level fitness.

In honey bee workers, the question of whether different physiological systems have different nutritional requirements has not been adequately studied. Maurizio (1954a) showed similar trends in hypopharyngeal gland and ovary development for bees fed different pollen diets. Haydak (1961), using similarly designed tests, showed that the development of hypopharyngeal glands was more sensitive to reductions in pollen quality than that of mandibular glands. If unequal partitioning of protein between developing hypopharyngeal glands and ovaries exists, it would affect our judgement of how these indicators should be used in making assessments of pollen quality.

Ambiguity also remains over the value of stored pollen as a protein source for honey bees. Most studies examining the nutritional value of pollen after periods of storage are confounded by the use of diets with mixed, and often unidentified, pollen species (Haydak 1961, 1963; Hagedorn and Moeller 1968; Dietz and Stevenson 1980). In addition, poor descriptions of the techniques used to store pollen and incomplete information about the conditions maintained during storage are common. Thus, it is difficult to assess the effects of specific storage parameters on pollen quality, and whether such effects are consistent among species.

The age of pollen used to feed honey bees can influence worker growth and development, or the production of brood by a colony. Workers fed dried pollen that is 1-year-old or older have smaller hypopharyngeal glands and lower rates of weight gain than

workers fed fresh pollen (Haydak 1961, Maurizio 1954a). Colonies fed diets composed of 2-year-old dried pollen rear less brood than colonies fed freshly dried pollen (Haydak 1963). The amount and age of pollen in formulated diets also can affect its utilization by workers. Hagedorn and Moeller (1968) determined that newly-emerged workers, fed pollen supplement containing small quantities of dried or frozen 1-year-old pollen, have similar hypopharyngeal gland development to those fed supplement mixed with fresh pollen. However, workers had smaller glands when they were fed supplements containing pollen stored for more than one year. The rate of thoracic weight gain in workers fed supplement containing dried pollen also decreases when the pollen component has been stored for extended periods.

In contrast, other studies show that the age of pollen fed to bees does not affect at least some of the physiological measures indicative of pollen quality. Ovarian development of queenless workers fed dried 1-year-old pollen (Pain 1961), and lifespan of caged bees fed dried 2-year-old pollen (de Groot 1953), is similar to that found in bees fed fresh pollen. Dietz and Stevenson (1980) showed that drying and freezing pollen can prevent its nutritional degradation to some extent. Brood production in colonies fed freshly collected, dried pollen does not differ from those fed dried frozen pollen. Dried frozen pollen can support limited brood rearing even after 11 years; without freezing, similarly-aged dried pollen is completely ineffective. The combination of drying and freezing is important as pollen stored by freezing is less adequate for long term brood production than pollen that is first dried and then frozen (Dietz and Stevenson 1980). However, drying pollen may not adequately prevent the degradation of lipids that are nutritionally important for honey bees or prevent changes in those lipids influencing the

palatability of pollen (Schmidt 1985). Freezing pollen, in combination with storage in an oxygen-reduced environment, might prevent degradation of pollen protein while preventing the oxidation of other non-protein constituents.

A nutritional comparison between fresh and 1-year-old stored pollen, using several identified pollen species, would provide a precise and meaningful comparison of the quality of pollen following storage and their utilization by different physiological systems. This would be useful information for beekeepers, who typically use the previous year's pollen to feed colonies, as well as for researchers interested in aspects of pollen nutrition and consumption.

In this study, I evaluated the quality of several single pollen diets and one pollen substitute for honey bees. The parameters I chose to measure were physiological indices of pollen utilization in newly-emerged workers: the development of hypopharyngeal glands and ovaries. I also determined if patterns of hypopharyngeal gland development differed from that ovaries, to ascertain whether one parameter was a more sensitive indicator of pollen protein utilization, or whether hypopharyngeal glands and ovaries utilized protein in a differential fashion. In addition, I examined the relationship between the quality of a pollen as a food source for bees and its protein content. Finally, I evaluated whether freezing pollen and storing it for one year in an oxygen-reduced environment caused a reduction in its quality.

Materials and Methods

Pollen Collection

During the summers of 1993 and 1994, pollen was collected from blooming trees

and field crops using honey bee (*Apis mellifera*) colonies fitted with O.A.C. pollen traps (Smith and Adie 1963). Four colonies, housed in standard Langstroth hive bodies, were placed in isolated plots of the following species: *Malus domestica* Borkh. (mixed var.) (apple), *Brassica rapa* L. var. 'Goldrush' (oilseed rape), *Phacelia tanacetifolia* L. var. 'Angelia' (phacelia), *Melilotus officinalis* (L.) Pall. var. 'Norgold' (yellow sweetclover), and *Helianthus annuus* L. var. 'Sigco 954' (sunflower). *Malus* pollen was collected from the orchards of the Agriculture and Agri-Food Canada Research Centre in Morden, Manitoba. Pollen from field crops was collected at the University of Manitoba Glenlea Research Station or on co-operating producers' farms in southern Manitoba.

Pine pollen was obtained by collecting male cones from pine trees, *Pinus banksiana* (Lamb.) (jack pine), from the Sandilands Provincial Forest in eastern Manitoba. Pollen was shaken from the cones after they were dried at 35° C for 1 d.

The following protocol was observed for the collection, handling and storage of all pollen samples to preserve the integrity of external pollen lipids, and to minimize oxidation and desiccation of pollen. Surfaces of O.A.C. pollen traps were rinsed with n-pentane, and the pollen collection trays of the traps were lined with aluminum foil, to ensure that pollen only came into contact with lipid-free surfaces. All laboratory tools and surfaces used for the handling and sorting of pollen were cleaned in a similar manner, and care was taken to avoid contacting pollen directly by hand. Pollen was separated from non-pollen debris by sieving and manual sorting, and corbicular loads were grouped on the basis of pollen load colour (Hodges 1984, Kirk 1994). Pollen from each colour cohort was mounted in glycerine jelly (Fægri et al. 1989) and examined under a compound microscope at 400× to confirm the identity of pollen species (Crompton and

Wojtas 1993). Fresh, undried pollen that was not immediately used for the preparation of pollen diets was readied for storage by placing it in glass vials (26 by 60 mm, 23 mL). After vials were filled with pollen, any air remaining was displaced with nitrogen. Vials were immediately sealed with teflon-lined screw-caps and frozen at -30° C.

Experimental Design

The experimental design consisted of two factors that were tested: the age of pollen (or pollen substitute) fed to bees, and its species. The age of pollen was defined as being 1-year-old or freshly-collected. One-year-old pollen (or pollen substitute) was collected during the previous summer and stored frozen, using the previously described protocol. Freshly collected pollen was collected and used immediately or was temporarily frozen until needed. This short period of freezing was necessary to preserve early-season (May) species (*Malus*, *Pinus*) under optimal conditions until late-season (August) species (*Helianthus*) could be collected, at which time all diets were evaluated simultaneously. Eight different pollen diets were tested, each formulated from a single pollen species or pollen substitute. Individual trials consisted of the eight pollen diets, of the same pollen age, fed to bees for a duration of 14 days. Trials were performed by staggering their starting dates by 2-day intervals. Six individual trials were performed, enabling all treatment combinations to be replicated three times.

Bioassay Cages

Worker bees were confined in bioassay cages and provided a single diet. Cages (12.7 by 17.5 by 6.4 cm) were constructed of 1.3 cm thick spruce plywood, and were

covered on one face with fibreglass screening (1.1 mm openings) (Fig. 2). Each cage contained a piece of plastic comb (10.1 by 14.9 cm) (Perma•Comb Systems[®], Woodland Hills, CA, USA), which formed the rear interior wall. Cages also contained a small (1.6 by 1.6 by 8.9 cm) plexiglass diet tray for feeding bees, and a 125 mL feeder bottle which provided a 2 M sucrose solution. Both the pollen diets and sucrose solution were fed *ad libitum*.

Diet Preparation

Six of the eight diets were prepared from the previously described pollen species, one was prepared from a commercial honey bee pollen substitute (Bee-Pro[®], Mann Lake Supply, Hackensack, MN, USA) and the final diet contained nothing. To simplify descriptions, diets prepared from pollen or the pollen substitute Bee-Pro[®] will be collectively referred to as pollen diets, hereafter. Honey bee-collected pollen was formulated into diets by mixing pollen with water to achieve a paste-like consistency. Each diet was prepared from a pooled sample containing equal amounts of pollen from each collection date. For Bee-Pro[®] and *Pinus* pollen, sucrose (30% w/w dry mass) was added before mixing with water. This amount is equivalent to the total weight of sugar normally found in bee-collected pollen, as determined over a wide range of plant species (Todd and Bretherick 1942; McLellan 1977; Solberg and Remedios 1980; Schmidt and Buchmann 1986). The bees receiving “nothing” were provided with an empty diet tray and a feeder containing 2 M sucrose.

Bioassay Protocol

Frames of *A. mellifera* capped brood were collected and incubated overnight at 30°C to obtain newly-emerged adult workers. Cohorts of 150 newly-emerged adult workers, collected within 12 h of emergence, were then placed in bioassay cages along with weighed portions of prepared diet mixtures and were incubated at 30 ± 1°C and 70% RH. To estimate the mass of water lost by diets to evaporation, equal portions of the same diet mixtures used in bioassay cages were placed in an additional set of diet trays. These trays were placed in the same incubator as their respective bioassay cages. All diet trays were reweighed, cleaned, and subsequently replenished with a known mass of freshly prepared diet mixture, on days 3 and 8. On day 14, the conclusion of experimental trials, diets were weighed a final time. Mortality of honey bees within treatments was evaluated by removing and counting dead bees from cages, on days 3, 8 and 14.

Hypopharyngeal glands were removed from randomly selected bees on days 0, 3, 8 and 14. These intervals were chosen to establish gland size. Hypopharyngeal glands become large and biosynthetically active by the time workers are three days old (Haydak 1957b, Brouwers 1982, Huang and Otis 1989) but decrease in size and activity with age (Crailsheim and Stolberg 1989, Huang et al. 1994, Lass and Crailsheim 1996). On day 0, 80 bees were selected from the pool of newly emerged workers to establish the extent of their gland development. On days 3, 8 and 14 of each trial, ten bees were removed from each bioassay cage, killed by crushing the thorax, and then decapitated. A median sagittal incision was made in each severed head and both hypopharyngeal glands were removed by dissection in phosphate buffered saline (PBS) (pH 7.3) (Dunbar 1987). Glands were placed in microcentrifuge tubes (1.5 mL) containing 0.1 mL of PBS, and

frozen until subsequent protein analysis.

On day 14 of trials, ovaries were dissected from 25 bees, randomly selected from each bioassay cage. These bees were used to establish a mean value for ovary development per treatment. Bees were killed by crushing the thorax, pinned in a dish containing *Apis* saline (Brouwers 1982), and dissected from the ventral side to expose their ovaries. Ovaries were examined *in situ* using a binocular dissecting microscope, and their stage of development was visually scored using a 5 point scale, modified from Velthuis (1970). A single, whole number score was assigned per ovary, after determining the stage of development to which the majority of its ovarioles belonged. Ovaries were classified as 0, undeveloped (completely resting ovary, small ovarioles close to each other); 1, oogenesis starting (cells swelling the apex of the ovariole and descending); 2, slight development (eggs distinguished from trophocytes with the nutritive follicle volume not exceeding that of the egg); 3, moderate development (egg volume exceeding that of the nutritive follicle); 4, highly developed (eggs fully elongated - “sausage-shaped” in appearance, with only a remnant of the trophocytes behind eggs).

Hypopharyngeal Gland Protein

Hypopharyngeal gland development was assessed using a modified Bradford dye-binding assay for total protein (Bradford 1976). Reagents for the Bradford dye-binding assay were prepared using coomassie brilliant blue G-250 dye (Pharmacia LKB Inc., Uppsala, Sweden) according to the protocol outlined in Peterson (1983). A bovine serum albumin (BSA) protein standard solution (0.56 mg/mL) was prepared from lyophilized crystals (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) and double-distilled water.

This solution was prepared with 1 mg/mL of sodium azide (Mallinckrodt Specialty Chemicals Canada, Inc., Mississauga, ON, Canada) and was kept frozen in 15 mL aliquots, until used.

For protein determination, the frozen hypopharyngeal glands were thawed and mechanically ground in their microcentrifuge tubes, using a pestle. Twenty-five microlitres of 50% (w/v) aqueous n-octyl- β -D-glucanopyranoside (OG) (ICN Biomedicals, Inc., Costa Mesa, CA, USA) was added to each sample to solubilize membrane bound-proteins (Fanger 1987). Individual samples were then mixed by vortexing, and were centrifuged for 10 min at $8,850 \times g$. A 10 μ L aliquot of the supernatant was then diluted by 10 \times in PBS (pH 7.3), or by an appropriate value so that the concentration of the each sample fell within the range of the standard curve. An appropriate amount of OG was added in diluted samples to achieve a final concentration of 0.2% (w/v) in the dye reagent (Fanger 1987).

Two 10 μ L aliquots of each diluted sample were pipetted into separate wells of a microassay plate. Standards, ranking in concentration from 0.05 to 0.5 μ g/ μ L of BSA, were prepared and added to microassay plates, along with reagent blanks. Dilute coomassie dye reagent (200 μ L) was then added to all wells containing samples and standards. The microassay plate was then agitated on an orbital plate shaker, and incubated at 20° C for 5 min. Optical densities of samples were read immediately in a microplate reader (Model 450, Bio-Rad laboratories, Hercules, CA, USA) set to 595 nm. Absorbances were plotted against the linear portion of the standard curve for calculation of protein concentrations in the unknowns.

Protein Determination of Pollen Diets

Duplicate samples of fresh and 1-year-old pollen diets (≈ 2.5 g, wet weight) were analyzed to determine total crude protein. Samples were weighed before and after drying in an oven at 70° C for 24 h, to ascertain dry weight and water content. Dried samples were ground with a mortar and pestle to achieve a powder-like consistency, and then stored in a desiccator until analyzed. Total nitrogen content of samples was determined using an elemental analyzer (Model FP-428, Leco Instruments Ltd., Mississauga, ON, Canada), calibrated against known nitrogen standards. To estimate total crude protein, nitrogen values were multiplied through by a conversion factor of 5.6 (Rabie et. al 1983).

Calculation of Diet Consumption

The net weight of pollen diet consumed within treatments was calculated for each treatment and time interval by weighing diet trays before and after bees consumed the diet and then correcting for the amount of water lost between time intervals. Water loss between time intervals was estimated using a duplicate set of diets placed inside incubators, which were not consumed by bees. Sugar content of bee-collected pollen diets (sugar is added to pollen during its collection by bees) was assumed to be equal (Schmidt and Buchmann 1986). Diet consumption within time periods was calculated based on the number of bees surviving at the midpoint of each interval, corrected for sampling loss. Consumption of diets over the entire experiment was calculated by dividing total consumption by the sum of bees surviving at the midpoint of each time period, weighted by the relative duration of the interval.

Statistical Analysis

Analyses were conducted on mean values of hypopharyngeal gland protein, ovary score and diet consumption for each bioassay cage, rather than for each individual bee, to provide comparisons based on the correct experimental unit. Mean consumption of diets and dietary protein over the entire experiment, as well as the extent of ovary development, were analyzed as factorial ANOVAs, with POLLEN AGE, REPLICATE and DIET as factors.

Differences in hypopharyngeal gland protein and diet consumption among time periods were examined using a split-plot design ANOVA with POLLEN AGE, REPLICATE and DIET as main plot factors. In these analyses TIME was treated as a repeated measure (SAS Institute 1989b), with DIET*REPLICATE(POLLEN AGE) used as the error term for testing between-subjects effects. Similarity in the response of individual diets among time periods was examined using tests of simple effects for DIET*POLLEN AGE, invoked using the LSMEANS statement with the SLICE option (PROC GLM, SAS Institute 1989b). Honey bee mortality was analyzed using the same design, but counts of dead bees per time interval were converted to cumulative proportions of the initial population. Proportions were then transformed using an angular transformation (Snedecor and Cochran 1980), prior to analysis. For all split-plot designs using TIME as a repeated measure, tests of sphericity were performed and, if necessary, probability levels were adjusted according to the assessed degree of failure for the Huynh-Feldt conditions to be met (Littell et al. 1996).

In all analyses, higher order interaction terms that did not partition significant or near significant portions of the variance were pooled into the residual error term; otherwise,

they and all hierarchical lower order interactions were retained in the model.

Comparisons between means were done either with single degree of freedom contrasts or least significant difference tests (LSD) (SAS Institute 1989b).

Results

Over the entire experiment, the average consumption of experimental diets was independent of any interaction between diet type and age ($F = 1.24$; $df = 6, 26$; $P = \text{NS}$) (Fig. 3). For all diets, consumption differed with the age of pollen used ($F = 6.55$; $df = 1, 26$; $P < 0.05$), with workers consuming slightly more fresh than 1-year-old pollen. This preference continued to remain evident after Bee-Pro[®] (a pollen substitute) was excluded from the previous analysis ($F = 4.31$; $df = 1, 22$; $P < 0.05$). The quantity of diet consumed also varied with the type of pollen fed ($F = 44.46$; $df = 6, 26$; $P < 0.0001$), as workers ate substantially more *Malus*, *Melilotus*, *Phacelia*, *Brassica* and *Helianthus* pollen than that of Bee-Pro[®] or *Pinus*.

Pollen consumption differed significantly among time intervals ($F = 661.21$; $df = 2, 36$; $P < 0.0001$) (Fig. 4). Patterns of consumption varied with the type of pollen bees were fed ($F = 26.51$; $df = 12, 36$; $P < 0.0001$), but not its age ($F = 2.58$; $df = 2, 36$; $P = \text{NS}$). Over all diets, maximum consumption occurred during days 0 - 3 ($F = 1199.96$; $df = 1, 36$; $P < 0.0001$), and was least during days 8 - 14 ($F = 723.83$; $df = 1, 36$; $P < 0.0001$). *Pinus* pollen was the only diet whose consumption did not vary with time ($F = 1.81$; $df = 2, 36$; $P = \text{NS}$). Within each time interval, Bee-Pro[®] and *Pinus* were consumed less than other pollen diets, over both ages of pollen.

Table 1 shows the total crude protein content of each pollen diet and its

accompanying water content. There was no linear relationship between the crude protein content of a pollen diet and its consumption by worker bees ($r^2 = 0.054$; $F = 0.69$; $df = 1, 12$; $P = \text{NS}$). Differences in crude protein between duplicate subsamples within diets ranged from 0.03 - 0.10% (w/w, dry mass). Estimates of the actual amount of protein consumed by bees in treatments were also calculated, by converting consumption values to dry mass quantities and multiplying by the appropriate crude protein conversion. Over the entire experiment, the mean consumption of dietary protein changed with the age of pollen fed ($F = 8.53$; $df = 1, 26$; $P < 0.01$), with greater amounts of protein consumed from freshly-collected than 1-year-old pollen diets (Fig. 5). Protein consumption also varied with the type of diet fed ($F = 40.97$; $df = 6, 26$; $P < 0.0001$), but this effect was influenced by the interaction between diet type and age ($F = 3.31$; $df = 6, 26$; $P < 0.05$). Workers consumed greater quantities of protein from *Phacelia* and Bee-Pro[®] diets when they were fresh, rather than 1-year-old ($F = 22.08$; $df = 1, 26$; $P < 0.0001$). Protein consumption from *Malus*, *Melilotus*, *Brassica*, *Helianthus* and *Pinus* diets remained similar between ages of pollen.

The amount of protein consumed from diets differed significantly among time periods ($F = 531.54$; $df = 2, 24$; $P < 0.0001$). Temporal patterns of protein consumption varied with the type of pollen diet fed to workers ($F = 23.51$; $df = 12, 24$; $P < 0.0001$), but not its age ($F = 2.68$; $df = 2, 24$; $P = \text{NS}$) (Fig. 6). Over all diets, most protein was consumed during days 0 - 3 ($F = 962.85$; $df = 1, 24$; $P < 0.0001$), while least was consumed during days 8 - 14 ($F = 584.92$; $df = 1, 24$; $P < 0.0001$). Protein consumption from *Pinus* pollen diets did not vary with time ($F = 0.64$; $df = 2, 24$; $P = \text{NS}$). Within each time period, less protein was consumed from *Helianthus*, Bee-Pro[®] or *Pinus* pollen

than from all other diets, over both ages of pollen.

Overall worker bee mortality, analyzed as a cumulative proportion of bees dying per cage, was similar in bees fed different ages of pollen ($F < 0.01$; $df = 1, 14$; $P = \text{NS}$), different pollen diets ($F = 2.27$; $df = 7, 14$; $P = \text{NS}$) and between different time periods ($F = 2.38$; $df = 2, 94$; $P = \text{NS}$). The mean cumulative proportion of dead bees at the end of the experiment was $3.6 \% \pm 0.5$ with values ranging from 0 - 16.0 %.

The overall analysis of samples from 1600 worker bees clearly shows that the type of pollen consumed by bees had a pronounced effect on their hypopharyngeal gland development ($F = 23.22$; $df = 7, 14$; $P < 0.0001$) (Fig. 7). Furthermore, these effects were consistent between freshly collected and 1-year-old pollen diets ($F = 0.54$; $df = 7, 14$; $P = \text{NS}$). The age of pollen used to feed bees did not affect the degree of hypopharyngeal gland development ($F = 0.28$; $df = 1, 14$; $P = \text{NS}$). For both ages of pollen, diets composed of *Malus*, *Melilotus*, *Phacelia* and *Brassica* pollen promoted greater hypopharyngeal gland development than diets prepared from *Pinus* or no pollen, with Bee-Pro[®] and *Helianthus* diets being intermediate.

The development of hypopharyngeal glands varied among time periods ($F = 14.40$; $df = 2, 74$; $P < 0.0001$), with temporal patterns being influenced by the type ($F = 2.64$; $df = 14, 74$; $P < 0.01$) and age ($F = 18.27$; $df = 2, 74$; $P < 0.0001$) of pollen consumed by workers (Fig. 8). For bees fed freshly collected pollen, patterns of hypopharyngeal gland protein changed considerably with diet ($F = 9.23$; $df = 14, 28$; $P < 0.0001$). On day 0, glands contained an average of $44.5 \pm 3.2 \mu\text{g}$ of protein, with values ranging from 14.2 - 80.5 μg . Glandular protein varied among time periods for workers fed fresh *Malus*, *Melilotus*, *Phacelia*, *Brassica* and *Helianthus* pollen, but was similar for those consuming

Bee-Pro[®], *Pinus* or no pollen. Bees fed *Malus* pollen produced glands that were largest during days 0 - 3. Those consuming *Melilotus*, *Phacelia*, *Brassica* and *Helianthus* diets produced glands that were smallest during days 0 - 3, peaking in size during days 3 - 8. Within individual time intervals, workers fed *Malus*, *Melilotus*, *Phacelia* and *Brassica* pollen consistently produced larger glands than bees fed other diets; workers fed *Pinus* or no pollen showed the least development.

For workers fed 1-year-old pollen, temporal patterns of hypopharyngeal gland development were also influenced by the type of diet workers consumed ($F = 2.18$ df = 14, 28; $P < 0.0001$) (Fig. 8). Hypopharyngeal glands from these bees contained an average of $38.9 \pm 2.9 \mu\text{g}$ of protein on day 0, with values ranging from 14.1 - 67.3 μg . For all diets, mean values for hypopharyngeal gland protein were greatest during days 0 - 3 ($F = 41.57$; df = 1, 28; $P < 0.0001$), however only those bees fed *Malus*, *Melilotus*, Bee-Pro[®] and *Helianthus* diets showed reductions in protein levels as the experiment progressed. Protein levels from bees fed the remaining pollen diets showed little variation over time, or possessed greater variability per time interval. The relative ranking of 1-year-old diets within time intervals showed some similarity to fresh pollen diets, but fewer consistent patterns among groups of diets were seen across time periods. Workers fed *Malus* and *Melilotus* diets during the 0 - 3 day interval produced larger hypopharyngeal glands than bees fed all other diets, and continued to produce larger glands than most remaining diets during the 3 - 8 day interval. Workers fed *Pinus* or no pollen had low hypopharyngeal gland protein during the 0 - 3 day interval, and during the 3 - 8 day and 8 - 14 day time intervals produced the lowest hypopharyngeal protein of all diets. All other 1-year-old diets were similar for hypopharyngeal gland development,

within all time periods.

The analyses of pooled ovary scores from 1200 dissections showed similar patterns to those indicated by hypopharyngeal gland development. The overall analysis showed that ovary development was greatly affected by the type of pollen fed to workers ($F = 10.39$; $df = 7, 30$; $P < 0.0001$) (Fig. 9). The age of pollen used to feed newly emerged workers had no effect on the extent of their ovarian development ($F = 0.72$; $df = 1, 30$; $P = \text{NS}$), and no interaction between pollen age and diet type existed ($F = 0.34$; $df = 7, 30$; $P = \text{NS}$). For both ages of pollen, diets composed of *Malus*, *Melilotus*, *Phacelia* and *Brassica* pollen promoted greater ovary development than diets prepared from *Pinus* or no pollen. Bee-Pro[®] and *Helianthus* diets produced intermediate ovary development.

The relationship between ovary score and hypopharyngeal gland protein is shown in Fig. 10. These two measures of protein utilization are well correlated ($r = +0.951$, $P < 0.0001$). The crude protein content of diets was found to be a good predictor of hypopharyngeal gland protein ($r^2 = 0.471$; $F = 12.48$; $df = 1, 14$; $P < 0.01$) and ovary score ($r^2 = 0.601$; $F = 21.07$; $df = 1, 14$; $P < 0.001$). However, stronger linear regressions were shown between protein consumption and hypopharyngeal gland development ($r^2 = 0.869$; $F = 92.92$; $df = 1, 14$; $P < 0.0001$) (Fig. 11a), and between protein consumption and ovary score ($r^2 = 0.905$; $F = 133.73$; $df = 1, 14$; $P < 0.0001$) (Fig. 11b).

Discussion

Measurements of hypopharyngeal gland and ovary development in newly-emerged worker bees appear to be reliable and sensitive indices of protein utilization, and when used together provide a good indication of the quality of pollen that has been consumed.

This conclusion is supported by a strong correlation between the amount of protein consumed from pollen diets and the extent of hypopharyngeal gland or ovary development.

Although the utilization of pollen protein by hypopharyngeal glands and ovaries closely paralleled each other, my results showed that there are important differences in the way that ovaries and hypopharyngeal glands assimilate protein from some pollen sources. Workers fed 1-year-old *Phacelia* pollen developed smaller hypopharyngeal glands than workers fed fresh *Phacelia* pollen, while bees fed other diets had no change in gland size between both ages of pollen. In contrast, ovary development for workers fed fresh or 1-year-old *Phacelia* pollen was similar, even though bees consumed less 1-year-old pollen. It is likely that decreased consumption of 1-year-old pollen limited the protein available for metabolic processes in these workers. As a result, protein may have been utilized according to a physiological priority, or sink, which favoured the development of ovaries over that of hypopharyngeal glands. If this is true, such differential utilization of protein would not occur under queenright conditions, where ovary development is naturally suppressed, but hypopharyngeal gland development is not (Jay 1968, 1970). My results suggest that nutritional evaluations performed under queenless conditions should use more than one physiological index of protein utilization. Such measures should include ovarian development.

The significant positive correlation between the crude protein content of pollen diets and hypopharyngeal gland or ovary development of workers indicates that, in the absence of a nutritional bioassay, crude protein content could be used as a general guideline for evaluating pollen quality. Although most species of pollen that have been quantitatively

analyzed exhibit similar amino acid profiles and contain the minimum levels of essential amino acids (de Groot 1953) necessary for normal honey bee growth and development (Gilliam et al. 1980; McCaughey et al. 1980; Solberg and Remedios 1980; Rayner and Langridge 1985; Wille et al. 1985; Day et al. 1990), protein content is important. The developmental rate of hypopharyngeal glands in workers is not related to the essential amino acid composition of the pollen consumed (McCaughey et al. 1980), but is correlated with the level of protein in the diet (Standifer et al. 1960; McCaughey et al. 1980) and the amount of protein that is ingested (Standifer et al. 1970; McCaughey et al. 1980). Furthermore, the addition of essential amino acids has not always proven to be successful at improving the nutritional status of specific pollen diets (de Groot 1953, Barker 1972, Loper and Berdel 1980b). Even for species such as dandelion (*Taraxacum officinale* Weber ex Wigg.), which does not support brood rearing because of amino acid deficiencies (Herbert et al. 1970, Loper and Berdel 1980b), crude protein content is characteristically low (9.9%) (Loper and Cohen 1987). Therefore, my results support the use of crude protein content as a parameter for evaluating the quality of a pollen diet.

Freezing pollen and storing it in an oxygen-reduced environment for up to one year did not degrade its nitrogen content, or change its nutritional value for honey bee workers. Although workers consumed slightly more fresh than 1-year-old frozen pollen, overall hypopharyngeal gland and ovary development did not differ between fresh or stored pollen diets. Although the crude protein content among species of pollen used in this study differed by up to 16%, protein levels for fresh and 1-year-old conspecific pollen differed by less than 1 % (except for *Phacelia* at 4.1%). These results are in agreement with studies that have reported little change in content of proteins, minerals,

carbohydrates, and lipids in pollen after storage by freezing (Youssef et al. 1978; Day et al. 1990). However, they are in contrast with reports of reductions in digestible proteins (Svoboda 1940), deterioration or lowered availability of proteins (Haydak 1963), decreased vitamin content (Hagedorn and Burger 1968), or increased mortality and loss of brood rearing capacity (Dietz and Stevenson 1980) of stored pollen. My 1-year-old pollen samples may have benefitted from a lower storage temperature (-30°C) than that used in other studies, and also the presence of an oxygen-reduced atmosphere. The pollen species chosen for this study may also have influenced the nature of my results. Lack of proper taxonomic identification of pollen species confounds the interpretation of results from many other studies, as mixed or unidentified pollen sources have often been utilized. My findings using pure pollen samples indicate that the use of 1-year-old frozen pollen for feeding supplements could be as nutritionally beneficial as fresh pollen if the storage protocols used in this study were followed.

Reduced palatability of stored pollen for worker bees may be responsible for the slight reduction in consumption of 1-year-old pollen relative to freshly-collected pollen observed in this study. The attractiveness and palatability of pollen is affected by its lipid composition (Louveaux 1959, Hügel 1962, Hohmann 1970, Doull and Standifer 1970, Doull 1974, Starrat and Boch 1971, Schmidt 1985, Schmidt et al. 1989). Although lipid composition changes when pollen is stored in the hive (Loper et al. 1980, van der Vorst et al. 1982), it has not been demonstrated whether such changes occur under other storage conditions. Changes in pollen lipids could explain slight reductions in pollen consumption in studies such as ours, where protein content has been shown to remain stable in storage. Reduced palatability of stored pollen occurs where pollen has been

dried and stored continuously at room temperature (Townsend and Smith 1969, Dietz and Stevenson 1980).

The species of pollen examined in this study, with the exception of *Pinus*, are usually collected in large quantities by honey bees. The relative differences in protein content among these species show that foraging honey bees collect pollen that varies greatly in quality. Mean crude protein values for *Malus domestica* (24.7%), *Melilotus officinalis* (24.0%), *Helianthus annuus* (14.9%), *Brassica napus* (25.2%) and *Pinus banksiana* (14.0%) are comparable to other studies that have analyzed bee-collected pollen, after their data are standardized for the protein conversion factor of 5.6. From these studies, it is apparent that protein may vary within a particular species, or a closely related group of species. Reported protein values for apple (*Malus pumila* Mill.) range between 22.7 - 24.6%, while those for *Trifolium* species vary between 17.6 - 18.4% (Standifer 1967; McCaughey et al. 1980). Oilseed rape pollen is reported to contain between 19.4 - 22.7 % protein for *Brassica rapa* (Todd and Bretherick 1942; Standifer 1967), 24.3 % for *Brassica napus* L. (Rayner and Langridge 1985) and 21.6 % for an unidentified mixture of both species (Stace 1992). In addition, *Helianthus annuus* pollen has been shown to contain 15.8 % protein (Rayner and Langridge 1985). Low protein values among various species of *Pinus* (1.9 - 12.1%) have also been reported for bee-collected and hand-collected samples (Svoboda 1940; Todd and Bretherick 1942; Day et al. 1990; de Groot 1953; Kleinschmidt and Kondos 1976; Solberg and Remedios 1980; Rayner and Langridge 1985). Published values for the crude protein content of *Phacelia tanacetifolia* pollen were not found; in this study it contained the highest level of crude protein (28.1%) of all natural pollen diets. Although variation in protein content occurs

between geographic locations and with different collection techniques (Loper and Cohen 1987), my reported values are similar to those of other studies. The tendency for honey bees to collect pollen from many different taxa may be an adaptation to prevent colonies from becoming dependent on a small number of inadequate protein sources, and lessen the impact of vitamin, mineral or lipid deficiencies, or toxin overloads (Schmidt 1984) associated with individual species.

An interesting finding in this study concerns the lack of relationship between the crude protein content of the diets tested and their relative consumption by newly-emerged workers. Based on the limited array of pollen species tested, this lack of relationship suggests that young worker bees, particularly nurses, may have no intrinsic mechanism through which they can discriminate the protein content of the diet that they are consuming. If nurse-aged workers were able to discriminate pollen protein and self-regulate dietary protein consumption, it would be expected that larger quantities of some pollen species, such as *Helianthus* and *Pinus*, would have been consumed to compensate for their low protein content (Waldbauer and Friedman 1991). In colonies, large increases in pollen consumption occur when the average level of protein in the pollen they collect decreases by $(x - 10)\%$, in an apparent attempt to meet protein requirements (Kleinschmidt and Kondos 1978). In this study, which used caged queenless workers, consumption did not increase with a decrease in pollen protein. *Helianthus* pollen, which is low in protein, was consumed in the same amounts as higher protein pollens, and *Pinus* pollen was consumed in small quantities. In contrast with my findings, Schmidt and Johnson (1984) showed a weak positive correlation between worker feeding preference and the protein content of pollen diets. Their results are, however, consistent in showing

that workers do not increase pollen consumption to compensate for reductions in dietary protein. Schmidt and Johnson's work also suggests that consumption may be influenced by physical or chemical cues that are unrelated to pollen quality.

Factors other than protein content may be important in determining the amount of pollen consumed by individual workers. The amount of pollen consumed is, in part, also a measure of how attractive and palatable a diet is. Bee-Pro[®], a pollen substitute containing no natural pollen, is as high in protein content as *Malus*, *Melilotus*, *Brassica* or *Phacelia* pollen. However, Bee-Pro[®] consumption was low relative to natural pollen. This may have occurred because Bee-Pro[®] lacks phagostimulants normally associated with the lipid components of pollen (Robinson and Nation 1968, Schmidt 1985). The possibility that *Pinus* pollen, an anemophilous species, lacks phagostimulants or contains repellents may also explain its low level of consumption. Many anemophilous species of pollen are readily collected by bees (O'Neal and Waller 1984), but others remain unpreferred or contain toxic compounds (Schmidt et al. 1987). This study demonstrates that *Pinus* pollen is not readily consumed by workers and supports the poor value of *Pinus* pollen for honey bee development and longevity as seen in other studies (Svoboda 1940, de Groot 1953). Its lack of nutritional value does not appear to be the result of deficient amino acid composition, as the supplementation of amino acids that are absent, or present in low concentrations in *Pinus* pollen, do not improve its quality (de Groot 1953). Therefore, the existence of compounds that reduce the palatability of *Pinus* pollen likely influence its utilization by honey bees.

Modern agricultural systems entail the use of large monocultures that severely limit floral diversity for bees. My findings suggest that the colony performance of honey bees

is not likely to be adversely affected if bees are limited to forage on monocultures of *Malus domestica*, *Melilotus officinalis*, *Brassica rapa* or *Phacelia tanacetifolia*.

However, honey bee colonies restricted to foraging on *Helianthus annuus* during its bloom period may suffer a slight loss of fitness. Pollen supplements made from Bee-Pro[®] might benefit from the addition of pollen, or phagostimulants from pollen, which bees normally prefer to consume. *Pinus banksiana* pollen is nutritionally deficient, and is not palatable to bees. Although sometimes marketed as a pollen substitute, it should not be used as a feed supplement for honey bees.

In conclusion, the development of hypopharyngeal glands and ovaries in newly-emerged honey bee workers is strongly correlated with the amount of pollen protein consumed from diets, and to a lesser extent, with the crude protein content of the diets themselves. For certain pollen diets, workers physiologically allocate protein in a differential fashion, promoting greater development of ovaries over that of hypopharyngeal glands. Over the species I have tested, the consumption of pollen by workers appears to be unrelated to its nutritional content, and may be more influenced by the presence or absence of phagostimulants or repellants. I also ascertained that pollen quality remains unaffected after storage for 1 year at -30° C in an oxygen-reduced environment. Although pollen quality is best evaluated in single-pollen nutritional bioassays, the crude protein content of pollen can be used as a general guideline for evaluating the quality of pollen collected by foragers.

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Table 1. Protein and water content of pollen diets

Pollen diet	Age of pollen	% protein ^a ,	% water,
		$\mu\text{g} / 100\mu\text{g}$ dry mass	$\mu\text{g} / 100\mu\text{g}$ wet mass
<i>Malus domestica</i>	Fresh	25.12	10.71
<i>Melilotus officinalis</i>	Fresh	24.15	23.48
<i>Phacelia tanacetifolia</i>	Fresh	30.10	14.56
<i>Brassica rapa</i>	Fresh	25.72	29.53
Bee-Pro [®]	Fresh	29.92	5.89
<i>Helianthus annuus</i>	Fresh	14.86	17.66
<i>Pinus banksiana</i>	Fresh	14.03	7.50
<i>Malus domestica</i>	1-year-old	24.29	11.50
<i>Melilotus officinalis</i>	1-year-old	23.92	13.88
<i>Phacelia tanacetifolia</i>	1-year-old	26.02	18.43
<i>Brassica rapa</i>	1-year-old	24.67	19.99
Bee-Pro [®]	1-year-old	29.89	5.80
<i>Helianthus annuus</i>	1-year-old	15.00	23.94
<i>Phacelia banksiana</i>	1-year-old	14.00	7.31

^aProtein determination for Bee-Pro[®] and jack pine was performed after the addition of 30% (w/w dry mass) sucrose.

Fig. 2. Bioassay cage used for experimental treatments, constructed from 1.3 cm thick spruce plywood and fibreglass screening. Not shown is Tergal[®] drapery lining material (200 μm openings) stretched across neck of sucrose feeder bottle. Plexiglass diet tray was inserted into opening at bottom side wall of cage. Unit of measure for all unlabeled dimensions is centimeters.

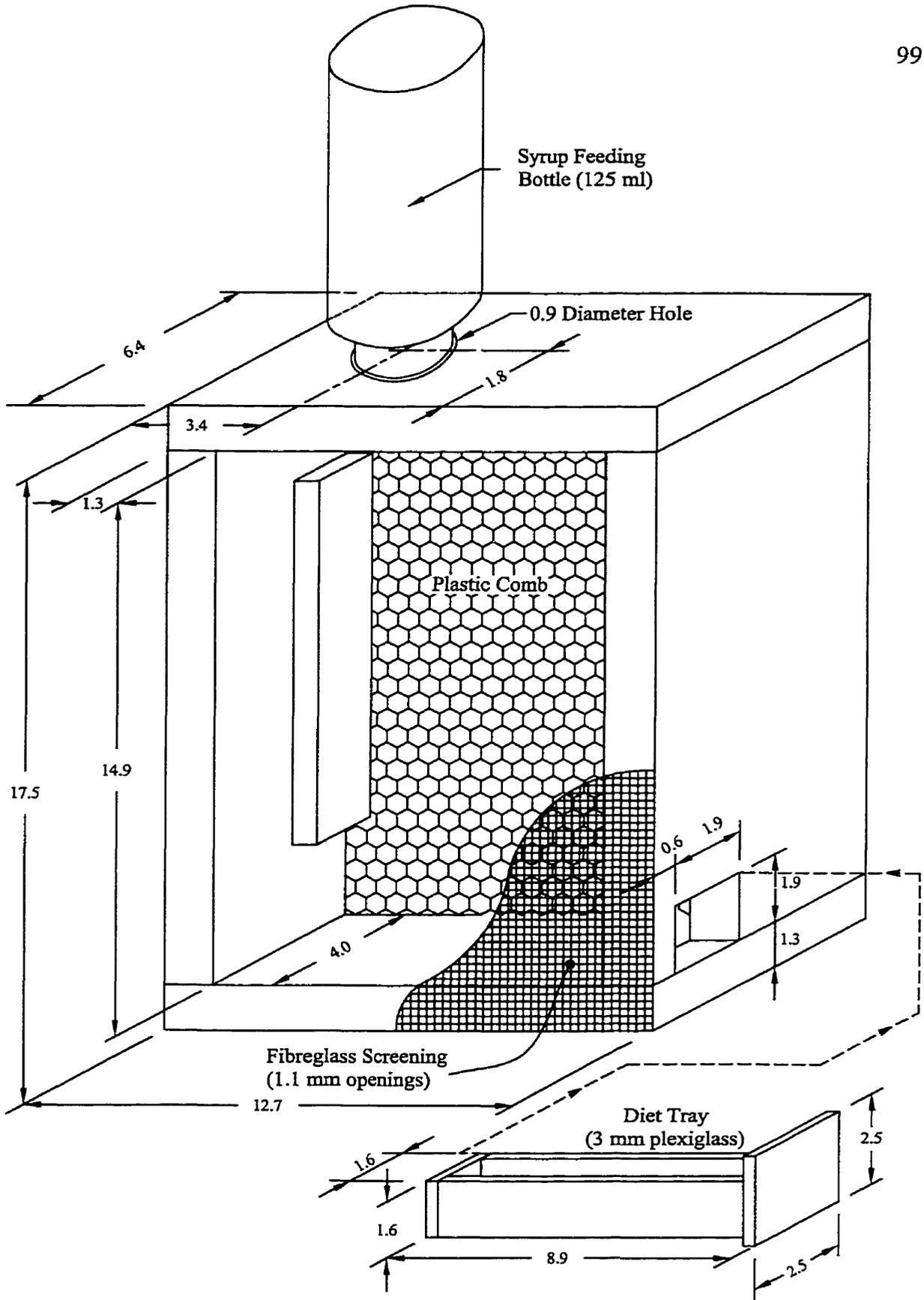


Fig. 3. Pollen consumption for worker bees fed freshly collected and 1-year-old pollen. Values represent the mean amount of pollen consumed within treatments per worker bee per day. Mean numbers of bees in treatments were calculated from the populations in cages at the midpoint of each experimental time interval, corrected for sampling loss and mortality, and weighted by the duration of the interval. Significant differences between diets within ages of pollen are indicated by different letters [LSD, $\alpha=0.05$ (experiment-wise error rate)].

Pollen Consumption (mg/bee/day, mean \pm se)

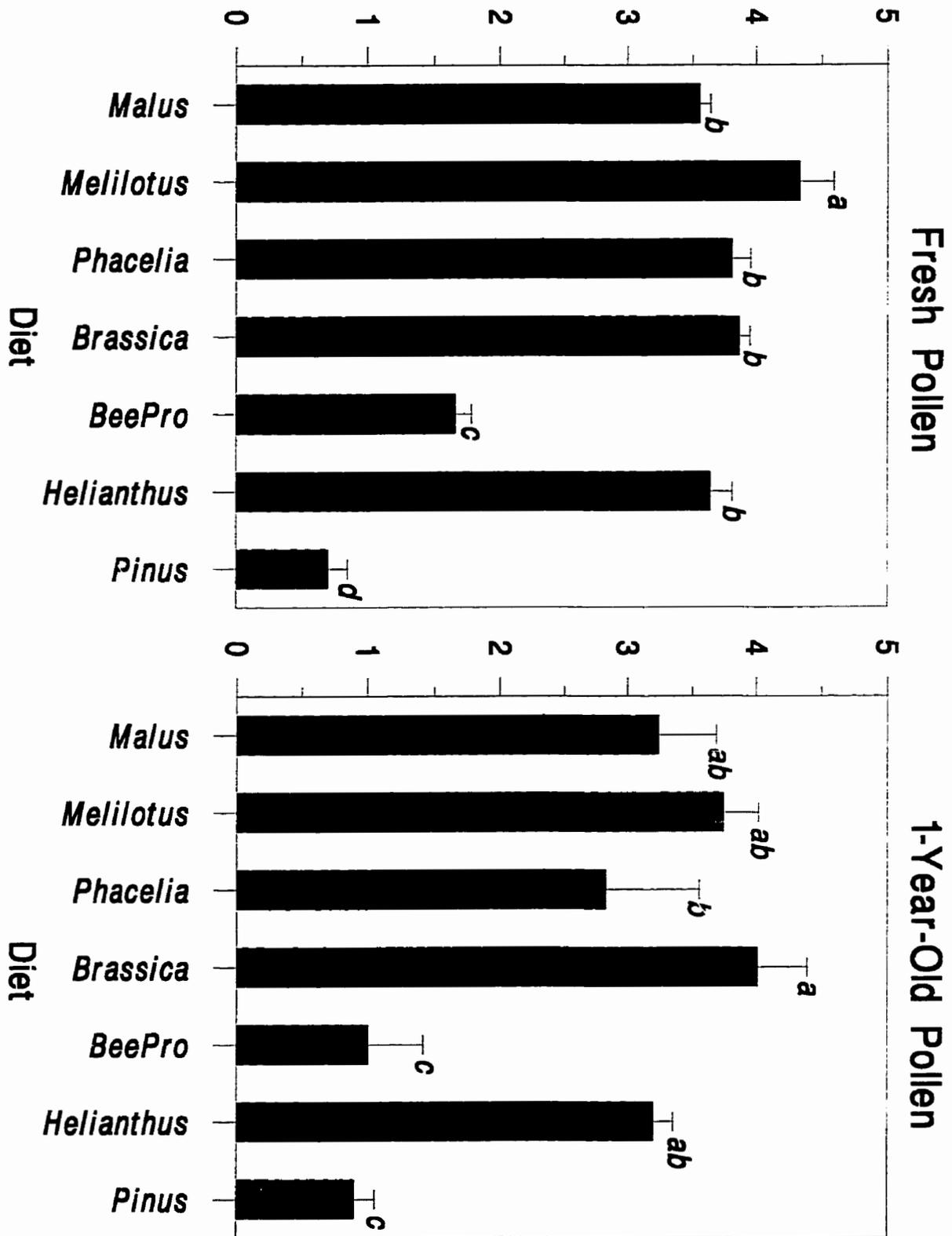
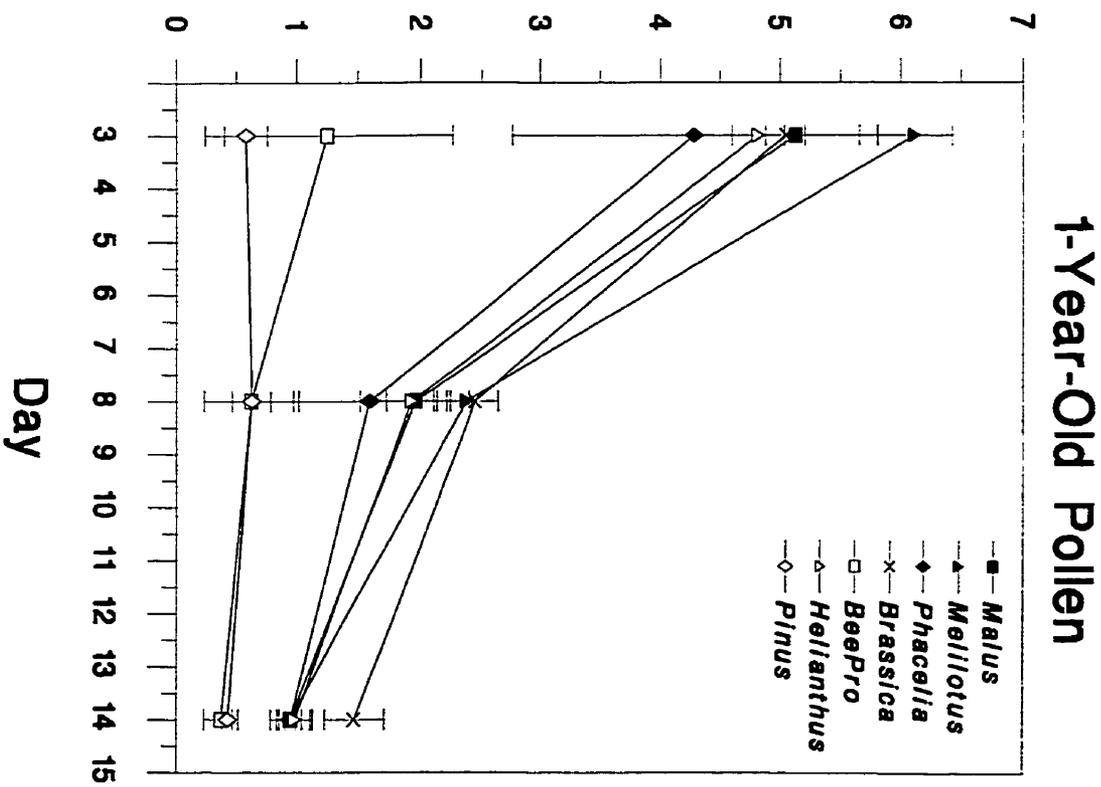
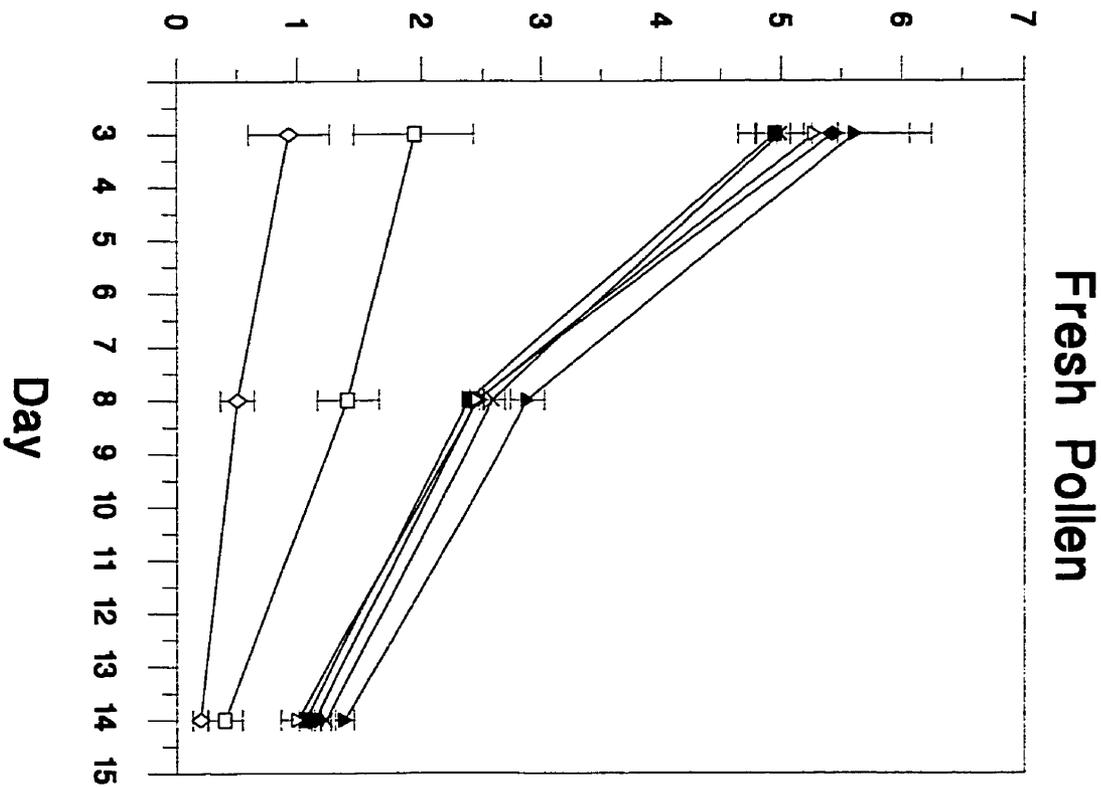


Fig. 4. Pollen consumption during each experimental time interval, for worker bees fed freshly collected and 1-year-old pollen. Values represent mean consumption per bee per day, based on populations in cages at the midpoint of the time interval, corrected for sampling loss and mortality.

Pollen Consumption (mg/bee/day, mean \pm se)



- Malus
- ▲ Mellilotus
- ◆ Phacelia
- × Brassica
- BeePro
- △ Helianthus
- ◇ Pinus

Fig. 5. Protein consumption for worker bees fed freshly collected and 1-year-old pollen. Values for consumption were calculated using the protein content of each diet. Mean numbers of bees in treatments were calculated from populations in cages at the midpoint of each experimental time interval, corrected for sampling loss and mortality, and weighted by the duration of the interval. Significant differences between diets within ages of pollen are indicated by different letters [LSD, $\alpha=0.05$ (experimentwise error rate)].

Protein Consumption (mg/bee/day, mean \pm se)

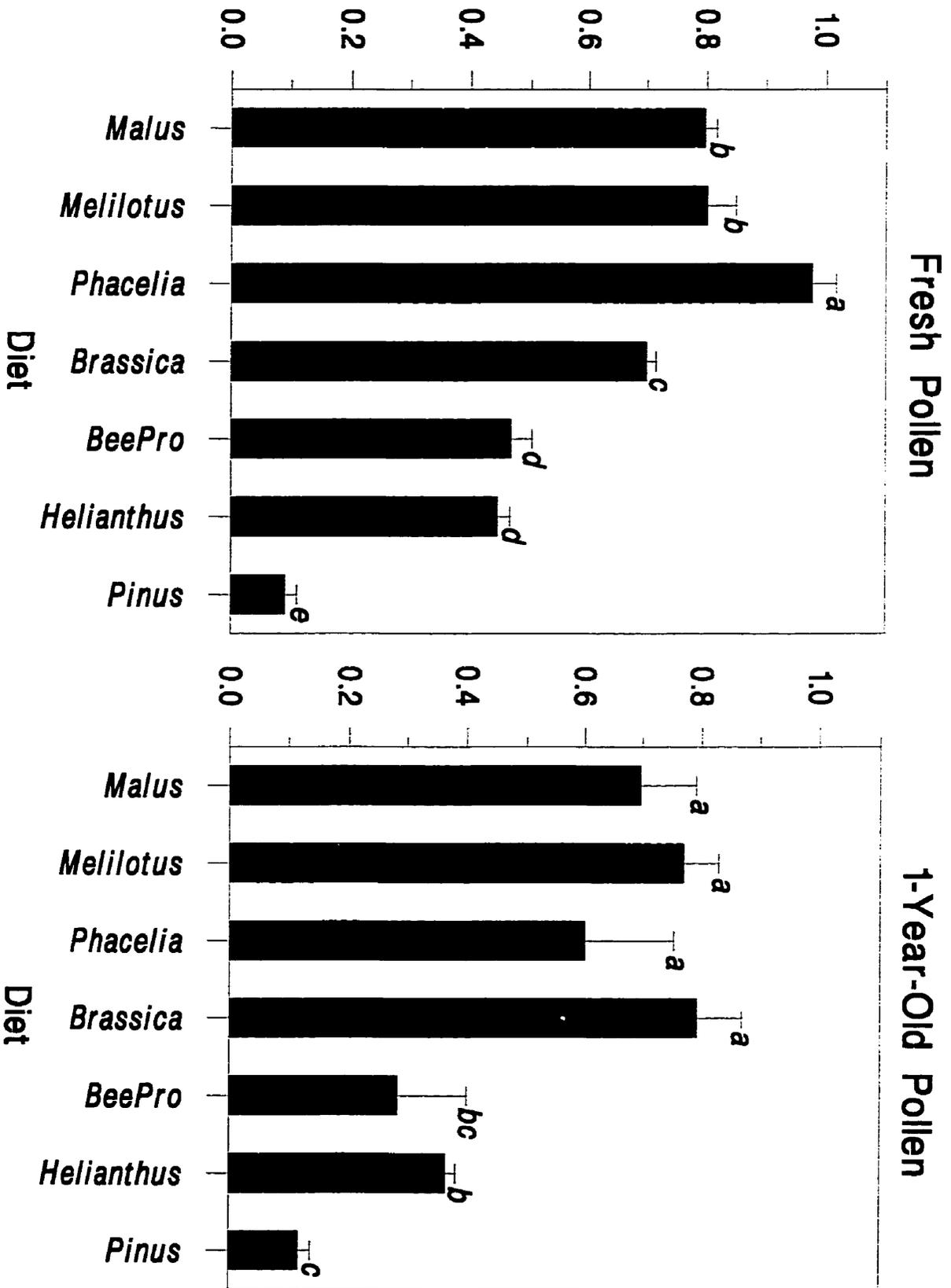


Fig. 6. Protein consumption during each experimental time interval, for worker bees fed freshly collected and 1-year-old pollen. Values for consumption were calculated using the protein content of each diet. Mean bee numbers per cage are based on populations at the midpoint of the time interval, corrected for sampling loss and mortality.

Protein Consumption (mg/bee/day, mean \pm se)

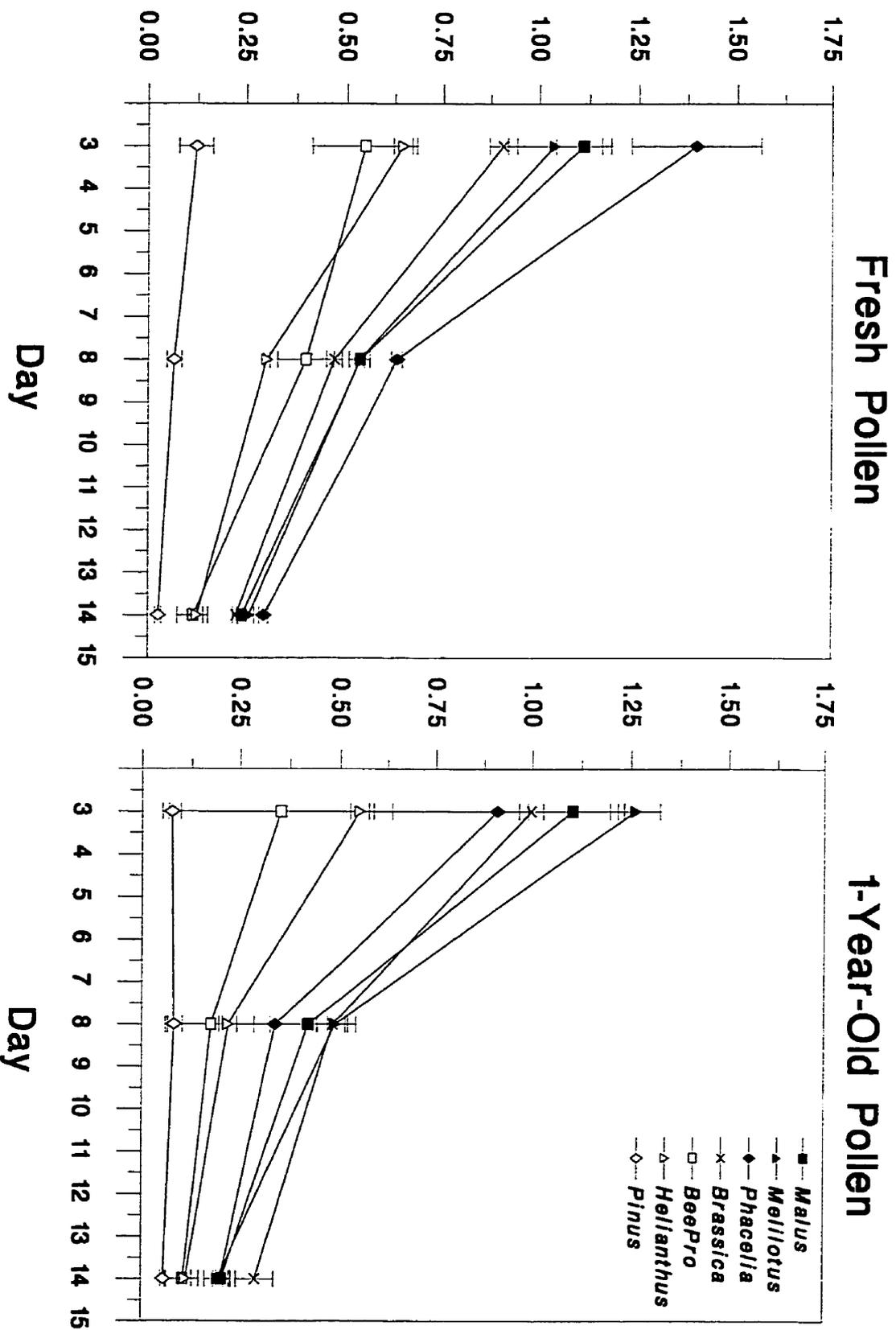


Fig. 7. Hypopharyngeal gland development for worker bees fed freshly collected and 1-year-old pollen. Values for hypopharyngeal gland protein represent mean development, averaged over the time intervals ending on days 3, 8 and 14. Significant differences between diets within ages of pollen are indicated by different letters [LSD, $\alpha=0.05$ (experimentwise error rate)].

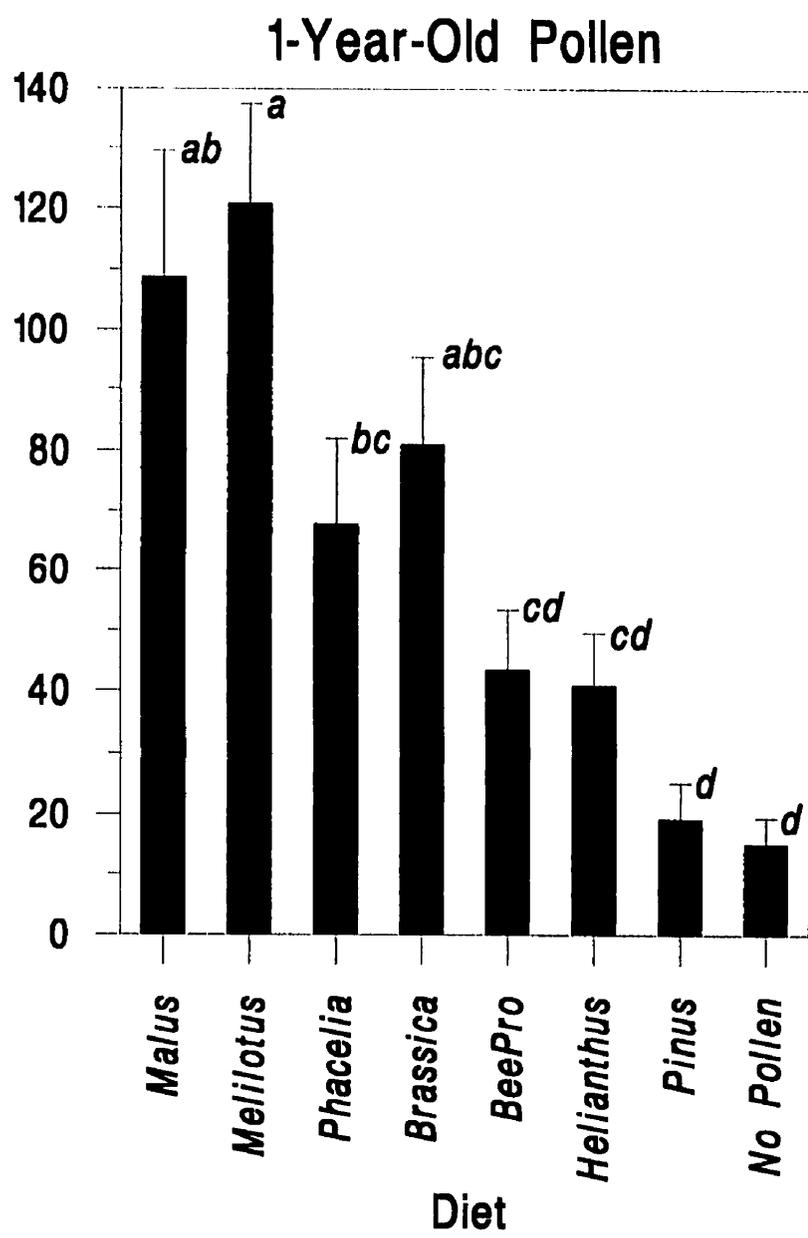
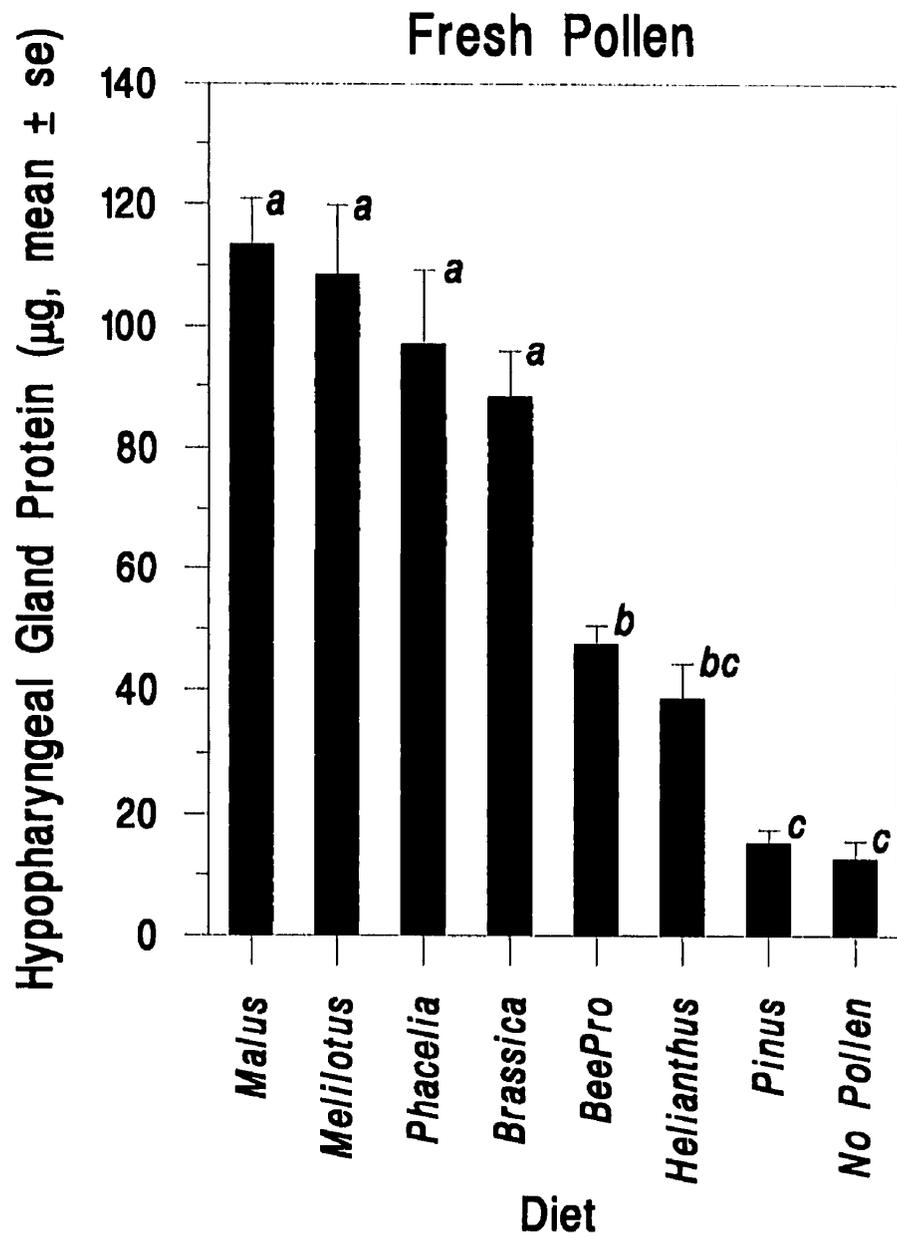
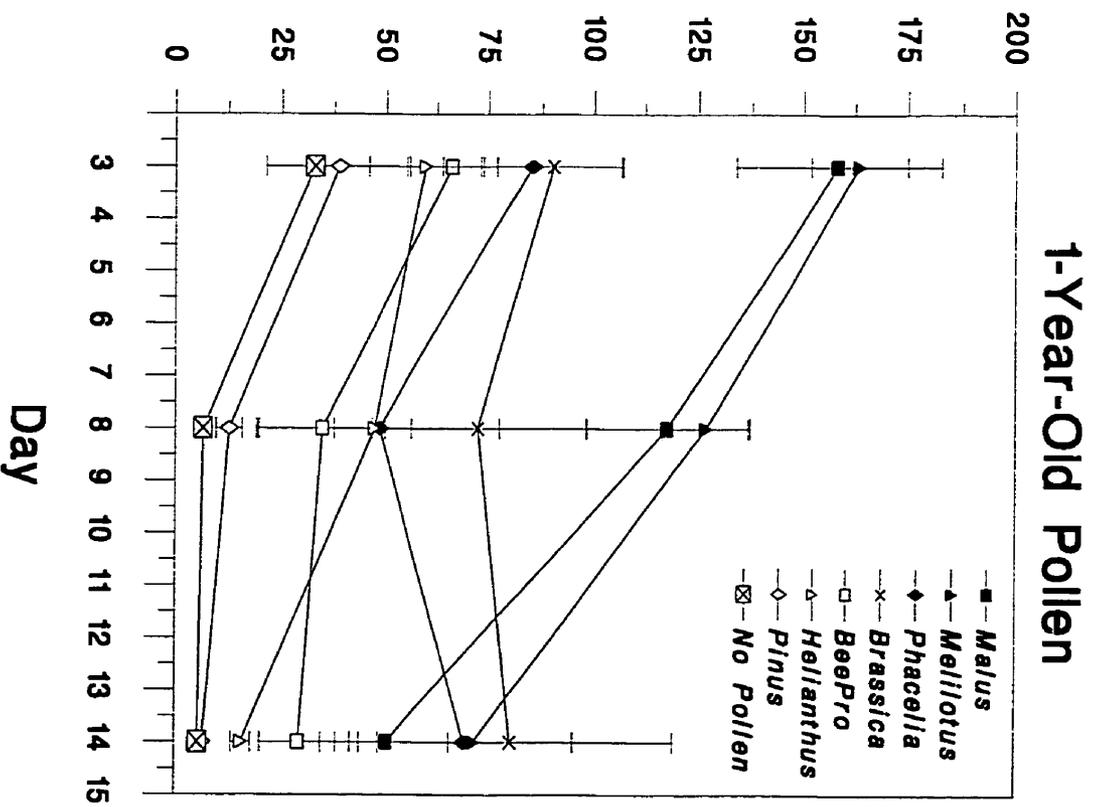
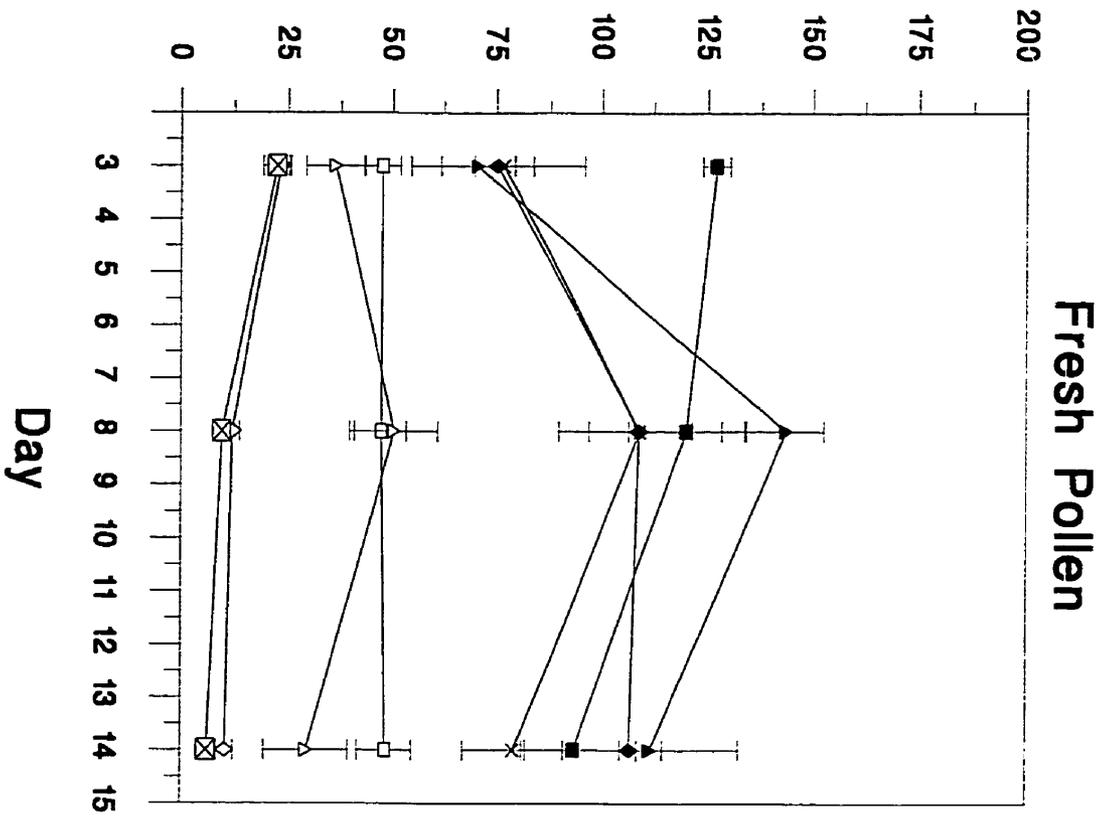


Fig 8. Hypopharyngeal gland development during each experimental time interval, for worker bees fed freshly collected and 1-year-old pollen.

Hypopharyngeal Gland Protein (μg , mean \pm se)



- Malus
- ▼ Mellilotus
- ◆ Phacelia
- × Brassica
- BeepPro
- △ Hellanthus
- ◇ Pinus
- ⊠ No Pollen

Fig. 9. Ovary development for worker bees fed freshly collected and 1-year-old pollen. Values for ovary score represent the mean extent of ovary development for bees on day 14 of the experimental period. Significant differences between diets are indicated by different letters [LSD, $\alpha=0.05$ (experiment-wise error rate)].

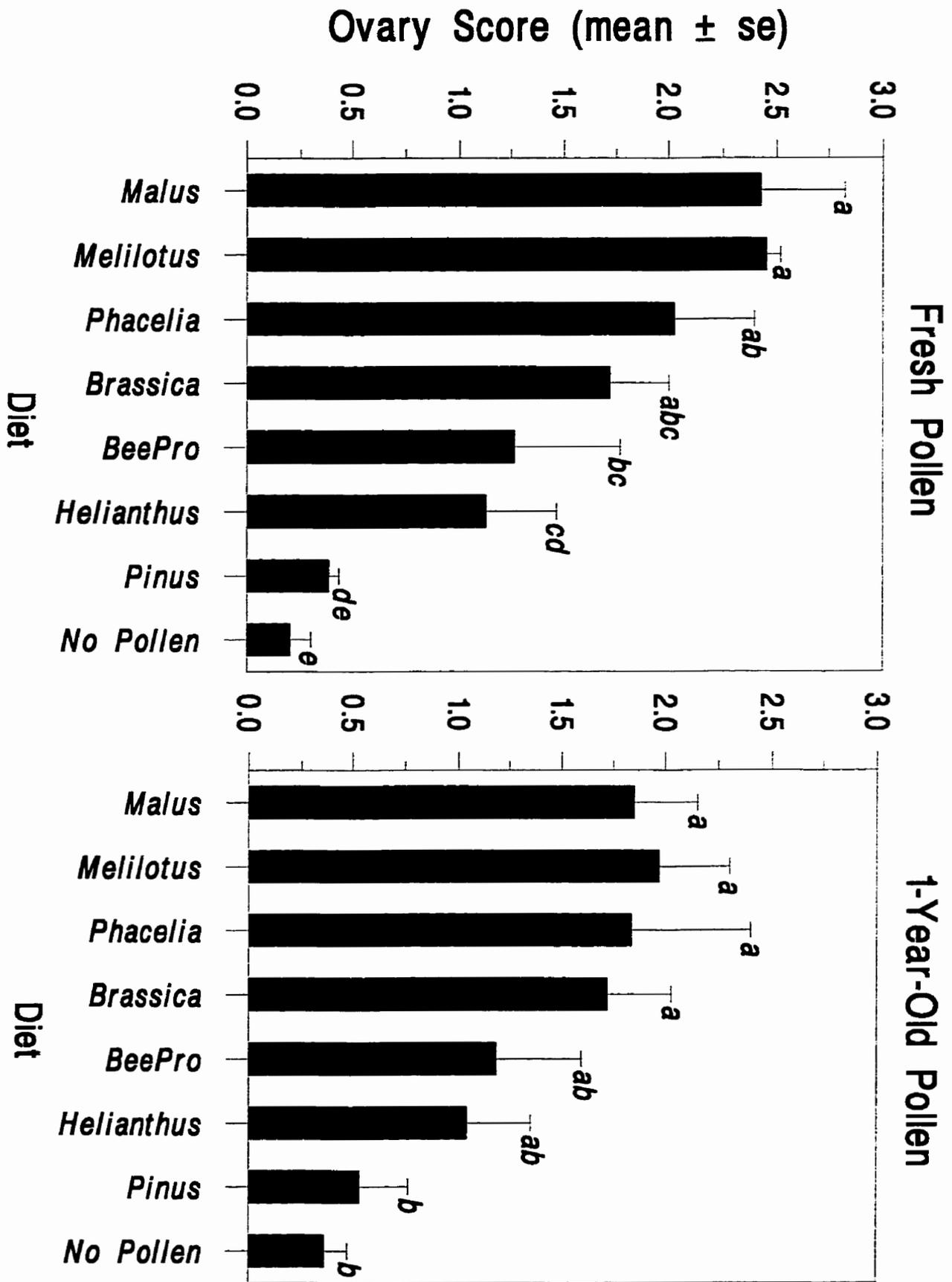


Fig. 10. Correlation between mean ovary score and hypopharyngeal gland protein for worker bees fed freshly collected and 1-year-old pollen. Values for hypopharyngeal gland protein represent mean development, averaged over the intervals ending on days 3, 8 and 14. Values for ovary score represent the mean extent of ovary development for bees on day 14 of the experimental period.

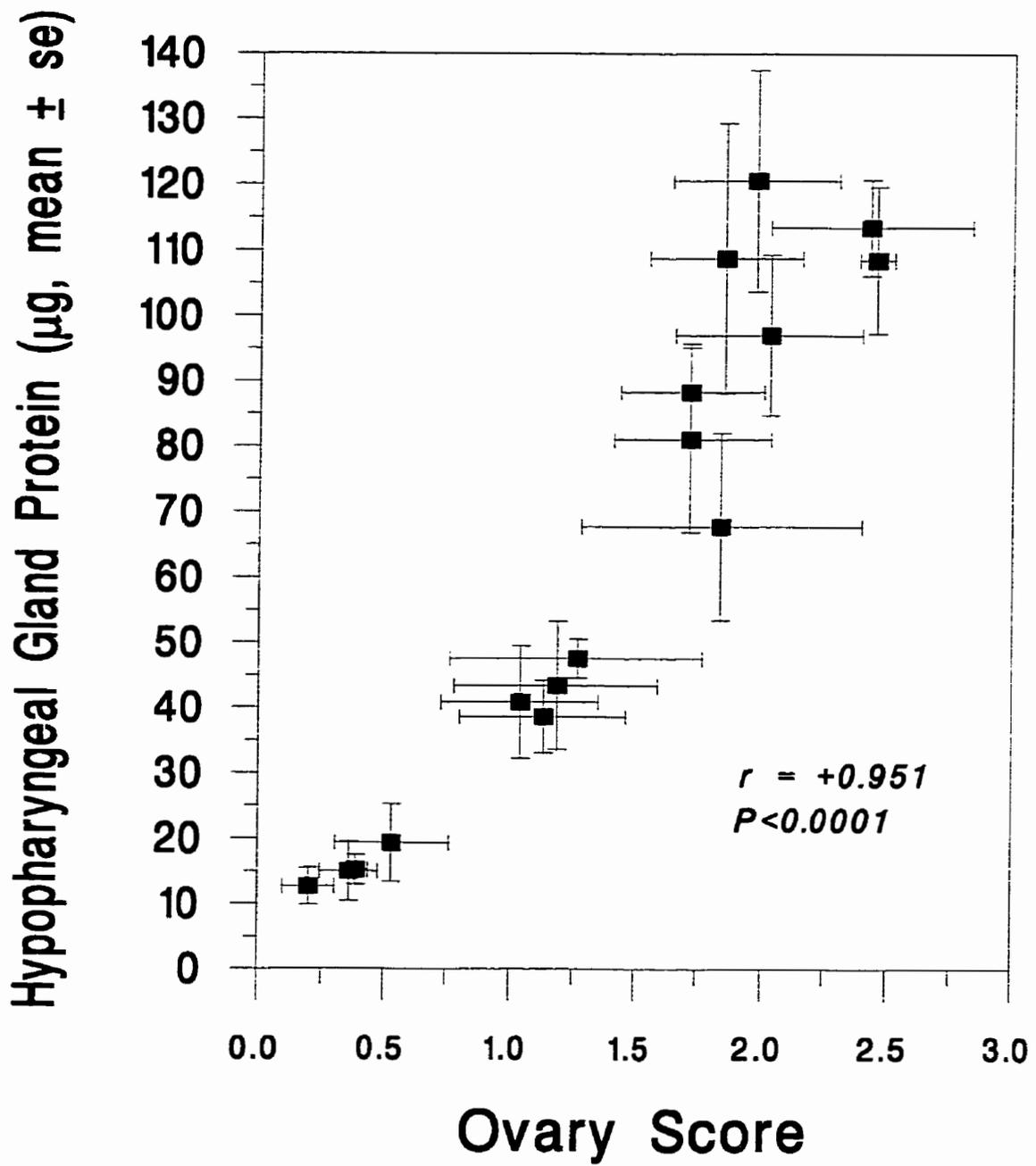
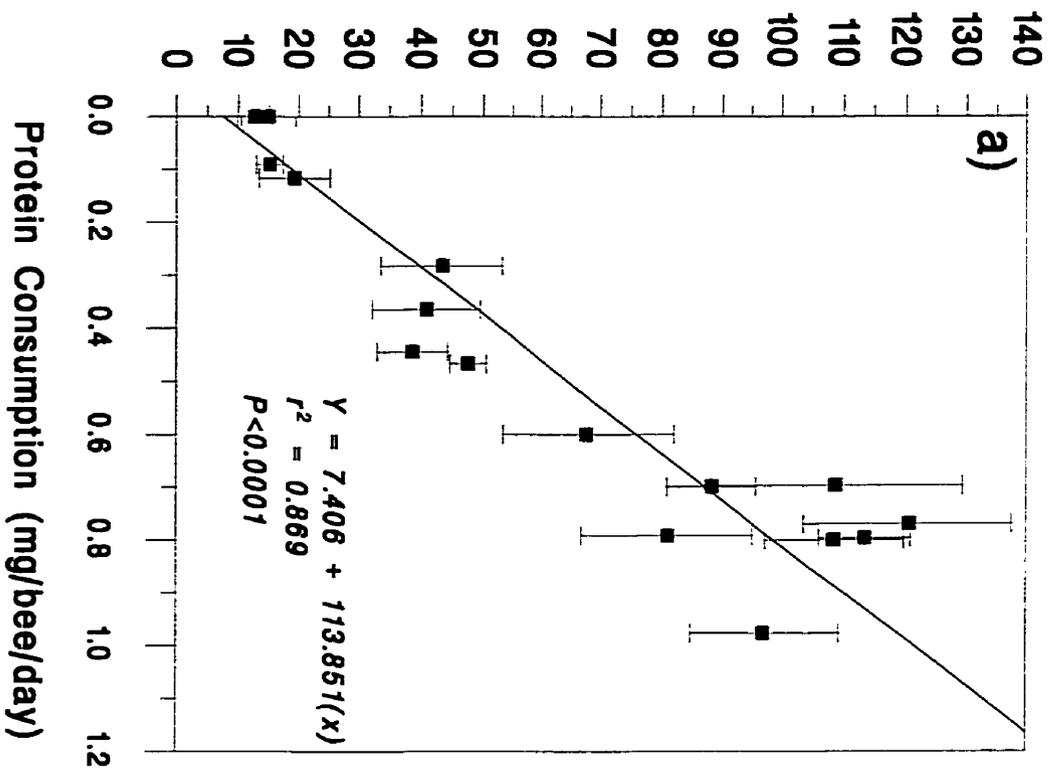
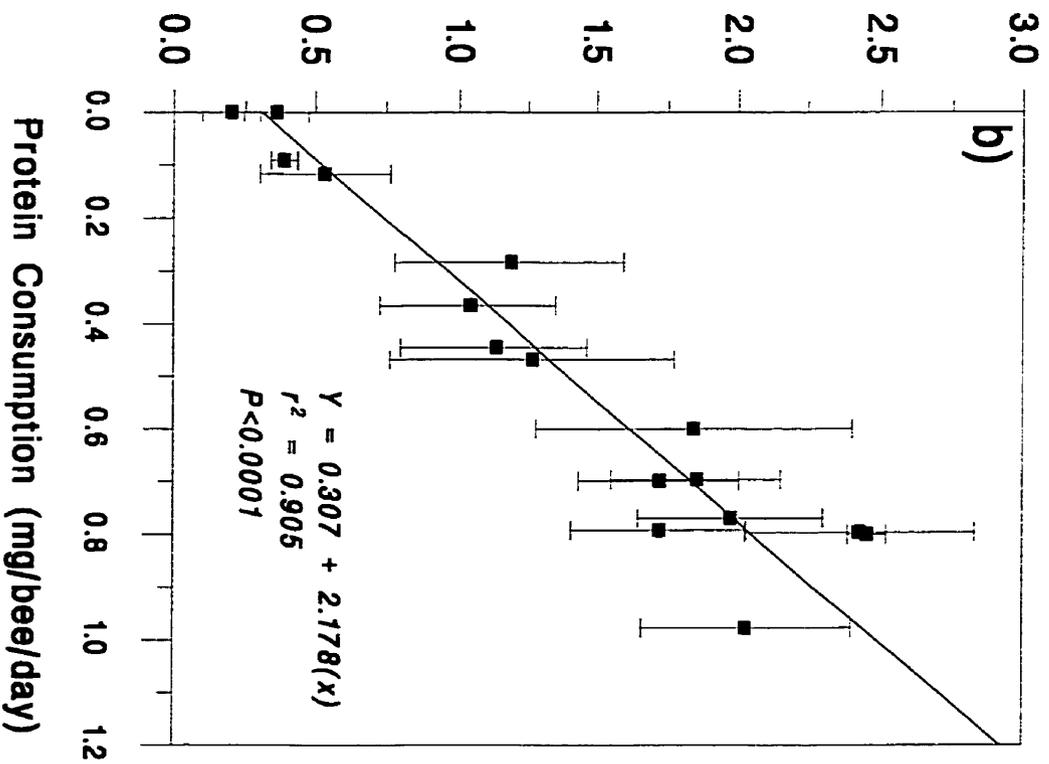


Fig. 11. Linear relationships between protein consumption per worker (from Figure 4) and indices of worker development, for freshly-collected and 1-year-old pollen diets. **a)** Hypopharyngeal gland protein. Values represent mean development, averaged over the intervals ending on days 3, 8 and 14. **b)** Ovary development. Values represent the mean extent of development on day 14 of the experimental period.

Hypopharyngeal Gland Protein (μg , mean \pm se)



Ovary Score (mean \pm se)



CHAPTER IV

**The Influence of Pollen Quality on Foraging Behaviour in Honey Bee
(*Apis mellifera* L.) Workers**

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The Influence of Pollen Quality on Foraging Behaviour in Honey Bee

***(Apis mellifera L.)* Workers**

Abstract

An experiment was conducted to determine whether honey bee (*Apis mellifera L.*) colonies respond to changes in the nutritional quality of their stored pollen reserve. Colonies were housed in vertical, three-frame observation hives situated inside a building, with entrances leading to the exterior. Queens were restricted to the upper two frames, which contained brood and honey, but no pollen. The lower frame of the colony was used for pollen deposition by foragers, and by manipulating the contents of this frame, two groups of treatments were created. In the first group, the size of the pollen reserve was varied to a low or high level, either by starving colonies of pollen or by providing them with a fully provisioned frame of pollen composed of mixed species. In the second group, the quality of the pollen reserve was manipulated by placing either low or high protein pollen supplements, provided *ad libitum*, within colonies. Before receiving treatments, all colonies were pollen starved for five days and all pollen foragers were marked. After applying treatments, foraging rates of colonies were determined four times per day and pollen loads were collected from experienced and inexperienced foragers for subsequent determination of weight, species composition and protein content. Each experimental trial was conducted for three days, and the entire experiment was replicated 11 times during the summer of 1996.

My results indicate that honey bee colonies respond to changes in the quality of their stored pollen reserve, and that these responses are similar to those for changes in pollen

quantity. Colonies responded to a decrease in the quality or size of their pollen reserve by increasing the proportion of pollen foragers in the foraging population, without altering their overall foraging rate. Colonies did not vary the breadth of floral species from which they collected pollen in response to experimental manipulations and exhibited no change in preference for the size of pollen grains collected. Individual pollen foragers also did not vary their effort during foraging trips in relation to the treatment applied to their colony. Individual foragers did not collect different weights of pollen loads, different numbers of species per foraging trip, and did not specialize in the collection of pollen with different nutritional values or sizes of grain in response to treatments. Individual foraging behaviour did, however, change markedly depending on the experience level of the forager. Foragers that were less experienced collected heavier loads, collected more species per foraging trip and specialized on pollen having a slightly greater protein content and grain size than more experienced foragers. Therefore, my results suggest that honey bee colonies respond to deficiencies in pollen quality by increasing the gross amount of protein returned to the colony, rather than by specializing on pollen having a greater protein content. Individual pollen foragers also appear to be insensitive to the quality of pollen they collect indicating that colony-level feedback is necessary to regulate the flow of protein to and within the colony. Colonies may respond to changes in the quality of their pollen stores by changing the ratio of inexperienced to experienced foragers within their foraging population.

Introduction

Pollen is an extremely important resource that honey bee colonies must collect. It

provides them with their only natural source of protein, which is needed for larval development, and fulfils other dietary requirements for lipids, sterols, vitamins and minerals (Herbert 1992). With the exception of water, nectar is the only other food item that honey bees require, but this resource is utilized in an entirely different fashion. Nectar is the major energy source of the colony, providing the raw fuel for the activities of all colony members. Not only are nectar and pollen utilized differently by honey bee colonies, but the way each is collected is also unique. However, our understanding of how colonies regulate pollen collection is poor relative to our knowledge of nectar collection.

Colonies typically recruit more foragers to the task of nectar collection than pollen collection, and only a small percentage of foragers collect both resources simultaneously (Parker 1926, Free 1960, Visscher and Seeley 1982). Behaviours of nectar and pollen foragers also differ: nectar foragers lap nectar from flowers and store it internally, whereas pollen foragers collect pollen externally by grooming it from their bodies and pressing it into their corbiculae (Hodges 1984). As a result, only nectar foragers receive direct qualitative information about the resource they are collecting, while they are collecting it, which allows them to optimize their foraging behaviour (Schmid-Hempel et al. 1985; Kacelnik et al. 1986; Schmid-Hempel 1986, 1987, 1991, 1993; Seeley 1986, 1994, 1995; Wolf and Schmid-Hempel 1990, Dyer and Seeley 1991; Waddington 1980, 1985; Núñez 1982; Varjú and Núñez 1991, 1993). Furthermore, colonies store more nectar than pollen. Based on conservative estimates, a generalized temperate honey bee colony collects about 120 kg of nectar per year, 70 kg of which is directly consumed during the summer, with the remaining 50 kg being converted to honey and accumulating

as a reserve (Rosov 1944, Seeley 1995).

In contrast, a similar colony would collect only 15-30 kg of pollen, almost all of which would be consumed, with a reserve of about 1 kg kept in the colony at any one time (Eckert 1942, Hirschfelder 1951, Jeffree and Allen 1957, Louveaux 1958, Seeley 1995). Thus, the small reserve of pollen in honey bee colonies makes them quite susceptible to sudden fluctuations in the supply of pollen in their environment. Because the production of brood and bees, and hence the overall fitness of the colony (Schmid-Hempel et al. 1993), is dependent on protein intake, it is paramount that honey bees tightly regulate their collection of pollen to meet their protein requirements. The regulation of pollen collection is therefore an inherently different process than nectar collection, and should be very sensitive to the flow of protein in the colony.

Previous studies have shown that honey bee colonies regulate their collection of pollen in response to changing demands for protein. Within the colony, the demand for protein is dependent on the rate and quantity of pollen consumed by nurses who, in turn, feed the developing brood. Any variation in the amount of brood within colonies results in corresponding adjustments to pollen foraging activity, in order to match pollen supply with demand (Filmer 1932; Cale 1968; Todd and Reed 1970; Al-Tikrity et al. 1972; Free 1967, 1979; Calderone 1993; Eckert et al. 1994; Pankiw et al. 1998b; Dreller et al. 1999). Colonies are also capable of responding to sudden decreases in protein intake resulting from the use of pollen traps. Under such circumstances, colony foraging effort is rapidly increased in proportion to decreases in the level of stored pollen within the colony (Lindauer 1952, van Laere and Martens 1971). Furthermore, colonies show compensatory responses after direct manipulations of the quantity of stored pollen by

changing the numbers of pollen foragers and their rate of pollen collection (Barker 1971, Free and Williams 1971, Moeller 1972, Fewell and Winston 1992, Camazine 1993, Dogterom and Winston 1999, Fewell and Bertram 1999, Dreller et al. 1999).

Two competing hypotheses have been proposed to explain how colonies regulate the quantity of pollen that they collect. In the “direct assessment” class of hypotheses, foragers determine a colony’s pollen demand by physically inspecting the amount of stored pollen and unsealed brood, or by detecting the intensity of odour produced by them (Free 1967, Pankiw et al. 1998b, Dreller et al. 1999). In the “indirect assessment” class of models, a different group of bees in the colony assimilates information about the need for pollen and then transmits that information to the pollen foragers. Camazine (1993) and Camazine et al. (1998) suggested that the quantity of protein produced by the hypopharyngeal glands of nurse bees acts as an inhibitory cue to regulate the amount of pollen being collected. High levels of protein received by foragers during their trophallactic exchanges with nurse bees inhibit them from collecting pollen, while low levels stimulate them to forage.

However, relatively little is known about whether individual bees or colonies respond to the protein content of the pollen that they are collecting. The protein content of pollen is a reliable and direct measure of pollen quality in the diet of honey bees (Pernal and Currie 2000) and is the major nutritional input that this resource provides to the colony. Pollen quality is also of considerable importance to colony fitness because protein content may vary considerably among different floral species (Todd and Bretherick 1942, Louveaux 1959, Stanley and Linskens 1974, McCaughey et al. 1980, Rayner and Langridge 1985, Winston 1987, Schmidt et al. 1987) and to a lesser extent,

even among different populations within the same species (Loper and Cohen 1987). Bumble bee foragers are sensitive to the availability of protein at a foraging site and the foraging costs associated with collecting it. As such, they forage in a manner that is consistent with maximizing the site-specific efficiency of protein collection (Rasheed and Harder 1997a, b). Fewell and Winston (1992) suggested that honey bee colonies choose pollen containing a higher nitrogen (protein) content when pollen stores within the colony are high, and accept lower quality pollen when stores are low. Waddington et al. (1998) associated differences in the recruitment dances of foragers with changes in the pollen offered at feeding stations, after it was mixed with an equal volume of cellulose powder. Presently, however, little other evidence exists to verify whether honey bee colonies have the ability to detect or respond to changes in pollen quality and the mechanism through which that might happen is unknown.

The question of whether honey bees, as colonies or as individual foragers, can detect or respond to changes in pollen quality is intriguing. The colony itself, though composed of thousands of autonomous individual members, functions as a coherent unit or “superorganism” (Seeley 1989, Wilson and Sober 1989). In order to maximize reproductive fitness, it would be reasonable to assume that colonies should specialize on only those pollens having high, or even moderate, levels of protein. However, many documented accounts describe honey bees collecting large quantities of pollen or pollen-like substances that have little or no nutritional benefit. Some examples include: sawdust, earth from swamps, coal dust, and animal feed (Root and Root 1935); rotted wood (Haydak and Tanquary 1943); road dust (Morse 1975); coal dust and flakes of paint (Fægri and van der Pijl 1979); epidermal hairs from plants, tar, and tree gum

(Johannsmeier 1981); and fungal spores (Shaw 1990). Further complicating a colony's choice of a protein-deficient pollen source is the fidelity that foragers develop to an easily-exploited food source over successive trips (Free 1963), coupled with their tendency not to sample other food sources during individual foraging bouts (Betts 1920, 1935; Percival 1947, Maurizio and Kollman 1949; Maurizio 1953, Free 1963). Honey bee foragers have clear preferences for some pollen types over others, both in natural settings (Linsley and McSwain 1947, Bohart 1957, Olsen et al. 1979, Jay and Jay 1984, Free 1993) as well as in controlled choice experiments (Levin & Bohart 1955, Doull 1966, Wahl 1966, Boch 1982, Schmidt 1982, Boelter and Wilson 1984). In addition, bees also show specific preferences for the type of pollen that they consume (Synge 1947, Purdie and Doull 1964, Campana & Moeller 1977, Boch 1982, Schmidt 1984, Schmidt and Johnson 1984, Schmidt et al. 1995, Pernal and Currie 2000). Among pollen-collecting foragers or those bees that consume pollen, experimental evidence suggests that the choice of pollen by bees is not influenced by the pollen's nutritional adequacy (Levin and Bohart 1955, Wahl 1966, Schmidt 1982, Free 1993). Pollen choice is, however, influenced by non-nutritional factors such as the age, moisture content, colour, pH, pollen grain size, physical configuration of the grains (Stanley and Linskens 1974, Jay 1986) and in particular, the presence of odours and phagostimulants (Louveaux 1959, Hugel 1962, Taber 1963, Lepage and Boch 1968, Robinson and Nation 1968, Hohmann 1970, Doull and Standifer 1970, Doull 1974, Boch 1982, Schmidt 1985). Therefore, the inability of honey bee foragers to be able to detect or choose pollen based on its quality appears to be at odds with foraging behaviours that would increase the fitness of the colony.

To examine whether honey bees can adjust their foraging behaviour in response to changes in pollen quality, responses must be examined both at the level of the colony and at the level of the individual forager. The total amount of protein being returned to a colony can be adjusted by a change in either the quantity of pollen being collected by foragers, the quality, or both. The rate of pollen intake to a colony can be modified by changes in the number or proportion of pollen-collecting specialists within the foraging population, and by changes in the effort of individual foragers. Individual foragers may increase their foraging effort by collecting larger pollen loads or by modifying their behaviour in such a way that the rate or efficiency of pollen collection is maximized. To achieve the latter, pollen foragers would have to optimize the time or energy expended to collect a given quantity of pollen against site-specific forage context variables.

Changes in the quality of pollen returned to the colony may also come about in different ways. Honey bee colonies may respond to reductions in pollen quantity or quality by recruiting foragers to floral patches having higher protein levels. Such a shift in foraging strategy could be detected by comparing the protein content of forager-collected pollen between colonies manipulated for different quantities or qualities of pollen in their stored reserve. Colonies may also increase the allocation of scouts in relation to protein demand. For instance, when protein levels in the colony are high, colonies may allocate more scouts to locate and exploit new and highly proteinaceous species of pollen. This increase in the breadth of sampling would be represented by an increase in the number of floral species being returned to such colonies. Similarly, qualitative changes in pollen collection could also result from the efforts of individual scouts or foragers to sample their floral environment more intensively per foraging trip.

Such changes could be detected by increases in the average number of pollen species collected per pollen load. Finally, the overall protein intake contributed by the quantitative and qualitative choices of foragers could be estimated by calculating the gross amount of protein collected per forager or the amount being returned to colonies per unit time.

The primary objective of this study was to compare changes in foraging behaviour resulting from manipulations of the quality of pollen stored in colonies with manipulations of the quantity of pollen stored in colonies. I also determined if such changes in foraging behaviour occurred at the level of the colony, the individual forager, or both. Finally, I compared the behaviour of two cohorts of foragers, those relatively inexperienced and those with known pollen foraging experience, to decide whether they differed in response to such manipulations.

Materials and Methods

Honey Bees and Observation Hives

In the spring of 1996, 50 nucleus colonies of honey bees were established. Hives were founded with mated Italian queens, commercially purchased from New Zealand, and were housed in Langstroth hive bodies. All colonies were located at the University of Manitoba Glenlea Research Station, 20 km south of Winnipeg, MB. These colonies were maintained as a pool of bees of similar genetic strain, from which colonies were chosen to stock experimental hives. The number of colonies established was in excess of the requirements of the experiment to allow for poor queen performance or premature colony death, and to facilitate the matching of colony size between treatments.

During experimental trials, honey bee colonies were housed in three-frame, vertical observation hives fabricated from construction-grade spruce framing lumber and glass (Figs. 12, 13). Twelve observation hives were constructed and installed at 61 cm intervals, along an east-facing wall inside the honey bee overwintering building at the University of Manitoba campus. Hives were designed to allow independent access to both sides of the full-depth Langstroth frames and permitted queen excluders (1.97 meshes cm^{-1} hardware cloth) to be inserted between frames. Entrances for colonies consisted of a 2.5 cm O.D. by 1.9 cm I.D. by 30 cm long PVC hose (Kuri Tec, Product #K010-1216, Kuriyama Corp., Brantford, ON.) which linked colonies to the exterior. Landing platforms (15.2 cm by 28.9 cm), were constructed from 1.9 cm thick fir plywood, and were installed on the exterior of the building below entrance tubes, 1 m above the ground. Coloured signs were placed above the hive entrances to minimize bee drifting.

Two weeks prior to the start of the first experimental trial, twelve colonies were transferred into experimental observation hives. Colonies were equalized for brood and honey, and were stocked to contain approximately 6,000 bees, each. Queens were restricted to the upper two frames of the colony, by the use of a queen excluder, resulting in the upper two frames being completely utilized for brood rearing with a minor amount of honey storage. An empty frame of dark brood comb was placed in the lowest position of the observation hives. As a result, it was the only frame in which pollen was stored.

Treatment Structure

In this experiment, I examined the foraging responses of honey bees to a

manipulation of their stored pollen reserve. A factorial design was employed (Mead et al. 1993), with two main factors being tested: 1. The stimulus mechanism influencing pollen foraging, and 2. The intensity of the stimulus. Each of these factors had two levels. For stimulus mechanism, one level was a manipulation of the “quality” of pollen stored in the hive, while the other was a manipulation of the “quantity” of pollen stored in the hive. For stimulus intensity, levels corresponded to either “high” or “low”. Therefore, four treatments, each corresponding to a manipulation of the stored pollen reserve, were used: quality high, quality low, quantity high and quantity low.

Experimental Trials

Six trials were performed between 12 July and 30 Aug 1996. Different colonies were used for each treatment. On five dates, two sets of treatments were run simultaneously and on one date only one set of treatments was used. Therefore, over the course of the summer, 11 replicates of each treatment were performed. Because a maximum of 8 of the 12 available experimental colonies were used at any one time, equally matched colonies could be selected at the start of each trial. Weak or failing colonies were replaced, and allowed two weeks to become accustomed to foraging from their observation hives before use in experimental trials. Most colonies were reused between successive trials, until they had been assigned to every treatment group. A small number of colonies were used in only two experimental trials. When reused, these colonies were assigned to treatments within the same stimulus mechanism factor but the opposite stimulus intensity levels (“high” or “low”).

Experimental Protocol

Trials consisted of a 5-day pre-experimental period followed immediately by a 3-day experimental period. At the start of the pre-experimental period, the areas of sealed and unsealed brood in the upper two frames of all 12 colonies were measured with a plexiglass grid, and colonies chosen for use in the trial were matched to ensure equal levels of brood and adult bees. During the pre-experimental period, all colonies chosen for experimental manipulations were pollen deprived in a manner similar to Camazine (1993). This was accomplished by removing the bottom frame from colonies each evening (between 2000 and 2200 hrs) and replacing it with an empty, dark brood frame. Pollen deprivation during the pre-experimental period ensured that any small amounts of pollen in the brood frames would be consumed, and kept the demand for pollen high prior to experimental manipulation. Typically, no cells containing pollen would be found in the upper two brood frames by the end of the of the pre-experimental period.

In addition to depriving colonies of pollen during the pre-experimental period, pollen foragers were also identified and marked. Pollen foragers returning to their colonies were captured, anaesthetized with gaseous CO₂, and painted on the top of their thorax with a different colour of model aircraft dope for each colony (Harris 1979). A pollen forager was identified by the presence of any visible amount of pollen on her corbiculae. To capture foragers, a strip of screening was placed on a downward angle in front of the entrance tubes of a colony, resting on the landing platform. This slowed the entrance of foragers to the colony, and caused bees to enter individually, rather than several at one time. Individual foragers were captured with forceps and placed in ventilated cages before anaesthetizing them. Marking was continuous during the periods of flight activity,

and an attempt was made to mark all of the known pollen foragers in each colony. Hence, marked pollen foragers that were collected during the subsequent experimental period were considered to be of a more experienced cohort than those pollen foragers collected without marks, which represented a less experienced, and presumably naive, group of foragers. Rates of drifting between experimental colonies were also determined by tabulating the number of foragers returning to colonies other than the one in which they had been marked.

Colony pollen stores were first manipulated on the evening of the fifth day of the pre-experimental period between 2000 and 2200 hrs, when foraging activity had ceased. The following morning constituted the first day of the experimental period. Colony stores were again manipulated during the evenings of the first and second day of the 3-day experimental period. Manipulations took place inside the overwintering building under red ambient lighting. Immediately prior to manipulations being performed, hive entrances were blocked, and smoke was used to drive bees onto the upper two frames. An excluder (2.76 meshes cm^{-1} hardware cloth) was then inserted between the middle and bottom frames, thereby confining almost all bees to the upper portion of the hive. This permitted the lower frames of colonies to be manipulated with minimal loss of bees. The few bees that did escape were recaptured from within the building and were replaced at the exterior hive entrance, after their colony was returned to its normal configuration.

For high quantity treatments, a pollen frame consisting of a large amount of stored pollen ($> 600 \text{ cm}^2$ per side), of mixed species (*Brassica napus* L., *Melilotus officinalis* (L.) Pall., *Melilotus alba* Desr. and *Trifolium* spp.), was placed in the bottom position of each hive. For low quantity treatments, an empty brood frame was placed in the same

lower position, as had been done for all colonies during the previous four days of the pre-experimental period. For high quality treatments, canola (*B. napus*) and yellow sweetclover (*M. officinalis*) pollen were collected using O.A.C. pollen traps (Smith and Adie 1963) located at the University of Manitoba campus. A mixture of these pollens was formulated into a supplement using high fructose corn syrup and equal portions were placed into “u”-shaped wax paper troughs (approx. 12.0 cm by 3.5 cm by 2.5 cm deep). For low quality treatments, similar supplements were made with jack pine pollen [*Pinus banksiana* Lamb.]), which were then placed in “v”-shaped plastic troughs (12.0 cm by 3.5 cm by 2.8 cm deep). Low and high quality pollen supplements were inserted into the space between the bottom and middle frames of colonies. The nutritional content of the pollen species used to formulate supplements was determined in an earlier study (Pernal and Currie 2000). Low and high quality supplements were provided *ad libitum*, and were replaced during the evenings of the first and second day of the experimental period. Frames used in manipulations of low and high quantity treatments were also replaced at the same time as the low and high quality supplements.

Consumption of low and high quality supplements was monitored daily, by weighing supplements at the time they were removed from colonies at the end of the day. Water loss of low and high quality supplements was evaluated by placing similar masses of the low and high quality diet mixtures into colonies not being used in a particular trial. The supplements used for calculating water loss were protected by screens to prevent consumption by bees, and were removed at the same intervals as the low and high quality diets in experimental colonies. Consumption estimates were corrected for evaporative water loss by comparison with unconsumed supplements. The plastic troughs used to

estimate water loss for jack pine pollen, by virtue of their “v” shape and protection from consumption, provided a greater surface area of supplement to be evaporated than troughs in treatment colonies. As a result, estimates of water loss in jack pine pollen supplements may have been slightly overestimated. Because the amount of water loss in these supplements was typically between 1 - 2 %, and never greater than 3 % of the original diet mass, a function to correct potential overestimation of water loss in low quality treatments was not applied.

Each day during the 3-day experimental period, returning pollen and nectar foragers from experimental colonies were counted during 5 minute intervals at 1000, 1200, 1330 and 1600 hrs to quantify foraging rates. In addition, marked (experienced) and unmarked (inexperienced) pollen foragers were collected from each colony, during morning and afternoon periods. I attempted to collect ten of each type of forager per colony; numbers less than ten were collected if a forager type was very infrequent in the foraging population and difficult to obtain during certain time periods. Collected foragers were placed in separate containers, frozen on dry ice, and stored in a freezer at -30° C until analyzed.

Analysis of Pollen Loads

Both pollen loads were removed from the corbiculae of frozen workers and weighed wet to the nearest 0.01 mg. One of the two pollen loads per forager was used to prepare slides for microscopic examination while the other was refrozen for subsequent protein analysis. Pollen load composition was determined by suspending pollen in water, agitating it, and then mounting it on glass slides with coverslips for examination. For

unifloral pollen loads, at least one representative of every species was mounted, unacetolyzed, in glycerine jelly (G-35, Fisher Scientific Co., Fairlawn, NJ.), corresponding to each colony, day and time period in which data were collected. Selected unifloral loads were also prepared by acetolysis and mounted in silicone oil (described below). All multifloral pollen loads, and those comprised of infrequently collected species, were mounted as unacetolyzed grains in glycerine jelly, with representative samples prepared by acetolysis. Preparation and mounting of pollen grains in glycerine jelly conformed to standard palynological techniques (Fægri et al. 1989), with the edges of coverslips being painted with a laquer sealant (glyceel, Gurr, High Wycombe, England) to prevent desiccation.

Pollen load preparations were examined using phase contrast or Nomarski differential interference contrast light microscopy (Photomicroscope II, Carl Zeiss, Germany). Routine identification was performed at 500 \times , while the identification of unknown or morphologically similar species was performed at 1000 \times with oil immersion. The identities of 100 pollen grains per preparation were ascertained using taxonomic keys and pollen atlases (Kapp 1969, Erdtman 1966b, Adams and Morton 1972, 1974, 1976, 1979; Bassett et al. 1978; Sawyer 1981, 1988; Fægri et al. 1989; Moore et al. 1991; Crompton and Wojtas 1993) and were verified against a set of reference slides prepared from known local flora. The size and morphology of pollen grains were used as primary taxonomic characters, however, the colour of pollen loads also provided a preliminary indication of species composition. Pollen load colour also provided valuable information for the identification of unknown species, or for closely related species that were difficult to distinguish by structural morphology (Hodges 1984, Kirk 1994). Furthermore, the

geographic distribution and bloom phenology of species were used to elucidate the identity of unknown pollen types (Scoggan 1957, Looman and Best 1979, Crompton and Wojtas 1993). Pollen grains were counted from a minimum of three microscopic fields, situated on opposite edges and the centre of sample preparations. Pollen grain equatorial and axial measurements were performed with a calibrated ocular grid.

Preparation of Reference Slides

A reference series of pollen slides was prepared to verify identifications made from bee-collected pollen. Pollen was hand-collected from local flora near the University of Manitoba and was also obtained from identified specimens housed in the University of Manitoba herbarium (WIN). For hand-collection, undehisced and newly-dehisced anthers were collected from blooming flowers. Later, anthers were placed in a small volume of water on a microscope slide and pollen was separated using fine forceps and a binocular dissecting microscope. Both herbarium and hand-collected pollen was suspended in 1.5 mL of water and passed through either a 45 μm or a 75 μm screen, depending on grain size, to remove any non-pollen debris. After desiccation using low heat, external lipid material was cleared from grains with diethyl ether or n-pentane, and a portion of pollen from each sample was mounted, unacetolyzed, in glycerine jelly (Fægri et al. 1989). The remaining portion of pollen from each sample was prepared by acetolysis (Erdtman 1960, Crompton and Wojtas 1993).

For acetolysis, 10 mL of a 9:1 mixture of acetic anhydride to concentrated sulfuric acid was added to the pollen. The suspension was slowly brought to a boil using a water bath, centrifuged (2,700 $\times g$) and supernatant decanted. This procedure was repeated,

each time with 10 mL of a different solvent, in the following sequence: distilled water, glacial acetic acid, 95% ethyl alcohol, absolute ethyl alcohol, and acetone. After the acetone was decanted, the remaining acetolyzed pollen grains were placed in spot plates containing silicone oil (60,000 viscosity, Johnson Instrument Sales Inc., Elmhurst, IL) and were mounted on microscope slides with glass coverslips. For species that did not have measurements listed in published studies, mean equatorial and axial diameters were determined from at least 20 grains.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was employed to confirm the identity of morphologically similar species, and to aid in the identification of samples difficult to ascertain by light microscopy. Non-acetolyzed pollen grains, cleared of all external lipids, were desiccated with low heat, and spread evenly over the surface of carbon adhesive tape (JBS #791, J.B. EM Services Inc., Pointe Claire, QC) attached to aluminum SEM stubs. Samples were sputter-coated (S150B, Edwards High Vacuum International, Wilmington, MA) with gold-palladium and then examined using a Stereoscan 120 SEM (S120, Cambridge Instruments (Canada) Inc., Montreal, QC). The SEM was equipped with a secondary emission detector, and an acceleration voltage of 30 KV was used. Images were digitally captured using an IBAS II image analyzer (Kontron Electronik, Newport Beach, CA) and were later output as composite images using a dye-sublimation printer or to Kodak[®] TMAX 100 print film.

Protein Determination

Total crude protein for pollen loads was calculated as the sum of the weight of protein contributed by each component species. The weight of protein contributed by each species was calculated as the product of species-specific protein constants and the portion of the mass that each species contributed to the load. Protein constants were determined from pooled pollen samples, comprised of several unifloral corbicular pollen loads subsampled from all collection dates during the experiment. Samples were weighed before and after drying in an oven at 70° C for 24 h to ascertain dry weight and water content. Dried samples were ground with a mortar and pestle to achieve a powder-like consistency, and then stored in a desiccator until analyzed. Total nitrogen content was determined using an elemental analyzer (Model FP-428, Leco Instruments Ltd., Mississauga, ON.), calibrated against known standards. To determine total crude protein, nitrogen values were multiplied by a conversion factor of 5.60. This factor is more accurate for pollen protein determinations than the 6.25 conversion, commonly used for other types of plant samples (Rabie et. al 1983).

The proportion of the total mass that an individual pollen species contributed to a multifloral pollen load was estimated using the relationship between the number of pollen grains in unifloral pollen loads of that species and the wet weight of such loads. This corrected for discrepancies between the size of pollen grains of different species and their relative contribution to the weight of pollen loads. Particle analysis techniques were used to determine such relationships for species that frequently occurred in this study. A model Z_{BI} Coulter Counter (Coulter Electronics, Inc., Hialeah, FL) was used to count pollen grains in unifloral loads. Calibration was performed with common ragweed pollen

(*Ambrosia artemisiifolia* L.), for precise determination of the volume of particles bracketed by each channel of the instrument. Lower and upper channel thresholds were adjusted for each species to provide a window, 10 μm above and below its mean equatorial diameter (Crompton and Wojtas 1993), in which pollen grains would be counted. Fifteen to twenty pollen loads of each species, over a wide range of masses, were selected for analysis. Loads were suspended in 15 mL of a weak aqueous electrolyte solution (1% sodium chloride), and were filtered through a 38, 53, or 75 μm screen, depending on grain size, to remove non-pollen debris. Samples were kept agitated by stirring, and a 500 μL subsample was drawn through the instrument's 100 μm diameter aperture tube for counting. Five replicate counts of each sample were performed and averaged. Pollen grain concentrations in sample suspensions were low enough to minimize the coincident passage of grains through the aperture; any corrections to counts for coincident passage were calculated as outlined in Sheldon and Parsons (1967).

The proportions of grains of each species were converted to weight proportions, using a standardized load size of 10 mg, then multiplied by sample mass to obtain the actual wet weight contributed by each species. After conversion to dry mass, weights were multiplied by species-specific protein constants to determine the weight of protein in each multifloral load. The weight of protein in unifloral loads was calculated in an analogous manner. For pollen loads comprised wholly, or partially, of infrequently collected species, protein was determined by direct elemental analysis of the sample.

Analysis of Pollen Grain Size

For analysis of pollen grain size, mean equatorial and axial diameters were used to calculate an approximate cross-sectional area for each species. To estimate cross-sectional area of grains having a prolate or subprolate shape, the product of the mean axial and equatorial diameters was used; for prolate spheroidal, spherical, or oblate spheroidal shapes, circular area was calculated using the largest diameter. To calculate the mean cross-sectional area for mixed loads, a weighted area mean was calculated based on the relative numeric abundance of each species present.

Statistical Analysis

Colony foraging rates were analyzed to examine differences in total foragers, pollen foragers or the proportion of pollen foragers returning to hives during 5 minute intervals. Counts of returning foragers were transformed using a square root transformation, and proportions of pollen foragers were transformed using an arcsine transformation (Snedecor and Cochran 1980). Data were analyzed as a split-plot analysis of variance (PROC GLM, SAS Institute 1989b), with REPLICATE, STIMULUS MECHANISM and STIMULUS INTENSITY as main plot factors, and DAYS AFTER COLONY MANIPULATION and TIME OF DAY as sub-plot factors. For these analyses, REPLICATE(STIMULUS MECHANISM*STIMULUS INTENSITY) was used as the error term for testing main plot factors. Data were pooled into morning (1000 and 1200 hrs) and afternoon (1330 and 1600 hrs) time periods, because low numbers of foragers were collected during the 1000 hr time period and because there were no interactions between TIME OF DAY and other model variables within morning and afternoon

periods.

Pollen foraging among experimental colonies was analyzed using several dependent variables that described pollen loads or their collection. These included: wet weight, percent protein, gross protein, pollen grain size, number of species collected per colony and number of species collected per pollen load. Variables in most analyses were analyzed directly, except for pollen grain size, where cross-sectional areas were log transformed (Snedecor and Cochran 1980). A split-split-plot ANOVA (PROC GLM, SAS Institute 1989b) was used, with REPLICATE, STIMULUS MECHANISM and STIMULUS INTENSITY as main plot factors, DAYS AFTER COLONY MANIPULATION and TIME OF DAY as sub-plot factors, and FORAGER EXPERIENCE as a sub-sub plot factor. For these analyses, REPLICATE(STIMULUS MECHANISM*STIMULUS INTENSITY) was used as the error term for testing main plot factors and DAYS AFTER COLONY MANIPULATION*TIME OF DAY*REPLICATE(STIMULUS MECHANISM*STIMULUS INTENSITY) was used for testing sub-plot factors.

For analysis of the number of pollen species collected per colony or per pollen load, a species was counted if it was present in any amount greater than zero. Analysis of the number of species collected per pollen load was performed after tabulation of the number of different species present in every load. However, analyses of pollen species collected per colony was done in a step-wise manner. To examine the main treatment effects on the number of species collected per colony, a model containing only the terms REPLICATE, STIMULUS MECHANISM and STIMULUS INTENSITY was used. In this analysis, the sum of different species collected per colony for each different replicate

and treatment combination was analyzed. In succeeding analyses, the variables DAYS AFTER COLONY MANIPULATION, TIME OF DAY and FORAGER EXPERIENCE were added to the analysis in a hierarchical fashion. For each succeeding step-wise analysis, the sum of different species sampled was thereby performed at more and more finite levels. This permitted an evaluation of the number of pollen species sampled by the colony in the following manner: among days within treatments, between time periods within days and treatments, and between forager experience within time periods, days and treatments. Counts of species collected were square root transformed prior to analysis (Snedecor and Cochran 1980).

Colony protein intake was calculated by determining the mean gross protein content of pollen loads for treatments within each time period, day and replicate of the experiment. These values were then multiplied by the corresponding pollen foraging rate during these periods. Colony protein intake rates were square root transformed (Snedecor and Cochran 1980) and analyzed using the previously described model for colony foraging rates.

Consumption of quality-manipulated supplements used in experimental treatments was analyzed as a split-plot ANOVA (PROC GLM, SAS Institute 1989b), with REPLICATE, STIMULUS MECHANISM and STIMULUS INTENSITY as main plot factors and DAYS AFTER COLONY MANIPULATION treated as a sub-plot factor. REPLICATE(STIMULUS MECHANISM*STIMULUS INTENSITY) was used as the error term for testing main plot factors. Diet consumption was calculated subsequent to correction for water loss.

For all split-plot and split-split-plot designs, tests of sphericity were performed and,

if necessary, probability levels were adjusted according to the assessed degree of failure for the Huynh-Feldt conditions to be met (Littell et al. 1996). Drifting of foragers among experimental colonies was analyzed by chi-square (PROC FREQ, SAS Institute 1989a).

Log-linear modelling (Bishop et al. 1975; PROC CATMOD, SAS Institute 1989a) was used to compare the diversity and number of species collected by colonies in treatments. To reduce model complexity, species occurring less than 10 times, from all pollen loads collected, were eliminated from the analysis. Preliminary models were constructed with the same variables used in the analyses of pollen foraging, including all higher order interactions. Non-significant model terms were eliminated from saturated models in a hierarchical fashion based on their chi-square probability and by comparing the maximum likelihood ratio statistic of saturated and reduced models.

For parametric analyses, comparisons between means were done with single degree of freedom contrasts and least significant difference tests (LSD) ($\alpha=0.05$, experimentwise error rate) (SAS Institute 1989b). Untransformed cell means for all parametric analyses are listed in Appendices 1 and 2. For all models, interaction terms that are not listed in the results section are not significant.

Results

Pollen Species Collected, Size of Loads and Mixing

Over the course of the experiment, 5477 sets of corbicular pollen loads were collected, that were comprised of 25 species. The number of occurrences of each species in pollen loads is shown in Table 2. For identification purposes, pollen grains of *B. napus* were not differentiated from those of *B. rapa*, and their numbers are listed

together. The six most abundant species comprised 96.2% of occurrences in all pollen loads. Pollen grain morphology of selected species, by SEM, is shown in Appendix 3.

The mean wet weight of pollen loads from the entire experiment was 10.58 ± 0.08 mg. Loads ranged in weight from 0.69 to 40.71 mg, with a median value of 9.58 mg and a mode of 4.21 mg; weights were normally distributed about the mean. Based on analysis of the dominant species (>80%) present in pollen loads (for those species having at least 10 loads in which they were dominant), the weight of pollen loads was significantly influenced by the species of pollen collected by foragers ($F = 121.7$; $df = 9$, 5378; $P < 0.0001$) (Table 3). Heaviest pollen loads were comprised of pollen from *B. napus*, *S. alba* and *T. hybridum* while loads comprised of pollen from *V. cracca*, *M. alba* and *S. arvensis* were the lightest; others were of intermediate weight. The weight of pollen loads was correlated with the number of pollen grains collected from a given species (Table 4). However, the weight of loads collected by foragers were neither correlated with the mean equatorial diameter of pollen grains ($r = +0.241$; $F = 0.49$; $df = 1$, 8; $P = \text{NS}$) nor their cross-sectional area ($r = +0.232$; $F = 0.46$; $df = 1$, 8; $P = \text{NS}$) suggesting properties other than the pollen grain size affect the quantity of pollen collected from different plant species.

Foragers collected a low percentage of loads with mixed species (Table 5). Across all samples, the number of pollen loads containing more than one species ranged from 1.39% to 2.83%, depending on the threshold chosen to record the presence of a secondary species. Thresholds of > 5% were used for analysis because contamination by residual pollen remaining on the bodies of bees from prior foraging trips, or from in-hive pollen transfer between bees, could influence results (Free and Williams 1972, DeGrandi-

Hoffman et al. 1986, Free 1993). The frequency of co-occurring species in loads is outlined in Table 6. For most dominant species, the percentage of mixing was low and the breadth of species co-occurrence increased with the number of pollen loads sampled. Higher percentages of mixed loads per dominant species and narrow breadth of co-occurring species were associated with lower sample sizes (< 20). The incidence of mixed loads between *T. hybridum* and *T. repens* is likely underestimated because of difficulty in identifying these species together in a mixed load.

A small percentage of marked foragers (7.2%) drifted between colonies. However, the rate of drifting between treatments did not differ ($\chi^2 = 0.986$, $df = 3$, $P = NS$).

Quantitative Foraging Responses

The wet weight of pollen loads varied with neither the stimulus mechanism applied to colonies ($F = 3.82$; $df = 1, 30$; $P = NS$) nor its intensity ($F = 0.28$; $df = 1, 30$; $P = NS$), and was not influenced by the interaction between these factors ($F = 0.84$; $df = 1, 30$; $P = NS$). Although no overall effect was detected among days after manipulation ($F = 0.17$ $df = 2, 179$; $P = NS$), the time of day during which pollen loads were collected did influence their weight, with foragers collecting heavier loads during afternoon than morning periods (11.78 ± 0.18 vs 8.23 ± 0.18 mg) ($F = 228.77$ $df = 1, 179$; $P < 0.0001$). Inexperienced foragers also collected heavier loads than experienced foragers (10.71 ± 0.22 vs 9.37 ± 0.20 mg) ($F = 48.95$ $df = 1, 223$; $P < 0.0001$) and this difference was evident during afternoon time periods ($F = 37.21$ $df = 1, 223$; $P < 0.0001$) (Fig. 14). No significant interactions between stimulus mechanism ($F = 1.08$; $df = 1, 223$; $P = NS$) or stimulus intensity ($F = 0.19$; $df = 1, 223$; $P = NS$) and forager experience were found.

Therefore, the sampling of pollen loads shows that inexperienced foragers collected larger loads than experienced foragers, and that pollen load size was not affected by the treatments applied to colonies.

Pollen foraging rates were greater in colonies receiving low stimulus intensities (pollen deprived or jack pine pollen supplement) than those receiving high stimulus intensities (full frame of mixed pollens or *M. officinalis*/*B. napus* pollen supplement) (7.70 ± 0.50 vs 6.02 ± 0.38 pollen foragers \cdot min⁻¹) ($F = 6.93$; $df = 1, 30$; $P < 0.05$). Pollen foraging rates were similar between levels of stimulus mechanism (quantity vs. quality) ($F = 0.57$; $df = 1, 30$; $P = \text{NS}$), and no interaction between stimulus mechanism and stimulus intensity was found ($F = 1.15$; $df = 1, 30$; $P = \text{NS}$). Pollen foraging rates did not vary among days after manipulation ($F = 2.63$; $df = 2, 179$; $P = \text{NS}$), but these rates were greater during afternoon time periods than mornings (10.06 ± 0.44 vs 3.65 ± 0.24 pollen foragers \cdot min⁻¹) ($F = 450.90$; $df = 1, 179$; $P < 0.0001$). In addition, significant interactions for both stimulus mechanism ($F = 5.98$; $df = 1, 179$; $P < 0.05$) and stimulus intensity ($F = 6.53$; $df = 1, 179$; $P < 0.05$) were found to occur with time of day. These interactions were the result of higher rates of pollen foraging during afternoon time periods for colonies receiving low stimulus intensity treatments ($F = 9.57$; $df = 1, 30$; $P < 0.01$) (Fig. 15). Whether colony pollen reserves were manipulated quantitatively or qualitatively, colonies responded by increasing their pollen foraging rates in response to high demand for protein.

The total foraging rate among colonies, as measured by the number of pollen and nectar foragers returning to the hive per minute, was similar between levels of stimulus mechanism ($F = 2.56$; $df = 1, 30$; $P = \text{NS}$) and intensity ($F = 4.01$; $df = 1, 30$; $P = \text{NS}$).

This rate (26.84 ± 0.81 foragers \cdot min⁻¹) was also independent of any interaction between treatment factors ($F = 2.35$; $df = 1, 30$; $P = \text{NS}$). The total rate of foraging did not vary among days ($F = 2.35$; $df = 2, 159$; $P = \text{NS}$), but did increase during afternoon time periods compared with mornings (36.02 ± 0.93 vs 17.66 ± 0.70 %) ($F = 727.22$; $df = 1, 159$; $P < 0.0001$). An interaction between time of day and stimulus mechanism also affected the total foraging rate ($F = 4.95$; $df = 1, 159$; $P < 0.05$). During afternoon periods, those colonies receiving the quantity stimulus mechanism treatments exhibited higher total rates of foraging than those receiving the quality stimulus mechanism treatments ($F = 5.33$; $df = 1, 30$; $P < 0.05$) (Fig. 16). No such difference existed during morning time periods ($F = 0.16$; $df = 1, 30$; $P = \text{NS}$). Most of the variation between the two levels of stimulus mechanism is attributable to the high foraging rate of colonies that were pollen starved (stimulus mechanism=quantity; stimulus intensity=low). Colonies receiving this treatment exhibited higher total foraging rates than the remaining three treatments in the experiment.

Overall, the proportion of pollen foragers (23.28 ± 0.60 %) was unaffected by the stimulus mechanism ($F = 0.22$; $df = 1, 30$; $P = \text{NS}$) or stimulus intensity ($F = 3.58$; $df = 1, 30$; $P = \text{NS}$) applied to colonies, or any interaction between these factors ($F = 0.42$; $df = 1, 30$; $P = \text{NS}$). The proportion of pollen foragers also remained similar among days after colony manipulation ($F = 2.39$; $df = 2, 159$; $P = \text{NS}$), however it did increase during afternoon time periods relative to mornings (27.24 ± 0.73 vs 19.32 ± 0.82 %) ($F = 116.64$; $df = 1, 159$; $P < 0.0001$). The interaction between time of day and stimulus intensity also affected the proportion of pollen foragers in the foraging population ($F = 6.21$; $df = 1, 159$; $P < 0.05$), whereby this proportion was increased for colonies receiving

low compared with high stimulus intensity treatments (29.76 ± 1.03 vs 24.72 ± 0.95 %) ($F = 9.54$; $df = 1, 30$; $P < 0.05$). No such differences were observed during morning time periods (19.71 ± 1.14 vs 18.94 ± 1.19) ($F = 0.20$; $df = 1, 30$; $P = \text{NS}$).

As previously described, the weight of pollen loads did not vary with the stimulus mechanism or the stimulus intensity applied to colonies. Therefore, the rate of pollen foraging can be considered to be an indirect measure of gross pollen intake. Temporal changes in colony pollen intake were investigated by analyzing the effects of treatments in relation to the day of the experimental period. Colonies responded to treatments differently, depending on the day of the experimental period examined ($F = 3.57$; $df = 2, 179$; $P < 0.05$), with maximum pollen intake occurring on day 3 ($F = 6.06$; $df = 1, 179$; $P < 0.05$), when averaged over all treatments. There was an interaction between the stimulus mechanism applied to colonies and the day of the experimental period ($F = 3.74$; $df = 2, 179$; $P < 0.05$), but no interaction was found between day of the experimental period and stimulus intensity ($F = 0.13$; $df = 2, 179$; $P = \text{NS}$) (Fig. 17). In low quantity treatments (stimulus mechanism = quantity; stimulus intensity = low;), a high pollen intake rate was maintained from the first day of the experimental period to the third day. Foragers in these colonies continued to respond to a high demand for pollen, as they had during the pre-experimental period of pollen deprivation. Remaining treatments had lower rates of pollen intake during days 1 and 2, and varied little among themselves. However, on day 3 of the experimental period, the pollen intake rate of the low quality treatment increased to that of the low quantity treatment, while the high quantity and high quality treatments remained unchanged. This temporal trend suggests that colonies respond to changes in the size of their pollen reserve more quickly than changes in its

quality.

Consumption of low and high quality supplements differed with treatment level, and among days of the experimental period (Fig. 18). Both diets were consumed in large quantities but, on an average daily basis, consumption of the high quality supplement was greater than that of the low quality supplement (24.6 ± 1.5 g vs 9.9 ± 0.9 g) ($F = 53.04$; $df = 1, 10$; $P < 0.0001$). The amount of supplement consumed by colonies varied between individual days ($F = 3.64$; $df = 2, 20$; $P < 0.05$), but patterns of consumption were similar between low and high quality treatments over days of the experiment ($F = 0.96$; $df = 2, 20$; $P = \text{NS}$). Greatest amounts of supplement were consumed on day 2 of the experimental period ($F = 6.87$; $df = 1, 20$; $P < 0.05$). Increased consumption on this day may account for a simultaneous decrease in pollen intake for colonies receiving the quality stimulus mechanism (Fig. 19).

Qualitative Foraging Responses

Analysis of the percent protein content of pollen loads collected by foragers showed that colonies did not respond to manipulations of their pollen stores by specializing on pollen species that differed in nutritional quality. Overall, the protein content of pollen loads (22.27 ± 0.04 % dry weight) did not vary among levels of stimulus mechanism ($F = 0.76$; $df = 1, 10$; $P = \text{NS}$) or stimulus intensity ($F = 1.07$; $df = 1, 10$; $P = \text{NS}$), and was not affected by the interaction between these two factors ($F = 2.76$; $df = 1, 10$; $P = \text{NS}$). The protein content of pollen loads also did not change among days after colony manipulation ($F = 0.61$; $df = 2, 179$; $P = \text{NS}$) or between morning and afternoon time periods ($F = 2.77$; $df = 1, 179$; $P = \text{NS}$). Inexperienced foragers collected more proteinaceous pollen

than did experienced foragers (22.36 ± 0.05 vs 22.17 ± 0.06 %) ($F = 8.24$; $df = 1, 221$; $P < 0.01$), but this effect varied with day of the experimental period ($F = 3.39$; $df = 2, 221$; $P < 0.05$) (Fig 20). Differences were observed only after day 1, when the protein content of pollen collected by inexperienced foragers increased sharply, and remained high.

Although the mean protein value and the wet weight of pollen loads were similar among experimental treatments, variation in the gross protein content of loads could exist. This is possible because of the demonstrated fidelity between the weight of pollen loads collected by foragers and the floral species visited. The gross protein content of pollen loads (1.88 ± 0.03 mg) did not, however, differ between levels of stimulus mechanism ($F = 4.41$; $df = 1, 10$; $P = \text{NS}$) or stimulus intensity ($F = 0.39$; $df = 1, 10$; $P = \text{NS}$) nor was it influenced by the interaction between these factors ($F = 0.62$; $df = 1, 10$; $P = \text{NS}$). Gross protein content also did not vary among days after colony manipulation ($F = 0.06$; $df = 2, 179$; $P = \text{NS}$). However, loads collected during afternoon periods contained more protein than loads collected during mornings (2.20 ± 0.04 vs 1.54 ± 0.04 mg) ($F = 200.36$; $df = 1, 179$; $P < 0.0001$). More protein per load was also collected by inexperienced than experienced foragers (2.01 ± 0.04 vs 1.74 ± 0.04 mg) ($F = 48.58$; $df = 1, 223$; $P < 0.0001$), however this effect was primarily the result of differences found during afternoon time periods (2.45 ± 0.05 vs 1.94 ± 0.04 mg) ($F = 35.32$; $df = 1, 223$; $P < 0.0001$).

The size of pollen grains collected by foragers, as measured by pollen grain cross-sectional area, did not vary among experimental treatments. Pollen grain size ($476.0 \pm 4.1 \mu\text{m}^2$) was consistent between levels of stimulus mechanism ($F = 1.53$; $df = 1, 10$; $P = \text{NS}$) and stimulus intensity ($F = 0.23$; $df = 1, 10$; $P = \text{NS}$), with no interaction between the

two factors ($F = 2.17$; $df = 1, 10$; $P = \text{NS}$). Grain size was consistent among days after colony manipulation ($F = 0.02$; $df = 2, 179$; $P = \text{NS}$), but foragers collected larger pollen grains during morning than afternoon time periods (486.5 ± 7.3 vs $466.2 \pm 4.03 \mu\text{m}^2$) ($F = 11.22$; $df = 1, 179$; $P < 0.001$). Variation in pollen grain size was also found in relation to forager experience. Inexperienced foragers collected larger pollen grains than experienced foragers (492.1 ± 6.5 vs $458.3 \pm 4.5 \mu\text{m}^2$) ($F = 23.17$; $df = 1, 221$; $P < 0.0001$) in both mornings ($F = 6.66$; $df = 1, 95$; $P < 0.05$) and afternoons ($F = 16.76$; $df = 1, 114$; $P < 0.0001$) (Fig. 21). However, no correlation was found between pollen grain cross-sectional area and crude protein content ($r = +0.138$, $P = \text{NS}$). An interaction between stimulus mechanism and forager experience was also found, indicating that inexperienced foragers collected larger pollen grains than experienced foragers in quality manipulated treatments ($F = 5.37$; $df = 1, 221$; $P < 0.05$) (Fig. 22).

The total number of pollen species collected by a colony's foraging force (7.30 ± 0.28) did not differ between overall levels of stimulus mechanism ($F = 0.06$; $df = 1, 30$; $P = \text{NS}$) or stimulus intensity ($F = 0.90$; $df = 1, 30$; $P = \text{NS}$), and no interaction between these two variables existed ($F = 2.66$; $df = 1, 30$; $P = \text{NS}$). There was, however, a significant interaction between stimulus intensity and the day of the experimental period ($F = 4.39$; $df = 2, 62$; $P < 0.05$) (Fig. 23). Colonies receiving low stimulus intensities steadily increased the number of species collected per day, until on day 3, this number exceeded that of the high stimulus intensity colonies. For high stimulus intensity colonies, the number of species remained consistent over the experimental period, with a slight decline on day 3. The number of pollen species collected per colony was also influenced by the time of day and the experience level of the forager. More species per

colony were collected during afternoon than morning periods (4.51 ± 0.10 vs 3.92 ± 0.15) ($F = 18.78$; $df = 1, 177$; $P < 0.0001$) and by inexperienced foragers ($F = 165.10$; $df = 1, 223$; $P < 0.0001$) (Fig. 24).

The number of pollen species collected per pollen load was analyzed to evaluate the breadth of flora sampled by individual bees while on foraging trips. No difference in the number of species per load (1.033 ± 0.004) was found among foragers from colonies receiving manipulations of either stimulus mechanism ($F = 0.11$; $df = 1, 10$ $P = NS$) or stimulus intensity ($F = 0.39$; $df = 1, 10$; $P = NS$). Similarly, the interaction between stimulus mechanism and stimulus intensity was not significant ($F = 2.92$; $df = 1, 10$; $P = NS$). The number of species collected per pollen load was similar among days of the experimental period ($F = 0.86$; $df = 2, 179$; $P = NS$), time periods ($F = 0.03$; $df = 1, 179$; $P = NS$) and levels of foraging experience ($F = 0.09$; $df = 1, 200$; $P = NS$). However, the number of species per load varied based on the interactions of stimulus intensity, time of day and forager experience ($F = 5.64$; $df = 1, 200$; $P < 0.05$) (Fig. 25). Variation was only observed in colonies receiving low stimulus intensities. During morning periods, experienced foragers collected more pollen species per load than inexperienced foragers, but this trend was reversed during afternoon periods.

Combination of Quantitative and Qualitative Responses

Estimates of the gross amount of protein being returned to colonies were derived by combining pollen foraging rate data with the gross protein content calculated for individual loads. Similar to the trends found for the rate of pollen foraging (pollen intake), the rate of protein intake (14.32 ± 0.81 mg · min⁻¹) was greater for colonies

receiving low stimulus intensities than those receiving high stimulus intensities (16.38 ± 1.33 vs 12.26 ± 0.91 $\text{mg} \cdot \text{min}^{-1}$) ($F = 7.30$; $df = 1, 30$; $P < 0.05$). No differences were found between levels of stimulus mechanism ($F = 0.17$; $df = 1, 30$; $P = \text{NS}$), and no interaction existed between levels of stimulus mechanism and stimulus intensity ($F = 1.87$; $df = 1, 30$; $P = \text{NS}$). Overall, protein intake was greater during afternoon than morning time periods (22.83 ± 1.17 vs 5.81 ± 0.43 $\text{mg} \cdot \text{min}^{-1}$) ($F = 538.56$; $df = 1, 179$; $P < 0.0001$), and time of day was found to interact with the level of stimulus intensity applied to colonies ($F = 8.57$; $df = 1, 179$; $P < 0.01$). When analyzed within time periods, increased rates of protein intake were found only during afternoon periods for those colonies receiving low stimulus intensities ($F = 10.45$; $df = 1, 30$; $P < 0.01$) (Fig. 26). Protein intake rate also varied with day of the experiment ($F = 5.77$; $df = 2, 179$; $P < 0.01$) (Fig. 27), with these trends being consistent among experimental treatments.

Analysis of Species Assemblages Collected by Colonies

Log-linear modelling was used to compare the diversity and number of species collected by colonies over the entire experimental period. Main effects and statistically significant interaction terms are listed in Table 7. No significant terms containing STIMULUS MECHANISM, STIMULUS INTENSITY were found, indicating that the species assemblages collected among treatments were similar. The overall abundance of the 11 species analyzed from pollen loads is presented in Fig. 28. The most frequently collected species were: *M. officinalis*, *T. hybridum*, *T. repens*, *B. napus* / *B. rapa*, *M. alba* and *A. lappa*. Each of the remaining species comprised less than 1.5 % of the total number of species occurring in pollen loads.

Although the collection of most species was relatively consistent throughout the progress of the experiment, some variation was detected among replicates (Table 7, line 1) (Table 8). Differences in the proportions of species collected among replicates represent seasonal differences in pollen collection by foragers, based on the temporal availability of pollen types. For example, *B. napus* / *B. rapa* pollen was most frequently collected during late July, when these species were at their peak abundance. Large proportions of *M. officinalis* were collected in all replicates, with slight declines evident only in late August. In contrast, *R. idaeus* pollen was only collected from replicates occurring late in August. Increased levels of *T. hybridum* were seen during July and, as the availability of other species diminished, peaked along with *T. repens* in late August. Within the same replicate, the proportions of *M. officinalis* and *Trifolium* spp. also appeared to vary inversely.

The collection of species by colonies was also influenced by the time of day (Table 7, line 5) (Fig. 29). During afternoon time periods, the overall frequency of species occurrences increased 26.2% over mornings, however the relative proportions of most species varied little between time periods. Exceptions included *B. napus* and *T. repens*, which were collected in greater proportions during morning periods, and *M. alba* and *T. hybridum* which were collected more frequently during afternoons.

The relative frequency with which pollen species were collected also varied with the experience level of foragers (Table 7, line 6) (Fig. 30). Experienced foragers collected a much larger proportion of *M. officinalis* pollen and a slightly greater proportion of *M. alba* pollen than inexperienced foragers, but collected similar or smaller proportions of all remaining species. Inexperienced foragers collected *A. lappa*, *B. napus*, *S. alba* and *T.*

repens pollen more frequently than experienced foragers. Experienced foragers appeared to concentrate their foraging strategies more intensely on *Melilotus* spp., while inexperienced foragers visited other constituent species more frequently.

The occurrence of species within replicates was also found to be influenced by the time of day during which foraging occurred (Table 7, line 7) (Table 9). Such trends represent the interaction of seasonal and temporal availability of pollen resources, or in isolated cases, local specialization of a colony's foragers. The latter may explain the large proportion of *A. lappa* pollen that was collected during the morning periods of replicate 1, even though replicate 2 was performed on the same date (17 July). Other trends are evident over replicates and between time periods. For example, greatest proportions of *B. napus* / *B. rapa* pollen were collected during July, principally during morning periods, while occurrences of *M. alba* were most evident late in the season, during afternoons. The proportions of *M. officinalis* pollen were slightly lower in replicates 1 - 3 during morning periods, and declined during late August for both time periods. *S. arvensis* was almost exclusively collected during morning periods across most replicates during mid- to late season. Occurrences of *T. hybridum* were not only greater during afternoon periods, but also started their late season rise by mid-August, occurring earlier than the analogous increases seen during morning periods. Also toward the end of the season, proportions of *T. repens* increased, however these increases were greater and started earlier in mornings than afternoons.

Interactions between the time of day and the day of the experimental period further affected the proportions of species collected by colonies (Table 7, line 8) (Table 10). The patterns of pollen collection among days of the experimental period and time periods

were inconsistent among most species. Smaller proportions of *A. lappa* and *M. alba* pollen were collected during morning periods on day 2, but collection of these species was similar among days during afternoon periods. *B. napus / rapa* pollen also showed slight decreases in the frequency of their collection on day 2, with this trend being similar between time periods. Proportions of *M. officinalis* pollen were greatest on day 2 during mornings, but in afternoon periods its frequency of collection gradually decreased over days. *S. arvensis* was collected in similar amounts among days in morning periods, but was only collected on day 2 during afternoons. The collection of *T. hybridum* was greatest on day 1 during morning periods and decreased on days 2 and 3, while its collection gradually increased over days during afternoon periods. Finally, the collection of *T. repens* gradually increased over days during morning periods, but in afternoons was similar between days 1 and 2 and decreased slightly on day 3.

The proportions of species collected by experienced and inexperienced foragers were also dependent on the time of day during which foraging occurred (Table 7, line 9) (Table 11). In both time periods, experienced foragers collected smaller proportions of pollen from *A. lappa*, *B. napus / rapa*, *S. alba* and *T. repens* than inexperienced foragers, however this trend was reversed for *M. alba* and *M. officinalis*. For *A. humilis*, a marginally greater proportion of pollen was collected by experienced than inexperienced foragers during morning periods, however none was collected by experienced foragers during afternoons. Similarly, no pollen was collected by inexperienced foragers during afternoon periods for *S. arvensis*. Proportions of *T. hybridum* pollen were similar between levels of foraging experience during morning periods, but during afternoons slightly more was collected by inexperienced foragers.

Discussion

These results clearly show that honey bees respond to manipulations of the quality of pollen stored in their hive causing a change in the dynamics of the foraging population. Colony response to reductions in pollen quality are similar to those found for pollen quantity. When pollen stores are of low quality or quantity, colony pollen intake is increased by the recruitment of greater numbers of pollen foragers. This change in the composition of the foraging population does not influence the overall rate of foraging, as measured by the combined number of nectar and pollen foragers returning to the hive. It does, however, increase the rate of pollen foraging and the rate of protein intake, as a direct result of the increased proportion of pollen foragers. Furthermore, differences seen in the behaviours of “experienced” and “inexperienced” foragers play an important role in a colony’s collection of pollen. Inexperienced foragers collect heavier pollen loads, select better quality pollen and sample their floral environment more extensively than more experienced foragers, however these effects are independent of the status of colony pollen stores.

The changes in colony-level foraging demonstrated in this study show that honey bees respond to a deficit in the quantity or quality of their pollen reserve in an analogous manner. Similar changes in the proportion of the foraging force devoted to pollen collection have been shown when colony manipulations create demands for pollen through manipulation of the size of pollen stores (Fewell and Winston 1992, Camazine 1993, Dogterom and Winston 1999, Fewell and Bertram 1999) or unsealed brood area (Free 1967, Eckert et al. 1994). These responses are characterized by an increase in the proportion of pollen foragers, while the total number of foragers, in most cases, are

unaffected. Page and Fondrk (1995) also showed that high pollen hoarding strain bees maintain increased levels of pollen intake, in part, by maintaining a high proportion of pollen foragers. Hence, a colony's ability to increase the proportion of pollen foragers appears to be a consistent mechanism of increasing pollen intake to satisfy a demand for protein in the colony. My results show that this response appears to be insensitive to the pathway by which the demand was created.

My data also show that colonies did not respond to protein shortages by specializing on pollen species of higher quality. Irrespective of the treatment applied, colony-level changes were not found for the percent protein or gross protein values of pollen loads collected by foragers. When pollen stores were of low quality or quantity, colonies showed no change in the size of pollen grains collected and did not sample their environment more broadly for sources of forage. This is shown by the lack of variation in the number of pollen species collected per colony and in the similarity of floral assemblages collected among treatment groups. Increased levels of pollen protein were only collected by inexperienced foragers, irrespective of their colony's need. Hence, any potential differences in the quality of pollen that might occur at the colony level would result from differences in numbers of inexperienced foragers recruited by colonies, rather than a generalized response of all foragers to colony need.

From my results, an important question needs to be addressed: why do relatively inexperienced foragers exhibit different pollen collection behaviours than experienced foragers? Several factors may account for these differences. First, the potential difference in age between these two foraging cohorts must be recognized as newly recruited foragers are generally younger than existing foragers (Winston 1987). Under

conditions of food deprivation, pollen intake is increased by the recruitment of precocious foragers into pollen collection (Fewell and Bertram 1999) and the age-related shift in the division of labour is accelerated (Schulz et al. 1998). Hence, colonies with low food quality or quantity probably recruited a high proportion of very young, inexperienced pollen foragers. The energetic constraints imposed on older foragers are quite different than younger foragers, because honey bees have a fixed lifetime energy budget for foraging (Neukirch 1982). As a result, bees do not maximize the rate at which they collect food, but instead maximize their energetic efficiency while they forage (Schmid-Hempel et al. 1985; Kacelnik et al. 1986; Schmid-Hempel 1986, 1993; Seeley 1986, 1994; Wolf and Schmid-Hempel 1990; Dyer and Seeley 1991). Another consequence of a forager's lifetime energy budget is that increased work effort is traded off with decreased life span (Schmid-Hempel 1987, Houston et al. 1988, Schmid-Hempel and Wolf 1988, Wolf and Schmid-Hempel 1989, Dyer and Seeley 1991). Therefore, the strategies used by energy-constrained older foragers to maximize energetic efficiency in relation to their remaining lifetime energy budget are likely to be different than for younger foragers. My "experienced foragers" may have collected smaller loads than younger, less experienced foragers to optimize their energetic efficiency, and respond to physical processes such as wing wear and degeneration of the flight mechanism (Baker 1976, Collatz and Wilps 1986, Kern 1986, Cartar 1992). Similar sharp declines in the foraging performance of nectar collectors are also seen as these bees approach senescence (Dukas and Visscher 1994). In contrast, the less constrained energy budget of novice foragers would better accommodate the greater time spent learning by younger foragers (Dukas and Visscher 1994). It would also permit them to sample their floral environment

more broadly, and provide a better energetic capacity to specialize on higher quality forage.

Differences in the behaviour of the experienced and inexperienced foraging cohorts may also have been related to the scouting and recruiting activity of colonies. Scouts find new sources of pollen and recruit other foragers (Seeley 1995). They typically account for 10% of the general foraging population, however their relative abundance is sensitive to foraging conditions and in times of dearth may comprise up to 36% of foragers (Seeley 1983, 1995). Scout bees are not of any specific age (zu Oettingen-Spielberg 1949, Dreller 1998), with both novice (Lindauer 1952) and experienced foragers (Seeley 1983, Seeley and Visscher 1988) engaged in scouting activities. In this study, inexperienced foragers were collected in a 2:1 ratio over experienced foragers. My data probably under-represent the true proportion of inexperienced foragers because of the greater effort spent trying to collect experienced foragers, that were present in smaller numbers particularly as the experiment progressed. Normal forager turnover likely contributed to the higher proportion of inexperienced pollen foragers, which would have progressively boosted the numbers of inexperienced foragers throughout the 3-d experimental period. Even when colonies are not experiencing food shortages, approximately 10% of the foraging population is replaced by novice foragers every day, in order to compensate for the death of older foragers (Sakagami and Fukuda 1968, Dukas and Visscher 1994, Seeley 1995). This would cause a natural, cumulative shift in the size of forager cohorts throughout the experimental period, increasing the size of the inexperienced cohort, while simultaneously decreasing the size of the experienced cohort. The numerical superiority of inexperienced foragers, of which many would be scouts, is important to consider

because the power of detection for each additional plant species would be affected by the number of scouts in each foraging cohort. This factor may have contributed to the larger number of species that inexperienced foragers collected per colony. However, the deletion of rare species from the analyses of pollen species assemblages collected by colonies had the effect of ameliorating such a sampling bias, and provided an additional assessment of the foraging patterns of these cohorts. These results reiterate the tendency of inexperienced foragers to visit less dominant floral species more frequently than experienced foragers, and are consistent with the premise that inexperienced foragers sample their floral environment more broadly.

The data from this study show that honey bee colonies responded to protein shortages by adjusting their rate of pollen intake, rather than specializing in the quality of pollen being collected. Three reasons could account for the lack of qualitative response observed at the colony level when the mechanism for such a response, increased recruitment of inexperienced pollen foragers, was clearly evident. First, the recruitment of inexperienced foragers in low stimulus intensity treatments may not have been great enough to effect detectable qualitative changes in colony-level responses. This may have resulted from the 5-d pre-experimental pollen starvation period, which could have severely depleted the supply of inexperienced foragers available to be recruited during the experimental period. Second, a portion of the newly-recruited pollen foraging specialists may have been comprised of nectar foragers that switched tasks to collect pollen (Free 1967, Fewell and Winston 1992, Eckert et al. 1994). These bees would not be as young as precocious pollen foragers, and therefore may have optimized their pollen-collecting behaviours in a manner similar to the experienced foraging cohort. Therefore, the true

magnitude of the inexperienced cohort may have been diluted by effects of such task-switched individuals. Third, sampling biases may have reduced the true contribution of inexperienced foragers to colony protein intake. Because my sampling protocol was not designed to determine the relative proportion of each experience cohort in the foraging population, it may have lacked the necessary sensitivity to detect such qualitative colony-level changes.

An important finding resulting from this study is that within levels of forager experience, the foraging effort of individual bees, as measured by the wet weight of their pollen load or other qualitative parameters of effort per foraging trip, was not affected by reductions in pollen quantity or quality. Similarly, Fewell and Bertram (1999) determined that foragers do not vary their individual foraging rates or change the size of pollen loads they collect in response to pollen storage levels. My data show that any changes in individual foraging effort are linked to the experience level of the forager. This clearly indicates that individual foragers having the same level of experience exert the same amount of effort to collect pollen, regardless of the colony state (Schmid-Hempel et al. 1993). It is possible that colonies may recruit different numbers of inexperienced foragers into the foraging population, in relation to colony need.

Fewell and Winston (1992) concluded that foragers in colonies with low pollen stores collect loads that are 19% larger than foragers in colonies with high pollen stores. Foragers also increase pollen load size in response to increased areas of unsealed brood (Ekert et al. 1994) and in colonies supplemented with synthetic queen mandibular pheromone (Higo et al. 1992). Although my data dispute that these changes are the result of increased effort on the part of individuals, the results of these studies are not mutually

exclusive with my findings. If we allow for the possibility that my sampling protocol under-represented the proportion of inexperienced pollen foragers in low stimulus intensity treatments, it is possible that increases in the average weight of pollen loads did exist over the entire foraging population. If true, my results would be congruent with the overall findings of Fewell and Winston (1992), but not with the hypothesis that individual-level effort is increased. I believe that the change in the weight of pollen loads detected by other studies are real phenomena, however I feel that their implicit assumption of an mean increase in the load weight for every forager is erroneous. The data in my study show that any increases detected actually reflect the compositional changes in the foraging population, which would contain a larger proportion of inexperienced foragers. As such, the average load size sampled over the entire forager population would increase, but as I have shown, individual effort between treatments within the inexperienced and experienced foraging cohorts would remain unchanged.

Other factors could substantially contribute to the changes in load size observed in some studies (Fewell and Winston 1992, Higo et al. 1992, Eckert et al. 1994). The fact that the weight of pollen loads varies greatly among plant species (Free 1993) was confirmed in this study, and it was also demonstrated that load weight was highly consistent within an individual plant species. It is therefore possible that individual foragers could serendipitously establish constancy (Free 1963) to floral sources from which they would collect heavier loads, in no direct relation to colony need. Similarly, pollen load weight of individual foragers could vary in response to such parameters as the distance a floral patch was located from a colony, the density of flowers within a patch or within an inflorescence, or the handling time necessary to collect pollen. Honey bee

nectar foragers vary load size in order to maximize their energetic efficiency (Schmid-Hempel et al. 1985; Kacelnik et al. 1986, Schmid-Hempel 1986, 1993), or to exchange food source information with nestmates more rapidly (Núñez 1982; Varjú and Núñez 1991, 1993). In addition, pollen foraging bumble bees maximize the efficiency of their pollen collection against energetic costs (Rasheed and Harder 1997a, b). Hence, it is reasonable to consider that honey bees may vary pollen load weight to optimize site-specific and species-specific efficiency of its collection. Furthermore, colonies simultaneously work several floral patches for pollen each day, with the distribution of bees among sites being frequently adjusted (Visscher and Seeley 1982). Therefore, a consistent, well replicated and rigorous sampling protocol for pollen loads is of paramount consideration to estimate the true impact of colony manipulations on individual foragers, in order to avoid biasing results by the foraging choices made by a limited subset of colonies or their workers. Furthermore, without information about the floral species composition of pollen loads and the potential shifts in species assemblages among colonies, differences in colony-level foraging strategy cannot be directly examined.

Evidence from this study unambiguously demonstrates that honey bee colonies match protein intake with protein need, suggesting that pollen collection is tightly regulated and sensitive to pollen quality. The collection of nectar by honey bees is also tuned to the conditions of the colony, and its collection has been described according to an energetic-based currency. (Cheverton et al. 1985; Schmid-Hempel et al. 1985; Seeley 1985b, 1986, 1994; Waddington 1980, 1985; Kacelnik et al. 1986; Stephens and Krebs 1986; Schmid-Hempel 1986, 1987, 1991; Wolf and Schmid-Hempel 1990; Seeley et al.

1991). Inherent to such models is the ability of the individual nectar forager to assess the profitability of a floral source and to adjust her behaviour to maximize the collection of this currency. While inside the colony, nectar foragers receive colony-level feedback that allows them to assess their colony's rate of nectar intake, and decide whether to continue to work the same floral patch and whether to recruit other foragers to it (Seeley 1986, 1994). I have demonstrated that honey bee colonies also forage in a manner consistent with the maximization of a proteinaceous currency. It is probable that this foraging strategy takes into account site-specific factors that influence the gross availability of pollen and the energetic costs associated with its collection. Such an economic motivation for the collection of pollen has been proposed for bumble bees (Rasheed and Harder 1997a, b) and is consistent with maximizing lifespan of the forager. However, the mechanisms and feedback influencing the behaviour of individual pollen foragers are likely to contrast greatly with those that influence nectar foragers.

The principal difference between the food gathering abilities of pollen and nectar foragers appears to be the inability of pollen foragers to assess the quality of the resource they are collecting. Although my experimental results demonstrate that pollen foragers collectively respond to the colony's demand for protein, individuals do not respond to colony-level reductions in pollen quality or quantity by specializing on more proteinaceous floral sources. This indicates that individual pollen foragers do not have the inherent ability to detect the quality of pollen they are collecting. In effect, pollen foragers respond to colony requirements in an "on or off" manner, with no consideration for the quality of the resource, but only for its profitability of collection. When colony demand for protein is great, colonies respond by allocating more pollen foragers resulting in an

increased quantity of pollen being returned to the colony. Thus, if overall pollen quality collected by foragers is low, then greater quantities will be collected to satisfy the protein demand signalled by the colony. In effect, my data show that honey bee colonies respond to changes in pollen quality in a quantitative manner.

The suggestion that individual pollen foragers cannot assess pollen quality is well supported by documented accounts of honey bees collecting and consuming pollen and pollen-like substances having little or no nutritive value (Root and Root 1935, Haydak and Tanquary 1943, Morse 1975, Fægri and van der Pijl 1979, Johannsmeier 1981, Shaw 1990, Pernal 2000a). Experimental choice tests also support the view that individual honey bee foragers do not possess the ability to assess pollen quality. Wahl (1966) showed that, given a choice of forage, bees collected worthless substances in addition to pollen substitutes with some nutritive value. Wahl further demonstrated that if more attractive pollen was made available, bees would not neglect worthless substances more quickly than pollen substitutes, suggesting that bees lacked the ability to discriminate quality. Schmidt (1982) examined pollen collecting preferences of honey bees in a greenhouse and determined that pollen odour was a crucial factor guiding pollen selection by foragers. Schmidt (1982) found no conclusive relationship between protein content and foraging preference. Although Levin and Bohart (1955) also concluded that protein content did not affect forager choice, some of their results have been used as evidence to suggest that honey bee collecting preferences and pollen protein content are directly correlated (Schmidt 1982, Fewell and Winston 1992). However, Levin and Bohart (1955) did not standardize pollen age for most of the species they tested so it is difficult to use their study to draw any useful conclusions about the relationship between pollen

protein content and its collection by bees. I have found no conclusive evidence demonstrating that the pollen choices made by honey bee foragers are the direct result of crude protein content. My ongoing research in this area (Pernal 2000a) also supports this position.

Pollen selection criteria used by honey bees may include such factors as pollen age, moisture content, colour, pH, pollen grain size, physical configuration of the grains and odour (Stanley and Linskens 1974, Jay 1986). These parameters are independent of the nutritive value of the pollen and could potentially influence its selection by foragers. Although the polylectic nature of honey bee foraging could ameliorate the effects of individuals becoming constant to a deficient protein source, the influence of these pollen selection criteria could potentially affect the rate of protein intake for a colony.

The criterion which appears to have the greatest proven effect on the pollen choices made by individual foragers is odour. Pollen odours are often distinct from, and more intense than, a flower's overall floral bouquet (von Frisch 1923, von Aufsess 1960). Furthermore, honey bees can differentiate the odour of pollen from the overall aroma of the flower, and can be conditioned to the odour of pollen alone (von Frisch 1923, von Aufsess 1960). In addition, honey bees use pollen odours to communicate the location of profitable food sources after returning to the hive (von Frisch 1967). Attractants and phagostimulants isolated from pollen have been shown to increase the choice or consumption of pollen and artificial diets for honey bees (Louveaux 1959, Hügel 1962, Taber 1963, Lepage and Boch 1968, Robinson and Nation 1968, Hohmann 1970, Doull and Standifer 1970, Doull 1974, Boch 1982, Schmidt 1985). The addition of pollen odour components to substrates having little or no nutritional value has been shown to

increase their selection or collection by foragers (Taber 1963, Hohmann 1970, Starrat and Boch 1971). Therefore, the importance of pollen odour must carefully be taken into account when evaluating the choices made by individual foragers. The influence of pollen odour, more than any other factor, is likely to have influenced the results of previous studies examining pollen choices of individual foragers. Without first controlling for this factor, the importance of the nutritional value of pollen to foragers cannot be correctly ascertained. Recently, an interesting study has suggested that pollen foragers modulate their threshold of dancing and their dance rate in relation to the quality of a pollen source (Waddington et al. 1998). Unfortunately, the true causes of these actions are difficult to interpret as neither the influence of pollen odour between pollen sources nor the factors that might affect the efficiency of pollen collection were standardized in the experiment. Pollen odour is a proven influence on pollen foraging behaviour. The interaction of pollen odour with protein content in the decisions made by individual foragers is worthy of careful investigation.

If individual pollen foragers are insensitive to pollen quality, then feedback about pollen quality at the level of the colony is critical if the colony is to match protein intake with protein need. However, determination of the mechanism by which workers perceive colony protein demand was not a focus of this study. Camazine (1993) suggested that pollen foragers obtain information about colony needs indirectly, during trophallactic contact with nurse bees. Nurse bees are the primary processors of pollen in a honey bee colony (Moritz and Crailsheim 1987, Crailsheim 1990a, Crailsheim et al. 1992, Szolderits and Crailsheim 1993), and they distribute proteinaceous material derived from pollen to other colony members (Crailsheim 1990a, Crailsheim 1991, Crailsheim et al.

1992, Crailsheim 1992, 1998). The cue received by foragers is inhibitory, and is based on the amount of protein disseminated by nurses from their hypopharyngeal gland secretions (Camazine et al. 1998). When the quantity of pollen stored in the colony is high, the amount of protein disseminated by nurses to foragers is great, and pollen collection is inhibited. However, if pollen storage levels are low, the amount of protein available for nurses to disseminate is small, and pollen foraging is stimulated. Crailsheim (1992, 1998) also suggested that the exchange of hypopharyngeal gland secretion between nurses might provide information, and that a reduced willingness to exchange or spend gland secretion when protein availability was low could provide nurses with information about protein availability in the hive. Although Camazine's mechanism was originally designed to explain foraging responses associated with fluctuations in the size of a colony's pollen reserve, it is based on a proteinaceous currency, and may also explain the responses of bees to pollen quality. Hence, if the nutritional content of the pollen reserve is low, the concentration of protein in the hypopharyngeal gland secretions of nurses would be decreased or the overall amount of proteinaceous food produced by nurses would be lessened. By either pathway, the quantity of protein being disseminated to foragers would decrease, thereby stimulating foraging. Conversely, if pollen stores are of high quality (and in sufficient supply), foragers would receive large enough quantities of protein to inhibit pollen foraging. Camazine's research also supports the hypothesis that foragers determine a colony's need for protein by "indirect assessment". Camazine (1993) showed that foragers did not require direct contact with stored pollen to become informed about colony needs, a finding consistent with this experiment. For my pollen quality treatments, pollen diets were presented within colonies using small troughs

situated between the middle and bottom frames. Incoming foragers had access to an empty brood frame situated in the lower third of the colony to deposit their loads, and were also permitted elsewhere in the colony. The empty lower frame provided an unrestricted storage area for the deposition of pollen loads, thereby minimizing the search time necessary for foragers to find an empty cell. If foragers used a direct method of assessing pollen stores, foraging rates would have been increased for both low and high stimulus levels of pollen quality treatments, as an empty pollen storage area would normally be associated with severe pollen shortage. However, this did not occur, which supports the hypothesis that protein flow influences pollen foraging rates. Finally, some of the temporal trends exhibited by colonies in this study also support Camazine's findings. Camazine showed that an overnight treatment of stored pollen given to a colony that was previously deprived of pollen was sensed by foragers quickly, and that changes in the dynamics of pollen foraging could be detected the following day. A similar, quick-acting change in pollen intake was found to occur on day 1 of this experiment for colonies that were supplemented with pollen during the previous evening. The addition of pollen took place after five days of pollen deprivation (Fig. 17). This study also showed that pollen intake rates for colonies supplied with the low quality diet took three days before they achieved the high level of pollen intake seen for the low quantity treatment on day 1. This slower rate of response for quality manipulated treatments may support a physiologically-based mechanism for the regulation of pollen foraging, whereby pollen stores must be consumed and insufficient protein flow perceived by foragers before foraging rates are maximized. Similar temporal lags in the allocation of foragers have also been shown to occur in relation to changes in colony

nutritional status (Schulz et al 1998). Hence, foraging responses to changes in pollen quality may not occur as rapidly as to changes in pollen quantity. Although the results of this study tend to support an indirect assessment hypothesis for the regulation of pollen foraging, I also acknowledge that certain concepts associated with “direct assessment” hypotheses, particularly the influence of odours produced from stored pollen or unsealed brood (Free 1967, 1979; Pankiw et al. 1998b), are consistent with my findings.

In conclusion, my research has examined several facets of honey bee foraging that were hitherto unexplored. I have conclusively demonstrated that honey bee colonies respond to changes in the nutritional value of their stored pollen reserve and that this response is similar to that seen for changes in the size of the pollen reserve. Colonies suffering decreases in the nutritional value of their pollen stores respond by increasing the proportion of pollen foragers while maintaining the same total rate of foraging. My data show that honey bee colonies do not respond to qualitative or quantitative changes in their stored pollen reserve by specializing on different assemblages of flora or on species having increased or decreased nutritional status. Moreover, the actions of individual foragers appear to be insensitive to the needs of the colony. Foragers did not alter the weight of the pollen load they collected, the type or number of species they sampled, or other qualitative parameters of the pollen they collected in response to colony-level changes. However, the foraging behaviours of inexperienced and experienced foragers were discovered to be considerably different. Inexperienced foragers exerted considerably more effort to collect pollen by collecting heavier loads, sampling more species per foraging trip and specializing on slightly more nutritious pollen. The actions of inexperienced and experienced foragers were not, however, linked to treatments

applied to their colonies. Honey bee colonies may respond to changes in the quantity and quality of pollen stored in their colony by changing the ratio of inexperienced to experienced foragers in their work force, although this remains unproven. Future work in this area should document changes in the recruitment of inexperienced and experienced foragers into the foraging population, before and after manipulations of colony state parameters. By integrating information about the changing proportions of each experience cohort in the population with pollen load analysis, the full quantitative and qualitative impact of foraging experience at the colony level can be elucidated.

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Table 2. Occurrence, protein content and water content of pollen species collected by honey bees, from analysis of corbicular pollen loads (N=5477)

Pollen species	Common name	Family	Occurrences in pollen loads	No. of pollen loads as dominant species ^a	% protein, $\mu\text{g} / 100 \mu\text{g dry mass}^b$	% water, $\mu\text{g} / 100 \mu\text{g wet mass}^c$
<i>Melilotus officinalis</i> (L.) Pall.	Yellow Sweetclover	Leguminosae	3048	3012	21.59	16.8
<i>Trifolium hybridum</i> L.	Alsike Clover	Leguminosae	867	856	23.54	17.4
<i>Trifolium repens</i> L.	White Clover	Leguminosae	502	478	25.36	18.4
<i>Brassica napus</i> L. / <i>B. rapa</i> L.	Oilseed Rape	Cruciferae	488	468	24.28	15.8
<i>Melilotus alba</i> Desr.	White Sweetclover	Leguminosae	323	302	20.67	17.4
<i>Arctium lappa</i> L.	Great Burdock	Compositae	209	196	17.83	20.7
<i>Sinapis alba</i> L.	Mustard	Cruciferae	81	67	24.91	17.6
<i>Rubus idaeus</i> L. var. <i>strigosus</i> (Michx.) Maxim	Raspberry	Rosaceae	42	42	26.34	19.5
<i>Sonchus arvensis</i> L.	Field Sow Thistle	Compositae	21	14	16.40	21.9
<i>Amelanchier humilis</i> Wieg.	Shadbush	Roasaceae	20	2	19.26	12.9
<i>Vicia cracca</i> L.	Tufted Vetch	Leguminosae	14	14	23.30	16.3

<i>Cornus stolonifera</i> Michx.	Red-Osier Dogwood	Cornaceae	8	5	16.04	18.6
<i>Zea mays</i> L.	Corn	Gramineae	8	7	15.23	15.7
<i>Cirsium arvense</i> (L.) Scop.	Canada Thistle	Compositae	5	1	17.83	9.9
<i>Cichorium intybus</i> L.	Chicory	Compositae	4	4	15.05	11.8
<i>Aster</i> spp. #1	Aster #1	Compositae	2	1	8.60	15.8
<i>Rosa acicularis</i> Lindl.	Prickly Wild Rose	Rosaceae	2	1	23.65	17.3
<i>Solidago canadensis</i> L.	Canada Goldenrod	Compositae	2	2	13.80	22.1
<i>Tilia americana</i> L.	American Linden	Tiliaceae	2	1	46.95	19.2
<i>Aster</i> spp. #2	Aster #2	Compositae	1	1	15.14	20.1
<i>Diervilla lonicera</i> Mill.	Bush-Honeysuckle	Caprifoliaceae	1	1	14.87	18.3
<i>Lotus corniculatus</i> L.	Bird's-Foot Trefoil	Leguminosae	1	1	30.80	24.0
<i>Potentilla fruticosa</i> L. ssp. <i>floribunda</i> (Nutt.) Elk.	Shrubby Cinquefoil	Rosaceae	1	1	18.18	23.6
<i>Vaccinium angustifolia</i> Ait.	Lowbush Blueberry	Ericaceae	1	0	16.93 ^d	24.8 ^e
<i>Xanthium strumarium</i> L.	Cocklebur	Compositae	1	0	5.65 ^d	16.7 ^e

^aSpecies comprising > 50% of the total number of pollen grains per load.

^bProtein determinations by elemental analysis of unifloral pollen loads, except where indicated.

^cWater content from unifloral pollen loads, except where indicated.

^dEstimated protein content calculated from multifloral pollen loads. Protein content was calculated as the difference between the total protein

content of the load and the protein contributed by other species, weighted in proportion to their cross-sectional areas.

“Water content of multifloral pollen loads.

Table 3. Wet weight (mean \pm SE) of corbicular pollen loads collected by honey bee foragers

Pollen species	N	Wet weight, mg
<i>B. napus</i> / <i>B. rapa</i>	466	15.46 \pm 0.35 ^a
<i>S. alba</i>	66	15.10 \pm 0.95 ^a
<i>T. hybridum</i>	849	13.63 \pm 0.22 ^{ab}
<i>T. repens</i>	472	12.47 \pm 0.29 ^b
<i>A. lappa</i>	196	9.59 \pm 0.32 ^c
<i>M. officinalis</i>	2976	9.10 \pm 0.09 ^c
<i>R. idaeus</i> var. <i>strigosus</i>	42	8.99 \pm 0.59 ^c
<i>V. cracca</i>	14	7.93 \pm 1.63 ^{cd}
<i>M. alba</i>	295	6.73 \pm 0.23 ^d
<i>S. arvensis</i>	12	6.55 \pm 1.20 ^d

Values calculated for pollen loads comprised of > 80% one species.

Means followed by the same letter are not significantly different (LSD, α = 0.05).

Table 4. Linear relationships between number of pollen grains (y) and wet weight of pollen loads (x), used for the calculation of protein content of multifloral pollen loads containing species frequently collected by foragers

Pollen Species	Relationship ^a	r^2
<i>M. officinalis</i>	$y = A1.080 + 6.671(x)$	0.943
<i>T. hybridum</i>	$y = -2.620 + 7.530(x)$	0.964
<i>T. repens</i>	$y = -2.008 + 5.366(x)$	0.945
<i>B. napus</i> / <i>B. rapa</i>	$y = A3.522 + 3.952(x)$	0.978
<i>M. alba</i>	$y = 1.305 + 6.756(x)$	0.952
<i>A. lappa</i>	$y = 0.563 + 1.022(x)$	0.955
<i>S. alba</i>	$y = A4.691 + 3.205(x)$	0.736
<i>R. idaeus</i> var. <i>strigosus</i>	$y = A1.284 + 4.231(x)$	0.995

^a y = number of pollen grains per corbicular pollen load ($\times 10^{-4}$); x = wet weight, mg.

Table 5. Frequency of pollen loads comprised of different numbers of species, based on different thresholds for recognizing secondary species in mixed loads

Threshold ^a	No. of pollen species per load				
	1	2	3	4	5
0%	5322 (97.17) ^b	136 (2.48)	17 (0.31)	1 (0.02)	1 (0.02)
5%	5376 (98.16)	100 (1.83)	1 (0.02)	0 (0)	0 (0)
10%	5401 (98.61)	76 (1.39)	0 (0)	0 (0)	0 (0)

^aA species comprising a proportion of the total number of grains in excess of the threshold indicated was recognized as a contributing species.

^bNumbers in brackets are percentages of the total number of pollen loads.

Table 6. Frequency of co-occurring species in mixed pollen loads^a

Pollen species	No. loads as dominant species ^b	Secondary species in mixed loads															No. mixed loads	% mixed loads ^c
		<i>M.o.</i>	<i>T.h.</i>	<i>T.r.</i>	<i>B.n.</i>	<i>M.a.</i>	<i>A.l.</i>	<i>S.a.</i>	<i>S.ar.</i>	<i>V.c.</i>	<i>Z.m.</i>	<i>R.a.</i>	<i>A.h.</i>	<i>C.s.</i>	<i>C.a.</i>	<i>T.a.</i>		
		<i>M. officinalis</i>	3012	•	8	16	4	19	2	0	2	0	1	1	1	1		
<i>T. hybridum</i>	856	8	•	3	0	0	0	0	0	0	0	0	0	1	2	0	14	1.6
<i>T. repens</i>	478	11	0	•	0	1	0	0	0	0	0	0	0	0	0	0	12	0.4
<i>B. napus^d</i>	468	1	0	0	•	1	0	0	1	0	0	0	0	0	0	0	3	0.6
<i>M. alba</i>	302	5	0	2	0	•	0	0	0	0	0	0	0	0	0	0	7	2.3
<i>A. lappa</i>	196	1	0	0	0	0	•	0	0	0	0	0	0	0	0	0	1	0.5
<i>S. alba</i>	67	1	0	0	0	0	0	•	0	0	0	0	0	0	0	0	1	1.5
<i>S. arvensis</i>	14	2	1	0	0	0	0	0	•	1	0	0	0	0	1	0	4	28.6
<i>V. cracca</i>	14	0	1	0	0	0	0	0	0	•	0	0	0	0	0	0	1	7.1
<i>Z. mays</i>	7	0	1	0	0	0	0	1	0	0	•	0	0	0	0	0	1	14.3
<i>R. acicularis</i>	1	1	0	0	0	0	0	0	0	0	0	•	0	0	0	0	1	100.0

^aMixed pollen loads are defined by the presence of at least two pollen species, each of which must compose >5% of the total number of grains in the load.

^bSpecies composing >50% of the grains in a pollen load are defined as the dominant species.

Percent mixed loads from all those in which a given species is dominant.

B. napus and *B. rapa*.

Species abbreviations: *M.o.*, *M. officinalis*; *T.h.*, *T. hybridum*; *T.r.*, *T. repens*; *B.n.*, *B. napus* / *B. rapa*; *A.l.*, *A. lappa*; *S.a.*, *S. alba*; *S.ar.*, *S. arvensis*; *V.c.*; *V. cracca*; *Z.m.*, *Z. mays*; *R.a.*, *R. acicularis*; *A.h.*, *A. humilis*; *C.s.*, *C. stolonifera*; *C.a.*, *C. arvensis*; *T.a.*, *T. americana*.

Table 7. Main effects and significant interaction terms from log-linear analysis of pollen species assemblages collected by colonies

Model term	df	χ^2	<i>P</i>
REPLICATE	100	606.42	< 0.0001
STIMULUS MECHANISM	10	5.69	NS
STIMULUS INTENSITY	10	3.82	NS
DAY	20	17.90	NS
TIME	10	116.97	< 0.0001
EXPERIENCE	10	55.98	< 0.0001
REPLICATE*TIME	100	125.93	< 0.05
DAY*TIME	20	34.59	< 0.05
TIME* EXPERIENCE	10	24.76	< 0.01

(TIME, time of day; EXPERIENCE, forager experience; DAY, days after colony manipulation; NS, non-significant).

Table 8. Occurrences of species in pollen loads, as proportions (%) of occurrences within replicates

Replicate	Date ^a	Pollen species											N ^b
		<i>A. humilis</i>	<i>A. lappa</i>	<i>B. napus</i>	<i>M. alba</i>	<i>M. officinalis</i>	<i>R. idaeus</i>	<i>S. alba</i>	<i>S. arvensis</i>	<i>T. hybridum</i>	<i>T. repens</i>	<i>V. cracca</i>	
1	17 July	0.29	6.45	9.68	7.04	55.43	0	0.59	0	19.06	1.47	0	341
2	17 July	0.31	0.31	10.70	4.89	58.72	0	0.31	0.31	22.94	0.92	0.31	327
3	25 July	0	5.55	22.66	4.75	52.46	0	0.16	0	12.84	1.58	0	631
4	25 July	0	3.19	16.57	2.40	54.09	0	0.40	0	20.36	2.99	0	501
5	31 July	0	7.72	11.31	1.44	67.15	0	0	0.18	6.82	5.39	0.18	557
6	10 Aug	2.26	4.70	3.38	3.57	64.85	0	7.33	0.75	5.08	6.58	0.75	532
7	10 Aug	1.11	1.77	7.54	2.44	62.75	0	5.32	0.67	11.09	7.32	0.67	451
8	15 Aug	0.19	4.73	8.90	6.06	59.85	0	1.14	0.38	9.85	8.33	0.38	528
9	15 Aug	0	1.65	5.99	8.88	59.92	0	1.24	0.83	10.33	11.16	0.83	484
10	28 Aug	0	2.38	0	9.51	38.78	5.65	0	0.45	25.11	17.98	0.45	673
11	28 Aug	0	1.69	0.51	10.85	33.22	0.68	0	0.51	26.78	25.76	0.51	590

^aFirst day of the experimental period within designated replicate.

^bTotal number of species occurrences within designated replicate.

Table 9. Occurrences of species in pollen loads, as proportions (%) of occurrences within replicates and time periods

R e p	Date ^b	Time period	Pollen species											N ^c
			<i>A. humilis</i>	<i>A. lappa</i>	<i>B. napus</i>	<i>M. alba</i>	<i>M. officinalis</i>	<i>R. idaeus</i>	<i>S. alba</i>	<i>S. arvensis</i>	<i>T. hybridum</i>	<i>T. repens</i>	<i>V. cracca</i>	
1	17 July	A.M.	0	19.12	23.53	0	39.71	0	2.94	0	13.24	1.47	0	68
2	17 July	A.M.	1.22	1.22	34.15	2.44	40.24	0	1.22	1.22	15.85	2.44	0	82
3	25 July	A.M.	0	6.19	32.99	3.44	45.70	0	0	0	10.65	1.03	0	291
4	25 July	A.M.	0	1.40	20.00	0.47	58.14	0	0.47	0	15.35	4.19	0	215
5	31 July	A.M.	0	8.81	17.62	1.76	61.67	0	0	0.44	5.29	4.41	0	227
6	10 Aug	A.M.	2.62	4.19	2.62	2.62	64.92	0	6.28	1.57	4.71	7.85	2.62	191
7	10 Aug	A.M.	1.61	1.08	4.84	1.08	66.13	0	5.91	1.61	9.68	8.06	0	186
8	15 Aug	A.M.	0.48	3.38	5.80	3.86	66.67	0	1.45	0.97	3.86	13.04	0.48	207
9	15 Aug	A.M.	0	0.86	6.87	3.86	63.95	0	2.15	1.72	5.58	15.02	0	233
10	28 Aug	A.M.	0	2.94	0	7.52	42.48	8.50	0	0.98	16.34	20.92	0.33	306
11	28 Aug	A.M.	0	1.24	1.24	7.88	35.27	0.83	0	1.24	15.35	36.93	0	241
1	17 July	P.M.	0.37	3.30	6.23	8.79	59.34	0	0	0	20.51	1.47	0	273
2	17 July	P.M.	0	0	2.86	5.71	64.90	0	0	0	25.31	0.41	0.82	245
3	25 July	P.M.	0	5.00	13.82	5.88	58.24	0	0.29	0	14.71	2.06	0	340
4	25 July	P.M.	0	4.55	13.99	3.85	51.05	0	0.35	0	24.13	2.10	0	286
5	31 July	P.M.	0	6.97	6.97	1.21	70.91	0	0	0	7.88	6.06	0	330
6	10 Aug	P.M.	2.05	4.99	3.81	4.11	64.81	0	7.92	0.29	5.28	5.87	0.88	341
7	10 Aug	P.M.	0.75	2.26	9.43	3.40	60.38	0	4.91	0	12.08	6.79	0	265
8	15 Aug	P.M.	0	5.61	10.90	7.48	55.45	0	0.93	0	13.71	5.30	0.62	321
9	15 Aug	P.M.	0	2.39	5.18	13.55	56.18	0	0.40	0	14.74	7.57	0	251
10	28 Aug	P.M.	0	1.91	0	11.17	35.69	3.27	0	0	32.43	15.53	0	367
11	28 Aug	P.M.	0	2.01	0	12.89	31.81	0.57	0	0	34.67	18.05	0	349

^aReplicate.

^bFirst day of the experimental period within designated replicate.

^cTotal number of species occurrences within designated replicate and time period.

Table 10. Occurrences of species in pollen loads, as proportions (%) of occurrences within days of the experiment and time periods

Day after colony manipulation	Time period	Pollen species											N ^a
		<i>A. humilis</i>	<i>A. lappa</i>	<i>B. napus</i>	<i>M. alba</i>	<i>M. officinalis</i>	<i>R. idaeus</i>	<i>S. alba</i>	<i>S. arvensis</i>	<i>T. hybridum</i>	<i>T. repens</i>	<i>V. cracca</i>	
1	A.M.	0.52	4.92	12.94	4.79	51.36	1.03	0.52	1.03	13.20	9.57	0.13	773
2	A.M.	0.80	2.40	10.93	2.27	58.27	1.73	2.53	0.67	8.00	12.13	0.27	750
3	A.M.	0	4.14	11.88	4.01	51.52	0.97	1.66	0.97	9.81	14.50	0.55	724
1	P.M.	0.09	3.23	6.98	6.98	59.23	0.43	0.85	0	14.13	7.83	0.26	1175
2	P.M.	0.71	3.82	5.77	6.93	53.73	0.27	1.51	0.09	19.27	7.64	0.27	1126
3	P.M.	0.09	3.94	6.84	7.50	50.61	0.56	1.78	0	23.52	5.06	0.09	1067

^aTotal number of species occurrences within designated day after colony manipulation and time period.

Table 11. Occurrences of species in pollen loads, as proportions (%) of occurrences within time periods and levels of forager experience

Time period	Forager experience	Pollen species											N ^a
		<i>A. humilis</i>	<i>A. lappa</i>	<i>B. napus</i>	<i>M. alba</i>	<i>M. officinalis</i>	<i>R. idaeus</i>	<i>S. alba</i>	<i>S. arvensis</i>	<i>T. hybridum</i>	<i>T. repens</i>	<i>V. cracca</i>	
A.M.	Experienced	0.86	2.06	10.48	4.64	61.51	1.03	0.52	0.69	10.65	7.39	0.17	582
A.M.	Inexperienced	0.30	4.44	12.43	3.36	50.99	1.32	1.92	0.96	10.27	13.63	0.36	1665
P.M.	Experienced	0	2.05	3.74	7.55	62.27	0.44	0.44	0.07	18.24	4.91	0.29	1365
P.M.	Inexperienced	0.50	4.74	8.44	6.84	49.48	0.40	2.00	0	19.22	8.24	0.15	2003

^aTotal number of species occurrences within designated time period and level of forager experience.

Fig. 12. Observation hive with honey bees.

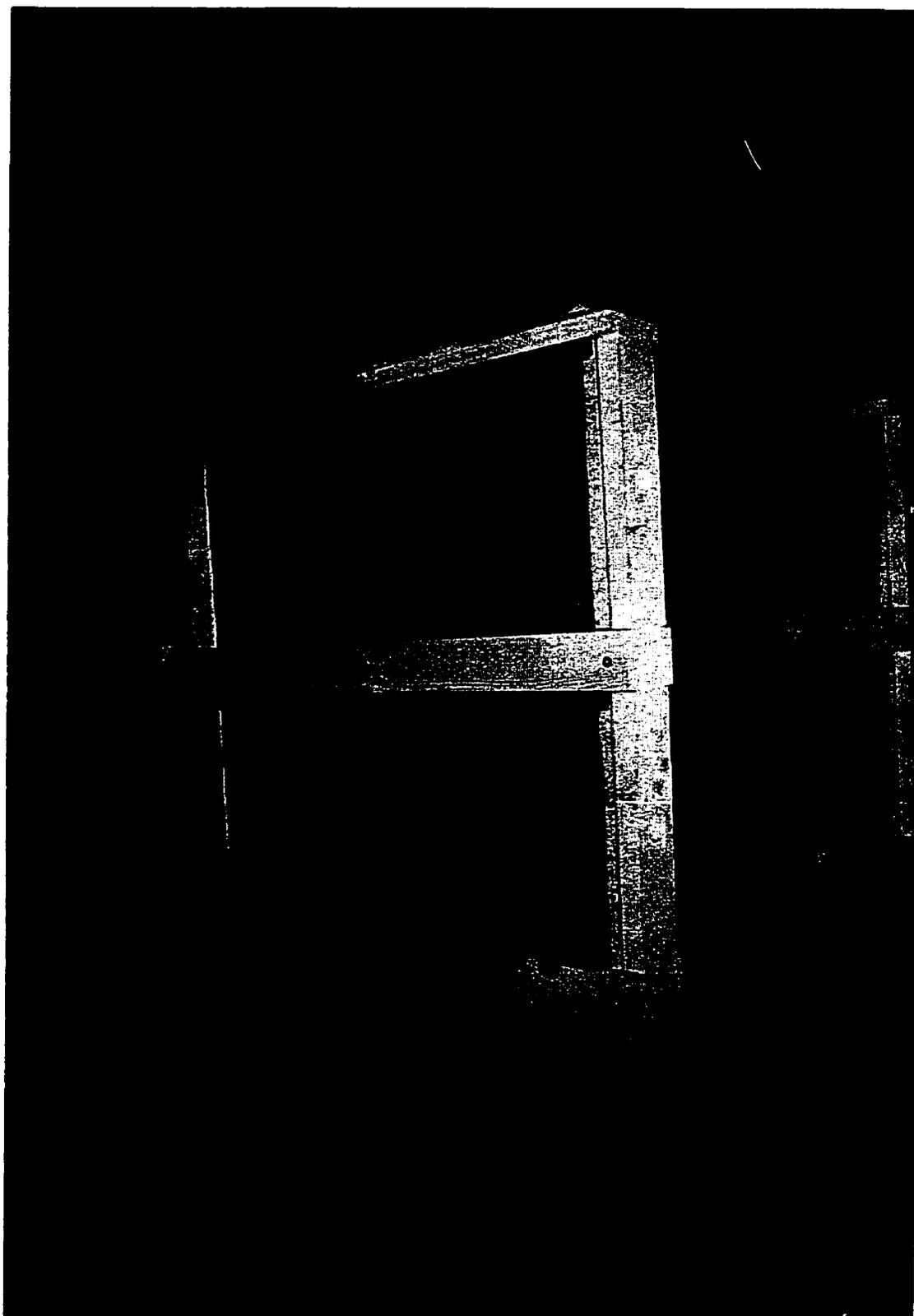


Fig. 13. Dimensions of observation hive components. Hives were designed to accommodate three full-depth Langstroth frames. Wood used for the assembly of hives was milled from construction-grade spruce framing lumber and wooden components were assembled with #8 wood screws. Vertical walls of observation hives were each comprised of three glass panes (not illustrated), 2 mm thick. The two uppermost panes measured 48.90 cm by 25.24 cm, and the bottom pane was 48.90 cm by 26.04 cm. Vertical grooves cut into wooden members (“TOP”, “BOTTOM”, “A”, “C” and “D”) held panes in place and permitted panes to be independently removed by sliding towards, and over, part “B”. Observation hives were mounted on a wooden base (30.48 cm by 59.53 cm) constructed from 1.91 cm thick fir plywood. Horizontal slots in parts “C” and “D” allowed excluders to be inserted and removed without opening the hive. Ventilation holes (2.38 cm) penetrated through the width of parts “A” and “B” and were covered with metal screening (7.09 meshes cm^{-1}) on the inside surface of the hive. The 2.38 cm entrance hole in part “A” was attached to an entrance tube, which led to the exterior of the honey bee overwintering building at the University of Manitoba.

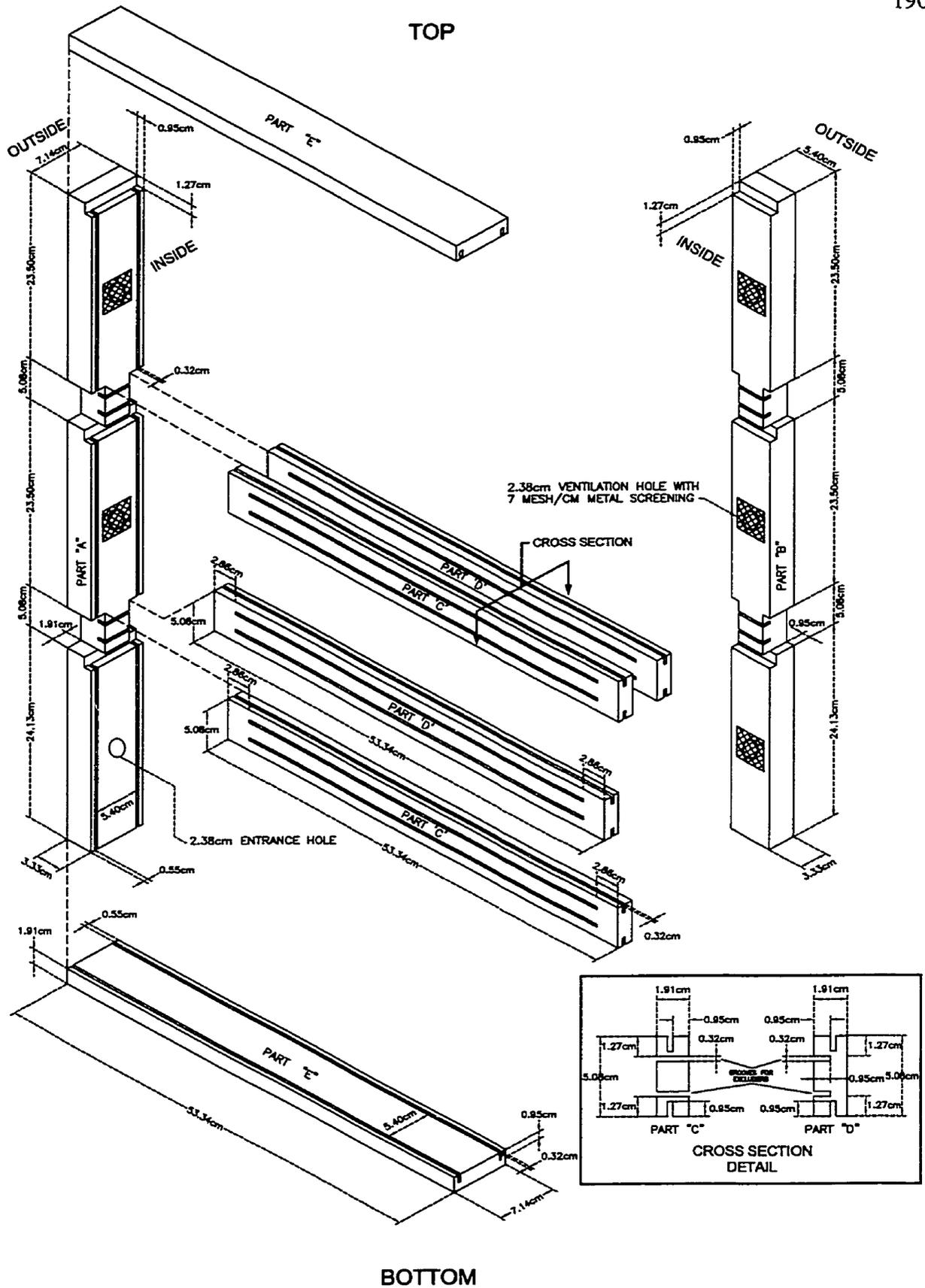


Fig. 14. Wet weight of pollen loads collected by experienced and inexperienced foragers during morning and afternoon data collection periods. Significance letters denote differences between time periods, or between experienced and inexperienced foragers within morning and afternoon time periods (single df comparisons, $\alpha = 0.05$). Vertical lines represent standard errors.

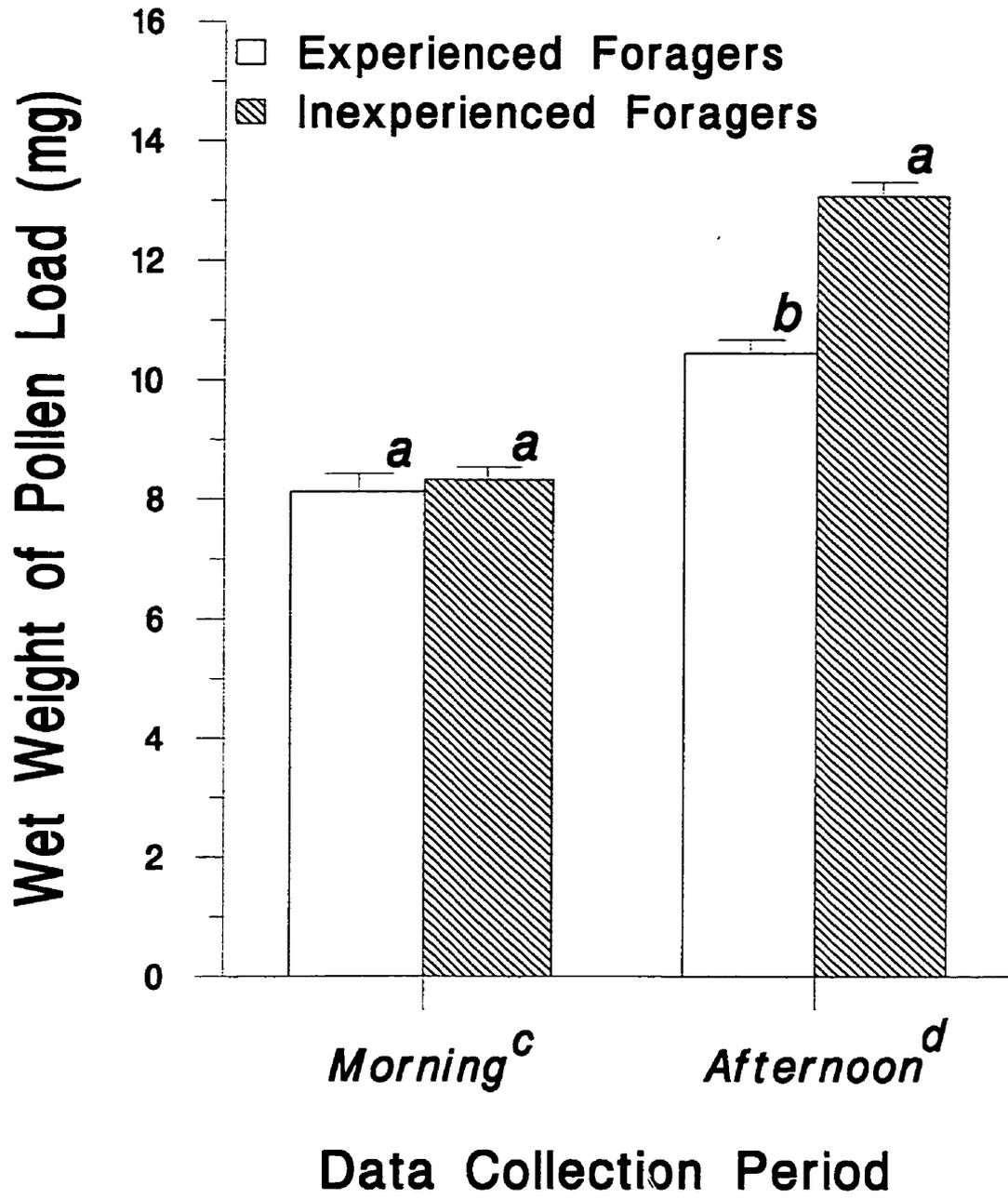


Fig. 15. Number of returning pollen foragers per min (pollen foraging rate) in experimental treatments during morning and afternoon sampling periods. Data plotted are non-transformed means and standard errors. Significance letters denote differences between levels of stimulus intensity within levels of stimulus mechanism and time of day (single df comparisons, $\alpha = 0.05$) (A.M., morning data collection periods, P.M., afternoon data collection periods).

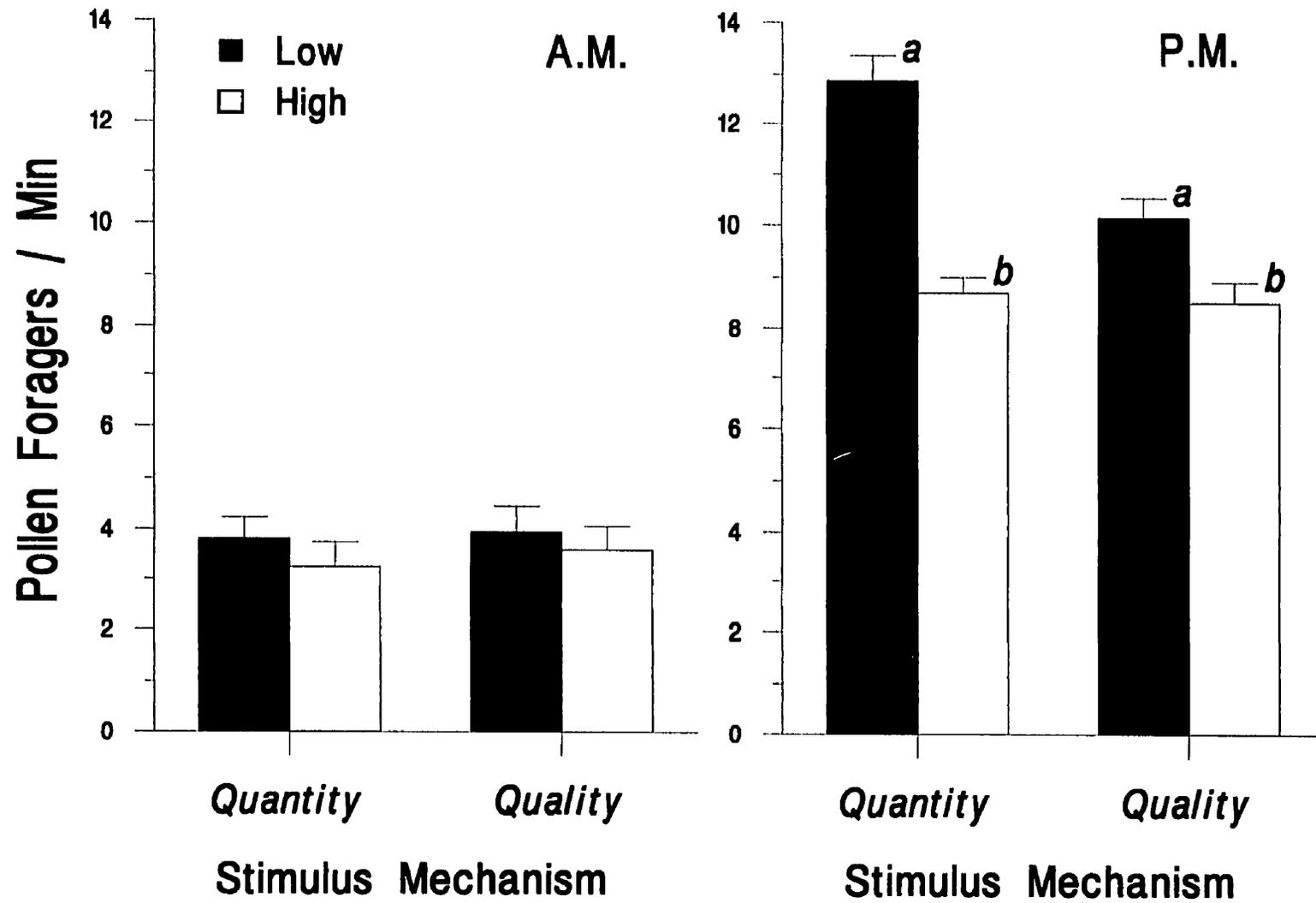


Fig. 16. Numbers of returning nectar and pollen forgers per min (total foraging rate) in experimental treatments during morning and afternoon sampling periods. Data plotted are non-transformed means and standard errors. Significance letters denote differences between levels of stimulus mechanism within time periods (single df comparisons, $\alpha = 0.05$) (A.M., morning data collection periods, P.M., afternoon data collection periods).

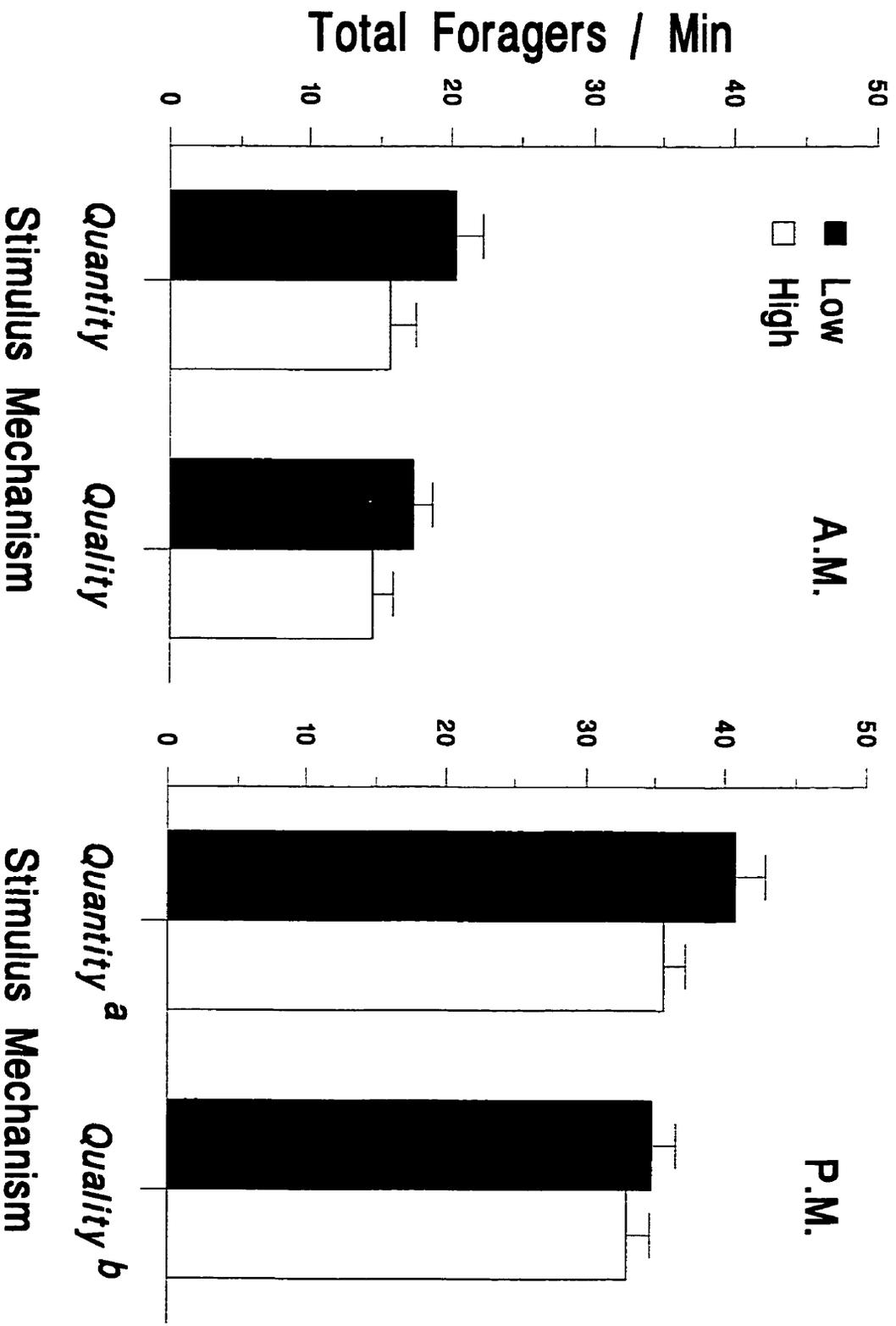


Fig. 17. Pollen intake rate for treatments by day of the experimental period. Data plotted are non-transformed means and standard errors.

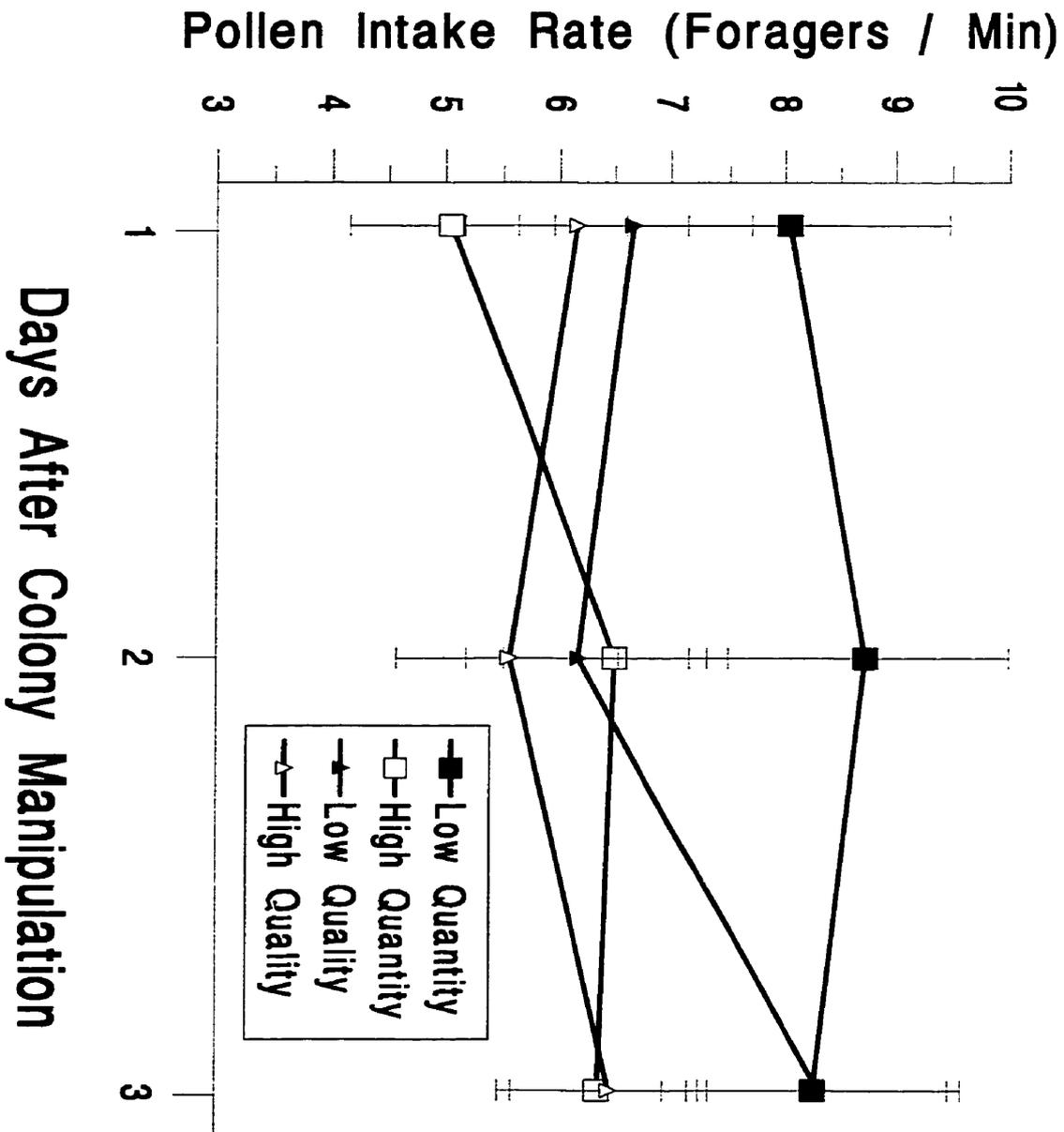


Fig. 18. Mean consumption of pollen supplements used in ‘low’ and ‘high’ quality treatments. Pollen supplements for high quality treatments were made from *M. officinalis* and *B. napus* pollen, while low quality pollen supplements were prepared from *P. baksiana* pollen. Significance letters denote differences between days within different levels of stimulus intensity (LSD, $\alpha = 0.05$). Vertical lines represent standard errors.

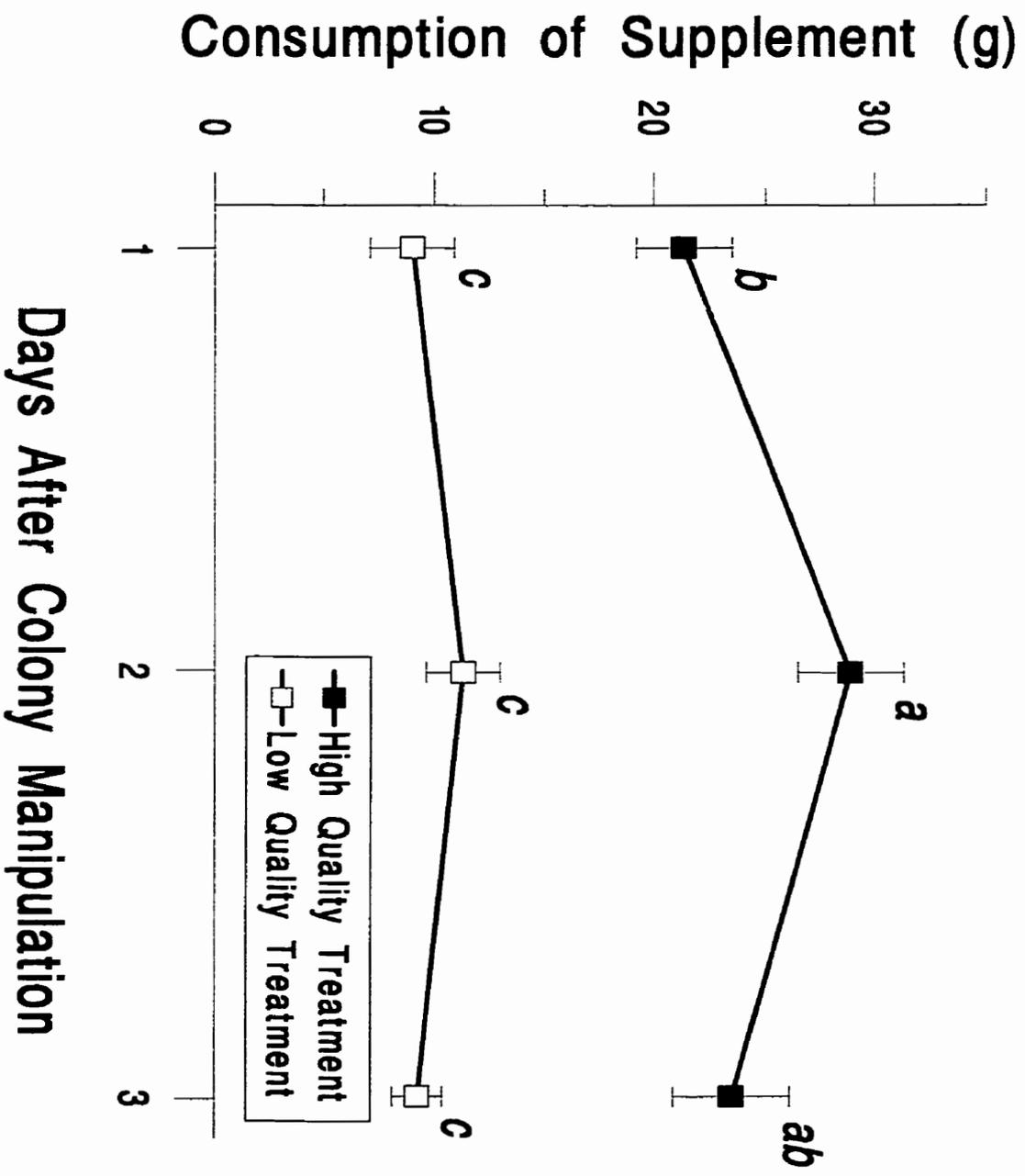


Fig. 19. Consumption of pollen supplements (bars) and changes in the pollen foraging rate of colonies (line). Data plotted for pollen foraging rate are non-transformed means and standard errors. Significance letters denote differences between days within consumption or pollen foraging rate (LSD, $\alpha = 0.05$). Vertical lines represent standard errors.

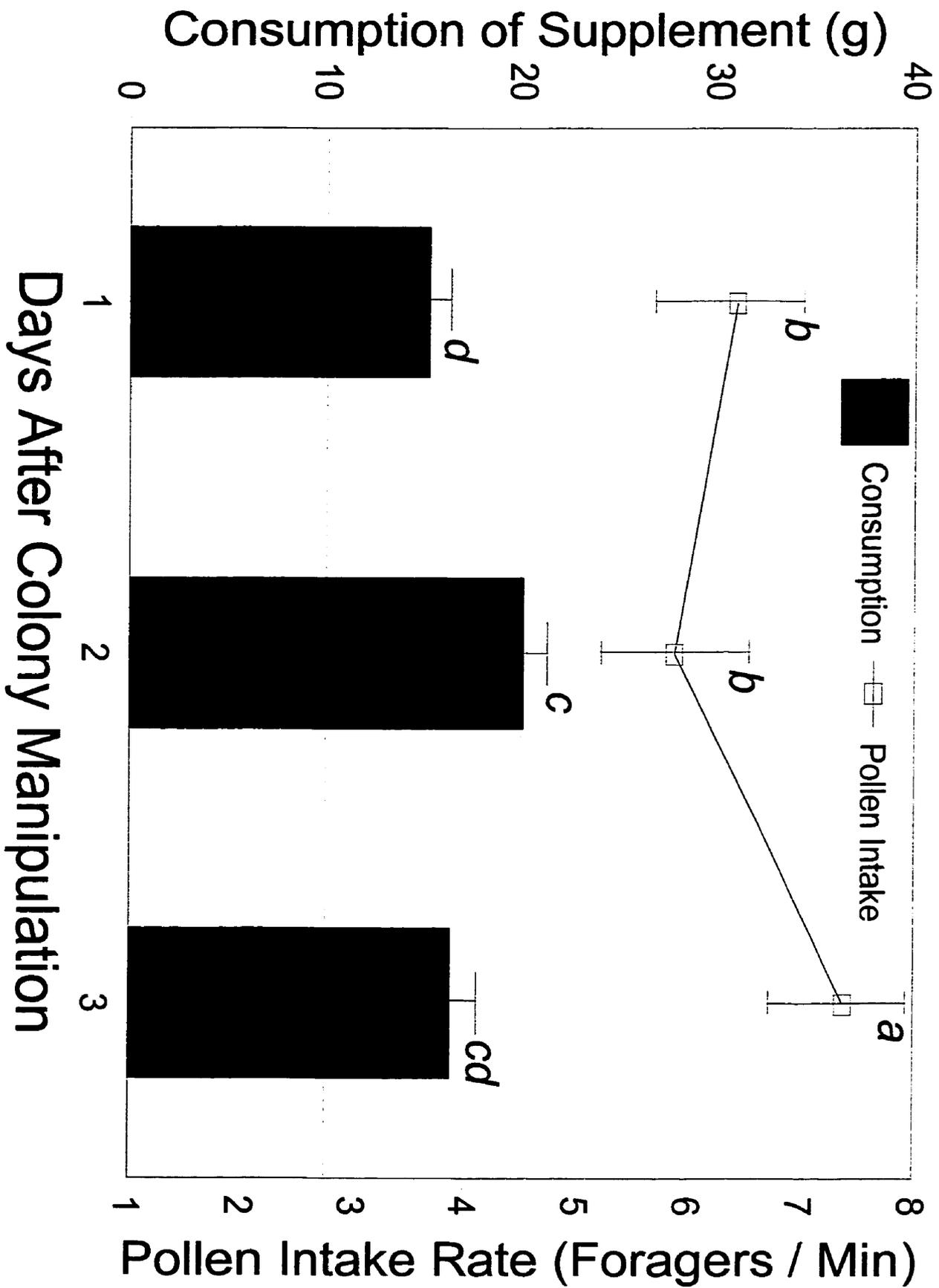


Fig. 20. Temporal changes in protein content of pollen loads (% dry weight) for experienced and inexperienced foragers. Significance letters denote differences between days within levels of forager experience (single df comparisons, $\alpha = 0.05$). Vertical lines represent standard errors.

Protein Content of Pollen Load (% Dry Weight)

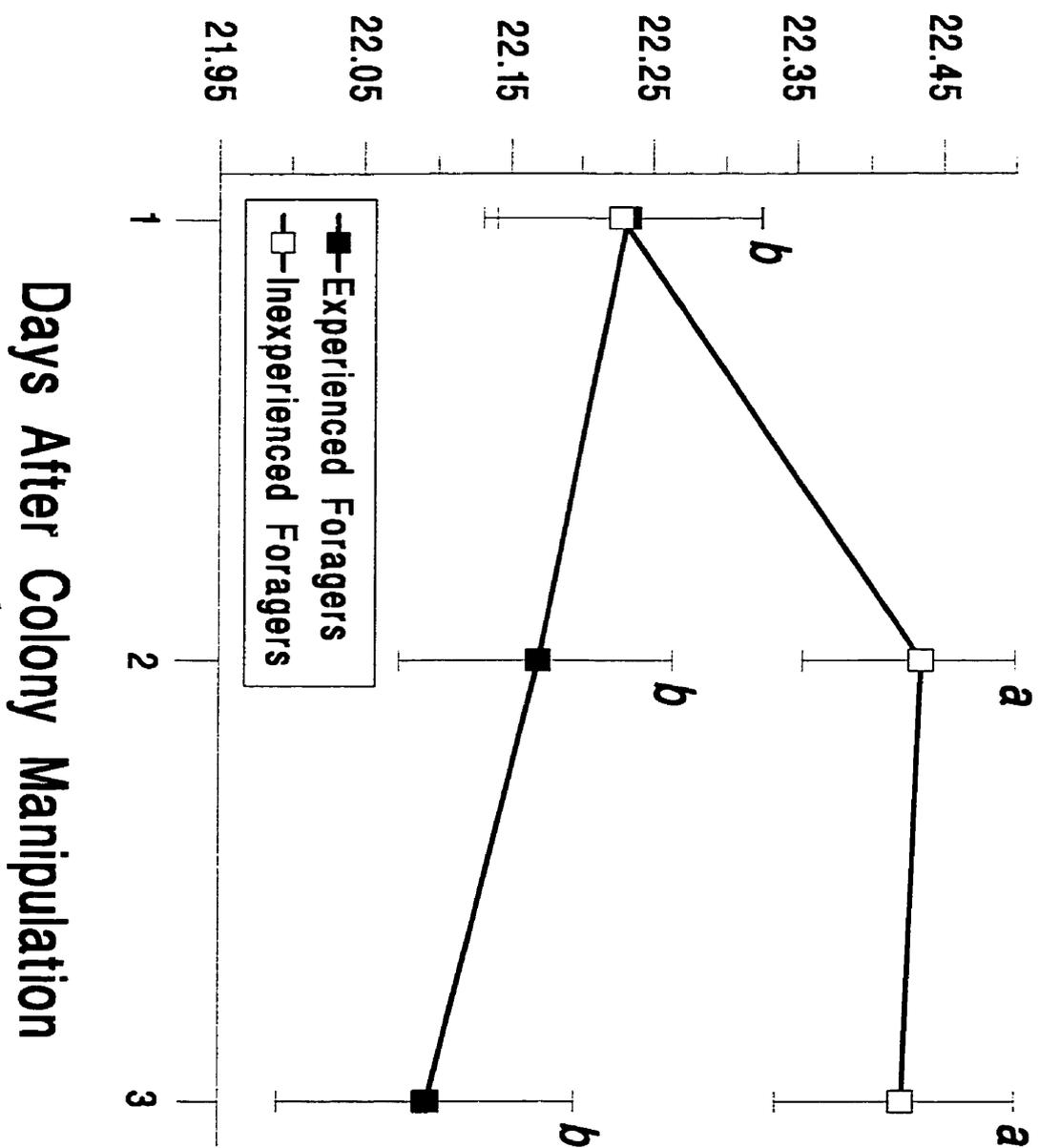


Fig. 21. Mean cross-sectional area of pollen grains in loads collected by experienced and inexperienced foragers during morning and afternoon data collection periods. Data plotted are non-transformed means and standard errors. Significance letters denote differences between time periods, or between experienced and inexperienced foragers within morning and afternoon time periods (single df comparisons, $\alpha = 0.05$). Vertical lines represent standard errors.

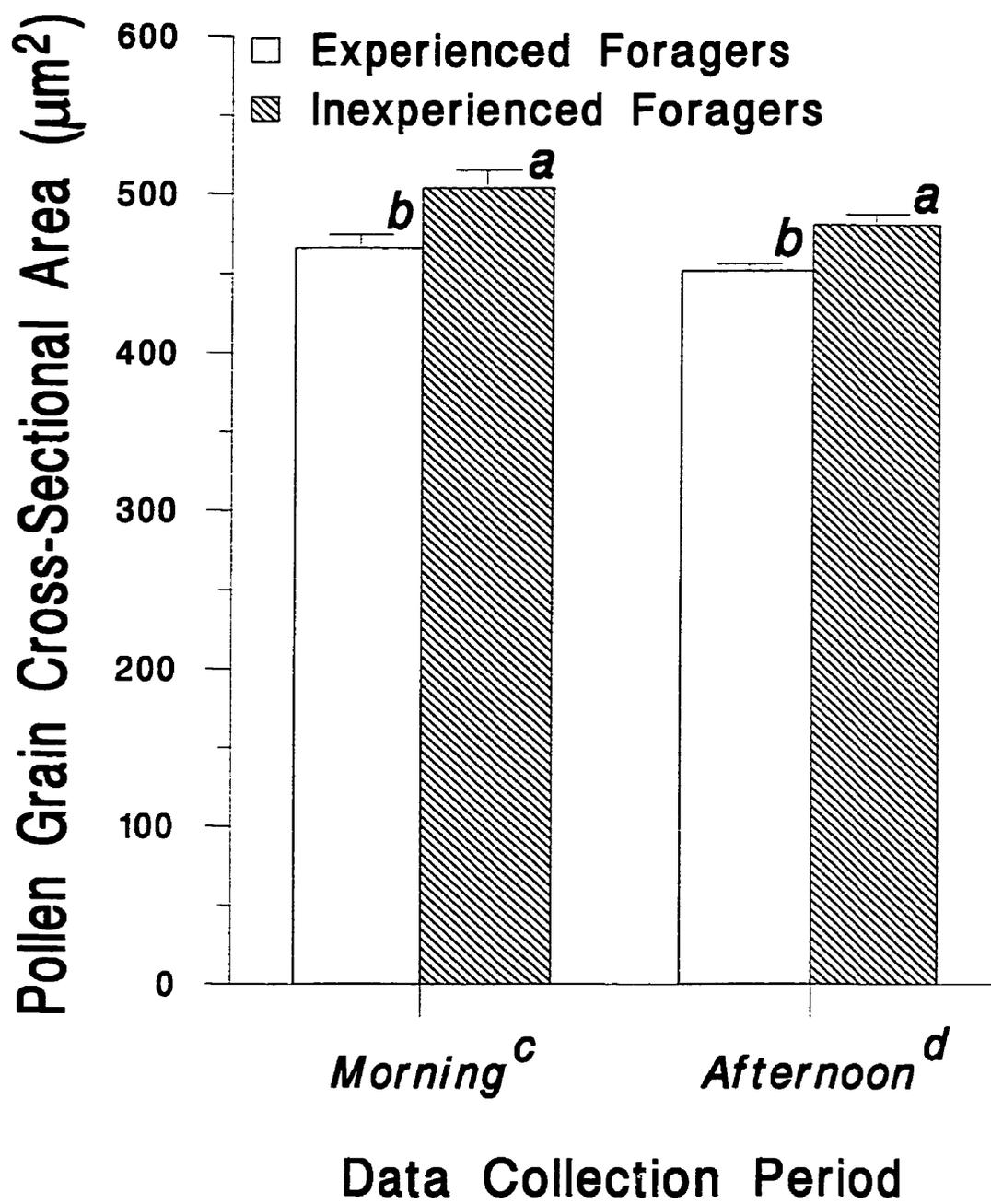


Fig 22. Mean cross-sectional area of pollen grains in loads collected by experienced and inexperienced foragers for quantity and quality manipulated treatments. Data plotted are non-transformed means and standard errors. Significance letters denote differences between experienced and inexperienced foragers within levels of stimulus mechanism (single df comparisons, $\alpha = 0.05$). Vertical lines represent standard errors.

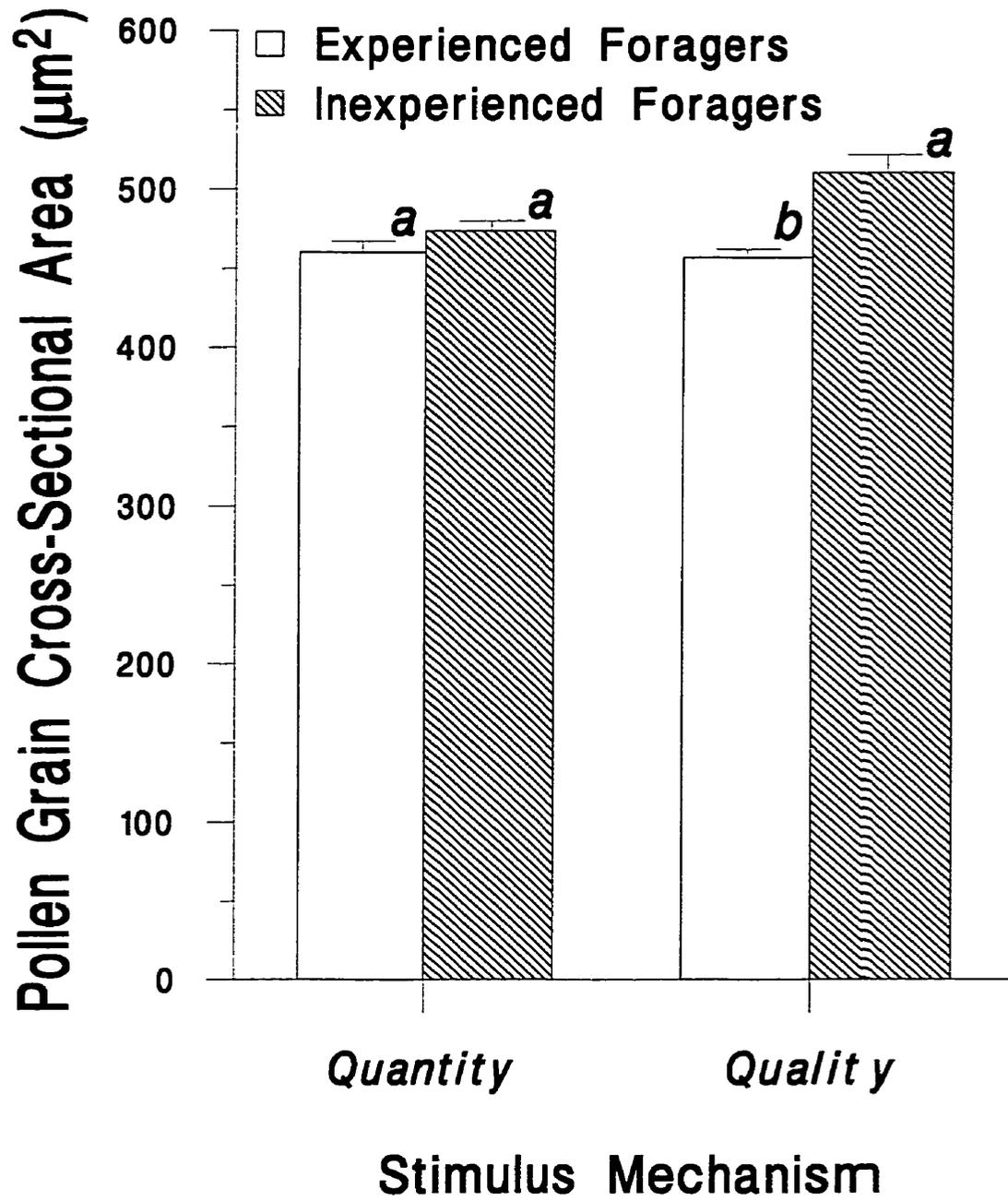
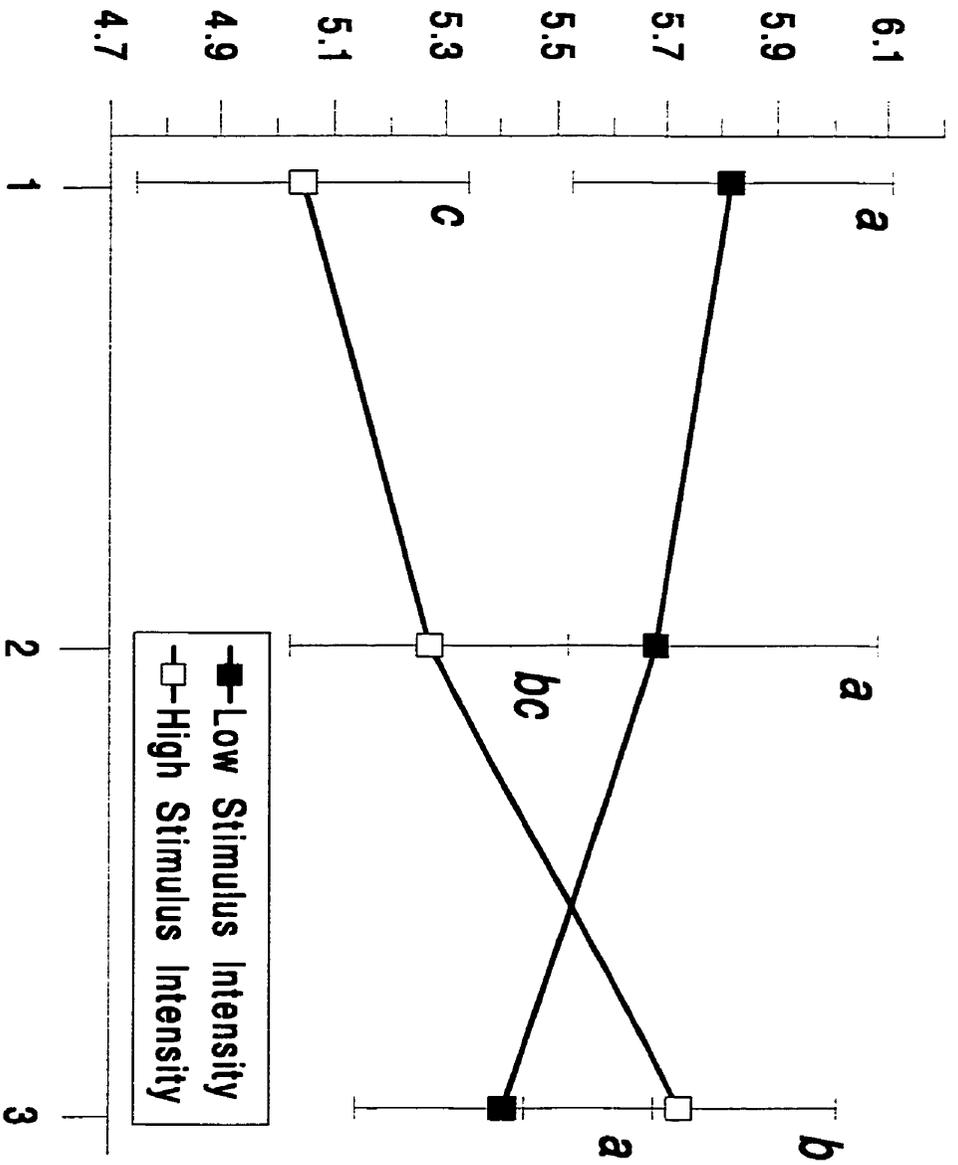


Fig. 23. Temporal changes in the number of pollen species collected by colonies receiving either low or high stimulus intensities. Data points are untransformed means and standard errors pooled over days of the experimental period, time of day and forager experience. Significance letters denote differences between days within levels of stimulus intensity (single df comparisons, $\alpha = 0.05$).

Pollen Species Collected / Colony



Days After Colony Manipulation

Fig. 24. Number of pollen species collected per colony during morning and afternoon data collection periods by inexperienced and experienced foragers. Data points are untransformed means and standard errors. Significance letters denote differences between data collection periods or between levels of forager experience within data collection periods (single df comparisons, $\alpha = 0.05$).

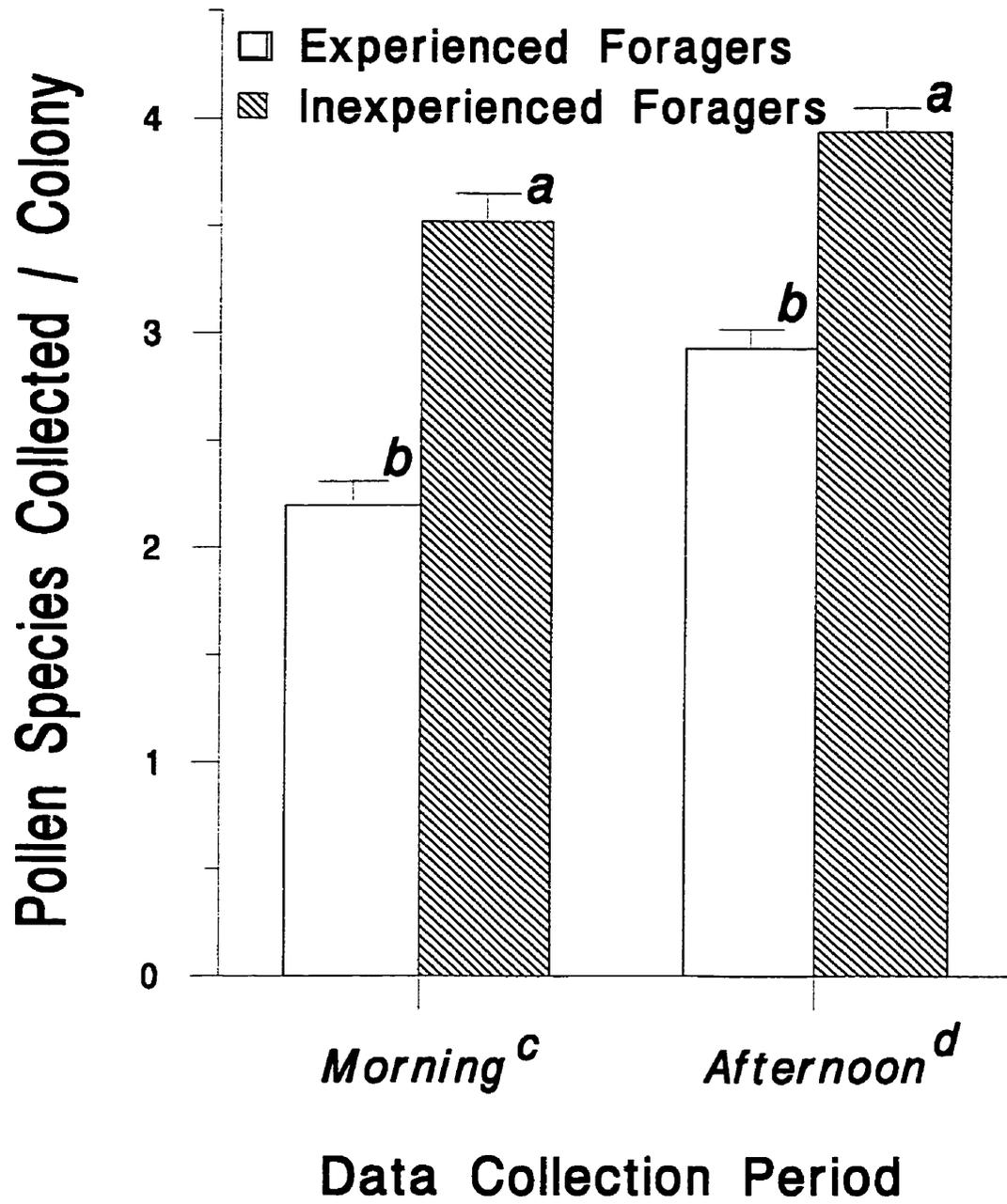


Fig. 25. Number of pollen species comprising individual loads of experienced and inexperienced foragers within levels of stimulus intensity and data collection period. Data points are untransformed means and standard errors. Significance letters denote differences between levels of forager experience within levels of stimulus intensity and time of day. (single df comparisons, $\alpha = 0.05$) (A.M., morning data collection periods, P.M., afternoon data collection periods).

Species Collected / Pollen Load

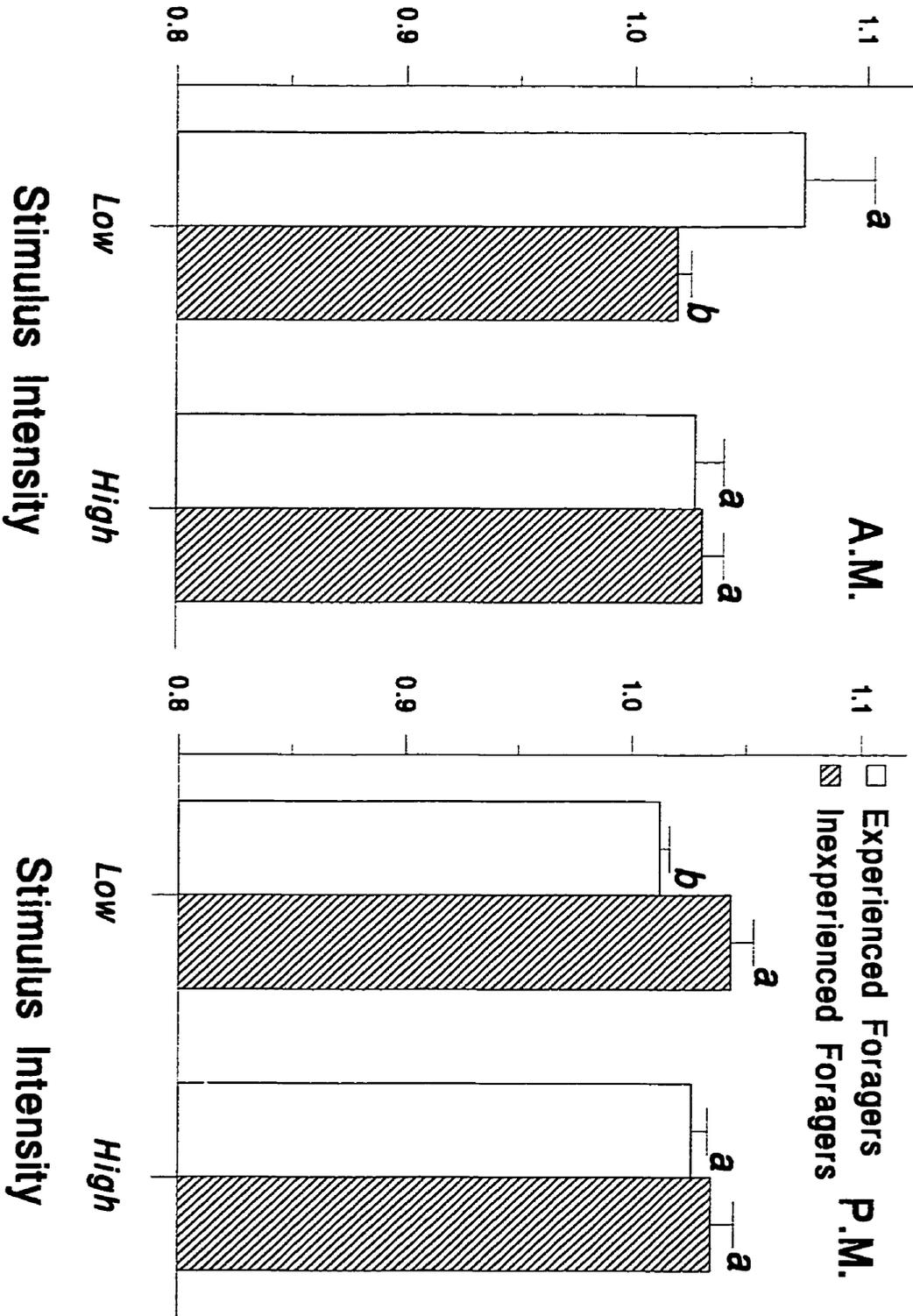


Fig. 26. Protein intake rate (mg / min) in experimental treatments during afternoon sampling periods. Data plotted are non-transformed means and standard errors. Significance letters denote differences between levels of stimulus intensity within levels of stimulus mechanism and time of day (single df comparisons, $\alpha = 0.05$) (A.M., morning data collection periods, P.M., afternoon data collection periods).

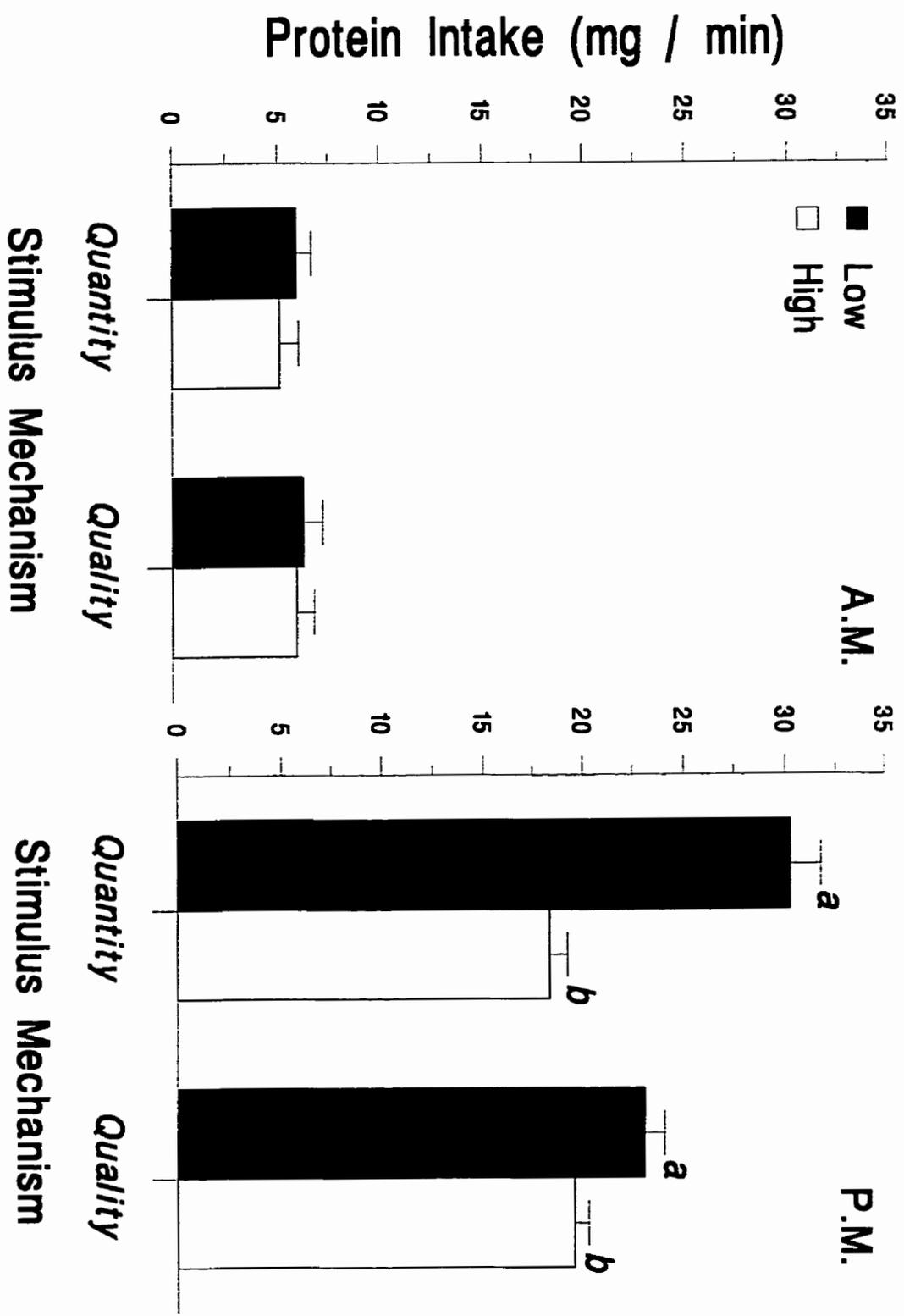


Fig. 27. Temporal changes in protein intake rate (mg / min) averaged over all experimental colonies. Data plotted are non-transformed means and standard errors. Significance letters denote differences between days of the experimental period (LSD, $\alpha = 0.05$).

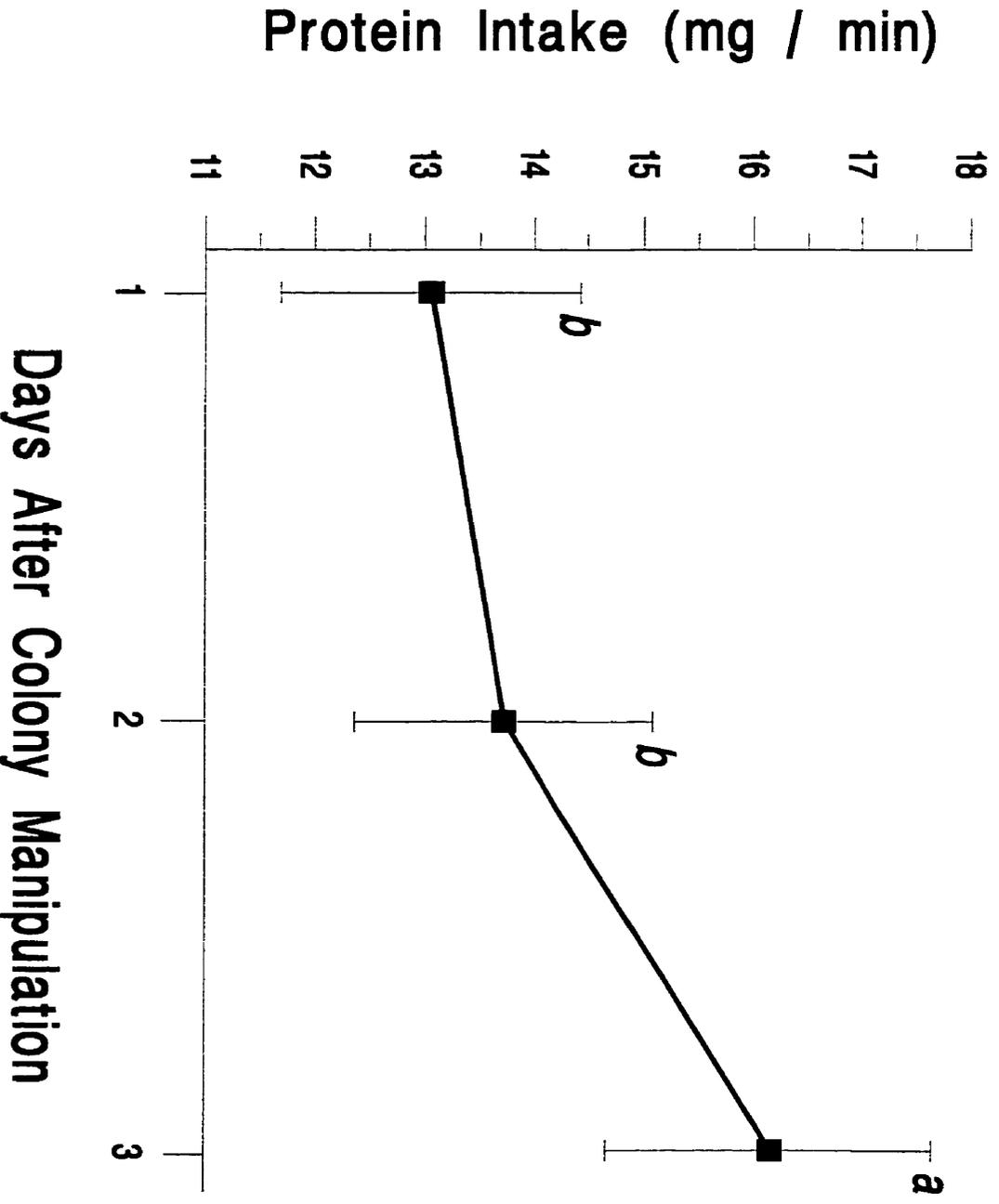


Fig. 28. Occurrences of species (N=5615) in pollen loads, as proportions of occurrences from all pollen loads examined. Only those species occurring at least 10 times, in all pollen loads examined, are plotted. (*B. napus* = *B. napus* + *B. rapa*).

Proportion of Occurrences in Pollen Loads (%)

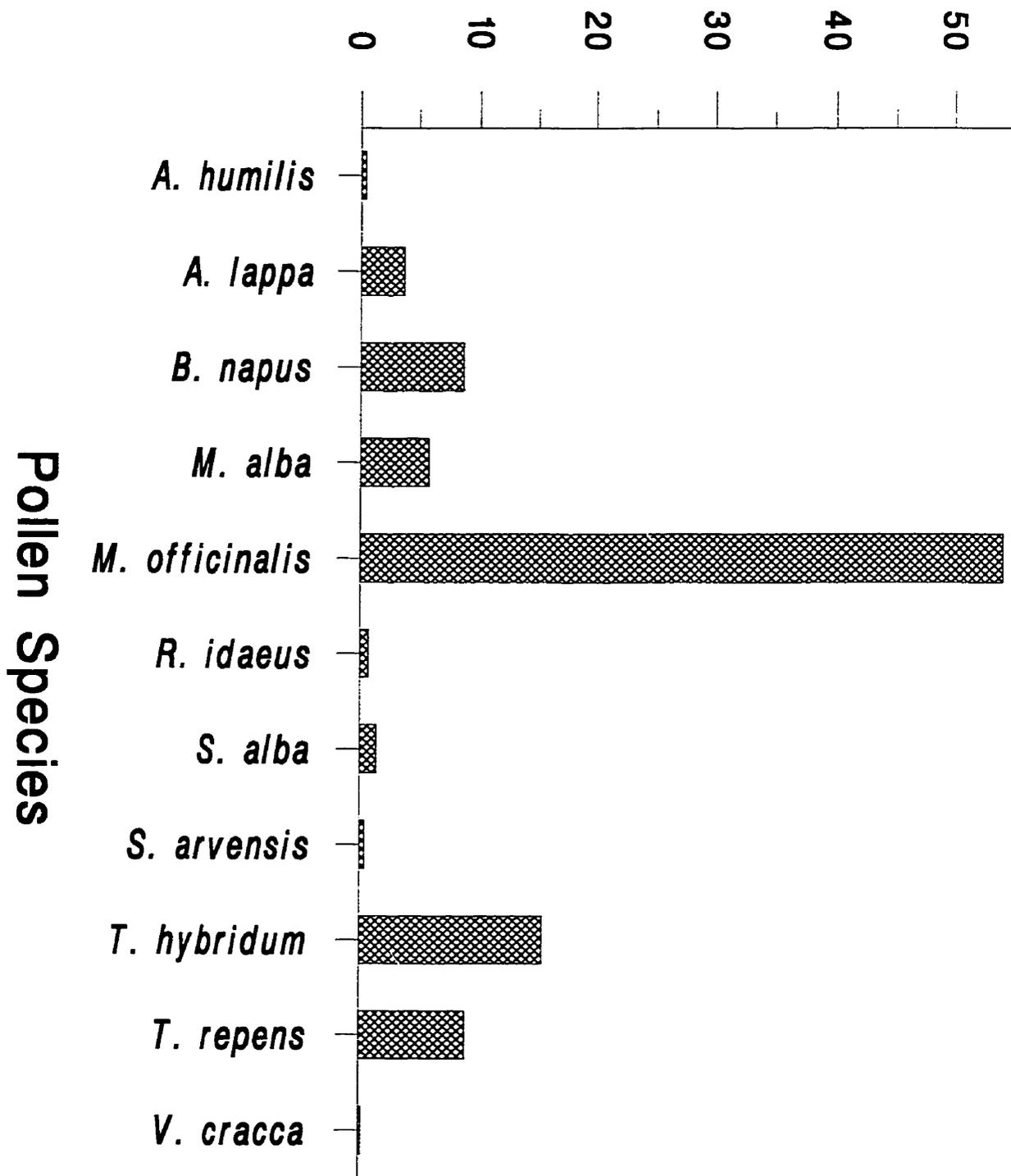


Fig. 29. Occurrences of species in pollen loads, as proportions of occurrences within morning (N=2247) and afternoon (N=3368) time periods. Only those species occurring at least 10 times, from all pollen loads examined, are plotted. (*B. napus* = *B. napus* + *B. rapa*).

Proportion of Occurrences in Pollen Loads (%)

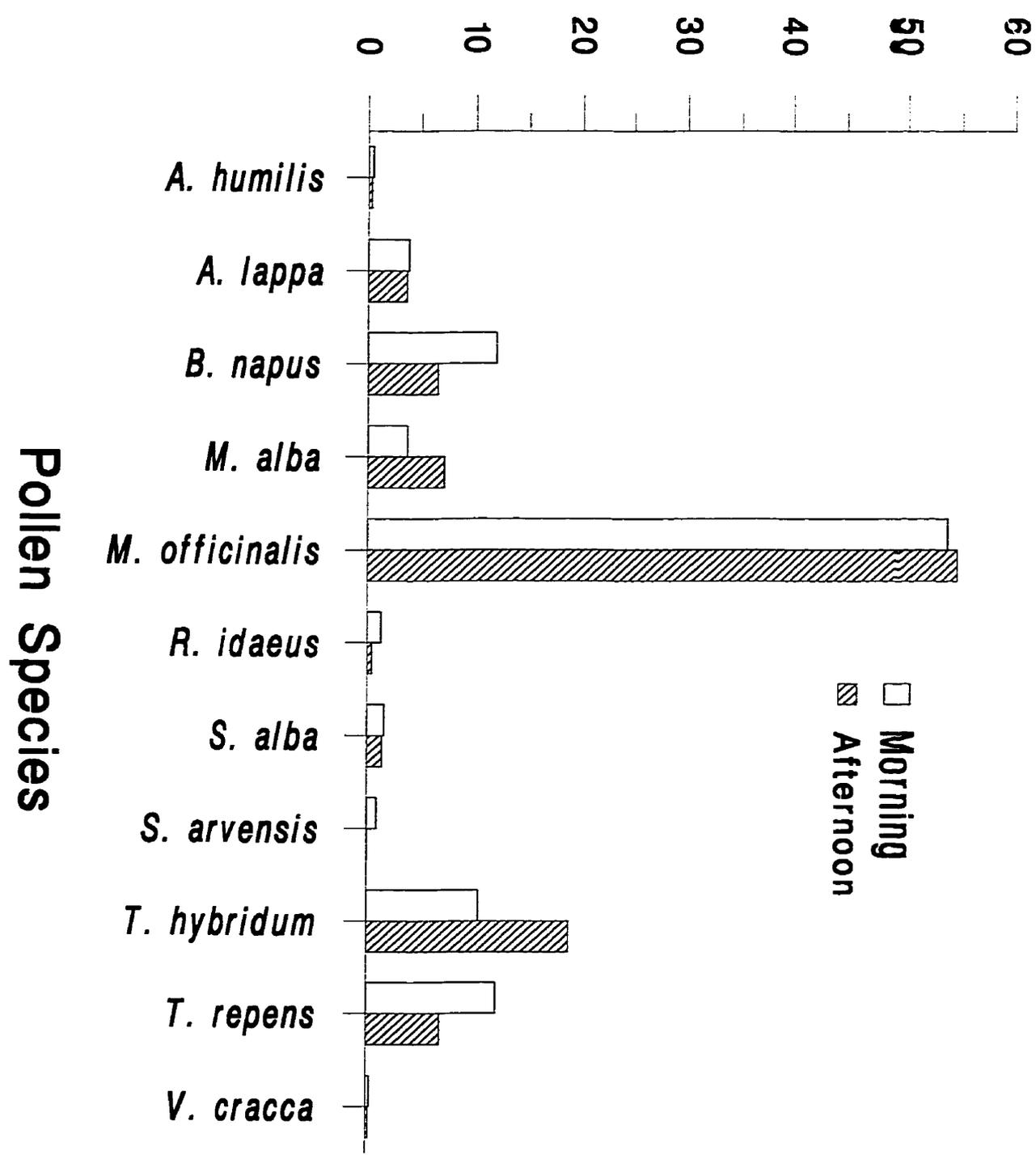
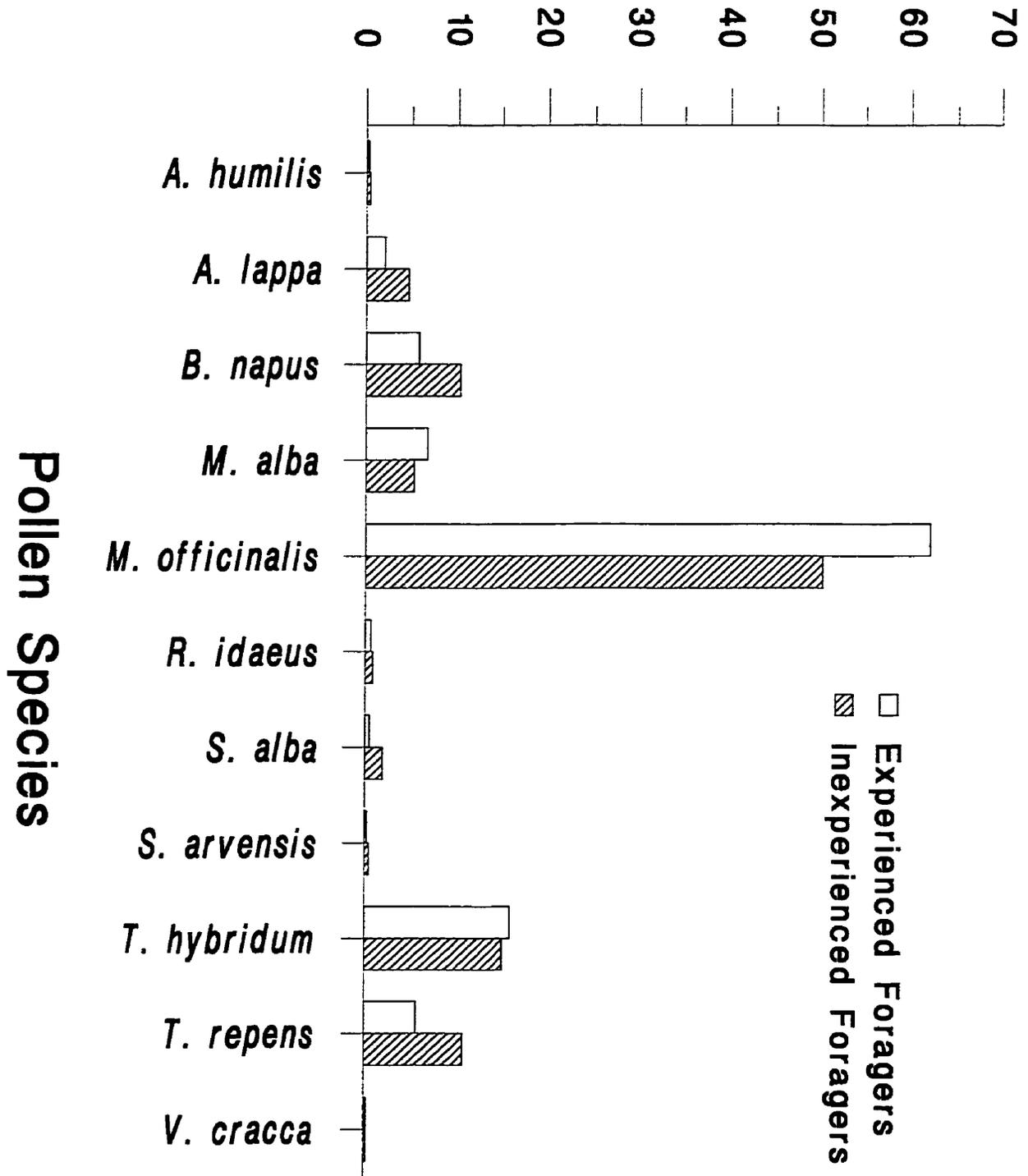


Fig. 30. Occurrences of species in pollen loads, as proportions of occurrences within experienced (N=1947) and inexperienced (N=3668) foragers. Only those species occurring at least 10 times, from all pollen loads examined, are plotted (*B. napus* = *B. napus* + *B. rapa*).

Proportion of Occurrences in Pollen Loads (%)



Appendix 1. Untransformed cell means (\pm SE) for parametric analyses not containing forager experience as a model term

Dependent variable analyzed	D a y	Time ^b	Quality manipulated treatments		Quantity manipulated treatments	
			High	Low	High	Low
Pollen foraging rate (pollen foragers \cdot min ⁻¹)	1	A.M.	3.90 \pm 1.15	3.60 \pm 0.90	2.54 \pm 1.05	3.38 \pm 0.89
	1	P.M.	8.42 \pm 1.31	9.73 \pm 0.35	7.56 \pm 1.01	12.70 \pm 1.86
	2	A.M.	2.86 \pm 0.60	3.27 \pm 0.59	3.67 \pm 0.82	4.05 \pm 0.57
	2	P.M.	8.25 \pm 1.50	9.08 \pm 1.44	9.34 \pm 1.37	13.43 \pm 1.92
	3	A.M.	4.05 \pm 0.62	5.01 \pm 1.10	3.50 \pm 0.82	4.03 \pm 0.73
	3	P.M.	8.88 \pm 1.30	11.64 \pm 1.50	9.23 \pm 1.02	12.52 \pm 1.79
Total foraging rate (nectar + pollen foragers \cdot min ⁻¹)	1	A.M.	17.09 \pm 3.08	16.43 \pm 2.34	13.69 \pm 2.60	18.93 \pm 2.59
	1	P.M.	34.38 \pm 2.29	34.21 \pm 3.13	33.35 \pm 2.72	43.26 \pm 3.80
	2	A.M.	16.92 \pm 2.07	17.01 \pm 2.13	16.62 \pm 2.18	20.88 \pm 2.42
	2	P.M.	30.85 \pm 3.43	33.25 \pm 3.69	36.98 \pm 3.79	38.49 \pm 4.14
	3	A.M.	18.27 \pm 2.34	18.43 \pm 2.66	16.55 \pm 2.19	21.16 \pm 2.73
	3	P.M.	33.53 \pm 3.32	36.96 \pm 2.26	36.59 \pm 1.73	40.43 \pm 3.26
Proportion of pollen foragers (%)	1	A.M.	21.49 \pm 3.73	19.69 \pm 2.73	14.28 \pm 3.00	18.23 \pm 3.11
	1	P.M.	24.43 \pm 3.04	28.13 \pm 2.46	22.41 \pm 1.91	28.89 \pm 2.60
	2	A.M.	16.84 \pm 1.96	18.93 \pm 2.52	21.04 \pm 3.05	19.59 \pm 1.96
	2	P.M.	25.16 \pm 2.24	27.07 \pm 2.68	25.77 \pm 2.49	34.21 \pm 2.14
	3	A.M.	21.52 \pm 1.94	23.37 \pm 3.79	18.44 \pm 3.35	18.42 \pm 2.58
	3	P.M.	25.79 \pm 2.48	30.43 \pm 2.62	24.76 \pm 2.07	29.84 \pm 2.69
Colony protein intake rate (mg \cdot min ⁻¹)	1	A.M.	6.33 \pm 1.88	5.52 \pm 1.24	4.39 \pm 2.18	5.02 \pm 1.33
	1	P.M.	19.45 \pm 3.77	19.94 \pm 2.79	14.38 \pm 1.65	29.49 \pm 5.79
	2	A.M.	4.11 \pm 0.77	4.60 \pm 0.69	4.75 \pm 1.00	6.45 \pm 1.10
	2	P.M.	19.39 \pm 3.32	20.93 \pm 3.39	19.66 \pm 3.62	29.70 \pm 4.60
	3	A.M.	7.36 \pm 1.56	8.70 \pm 2.38	6.18 \pm 1.47	6.31 \pm 1.51
	3	P.M.	20.02 \pm 3.11	28.36 \pm 3.75	21.11 \pm 2.64	31.23 \pm 6.08

Appendix 1. (continued). Untransformed cell means (\pm SE) for parametric analyses not containing forager experience as a model term

Dependent variable analyzed	D a y	Time ^b	Quality manipulated treatments		Quantity manipulated treatments	
			High	Low	High	Low
Consumption of diet supplements (g)	1	A.M.+P.M.	21.37 \pm 2.14	9.03 \pm 1.91	N/A ^c	N/A
	2	A.M.+P.M.	28.96 \pm 2.42	11.36 \pm 1.68	N/A	N/A
	3	A.M.+P.M.	23.58 \pm 2.60	9.28 \pm 1.13	N/A	N/A

^aDays after colony manipulation.

^bTime of day.

^cNon-applicable.

Appendix 2. Untransformed cell means (\pm SE) for parametric analyses containing forager experience as a model term

Dependent variable analyzed	D	a	Time	Quality manipulated treatments				Quantity manipulated treatments			
				High		Low		High		Low	
				Exp. ^c	Inexp. ^d	Exp.	Inexp.	Exp.	Inexp.	Exp.	Inexp.
Wet weight of pollen loads (mg)	1	A.M.	9.12 \pm 1.01	8.99 \pm 1.02	9.38 \pm 1.41	8.99 \pm 0.60	7.89 \pm 0.64	8.89 \pm 0.58	8.73 \pm 1.13	8.01 \pm 0.72	
	1	P.M.	10.21 \pm 0.71	13.73 \pm 1.03	9.57 \pm 0.55	12.55 \pm 0.65	10.05 \pm 0.74	11.15 \pm 0.83	10.09 \pm 0.89	13.05 \pm 1.00	
	2	A.M.	6.61 \pm 0.91	7.91 \pm 0.70	7.50 \pm 0.66	8.20 \pm 0.66	8.63 \pm 0.72	6.42 \pm 0.69	8.69 \pm 0.58	8.32 \pm 0.75	
	2	P.M.	10.72 \pm 1.26	14.14 \pm 0.60	11.83 \pm 0.81	12.97 \pm 0.85	9.97 \pm 0.71	11.75 \pm 0.88	11.11 \pm 0.71	12.90 \pm 0.49	
	3	A.M.	9.08 \pm 1.49	9.02 \pm 0.80	7.64 \pm 1.00	8.72 \pm 0.68	8.45 \pm 0.82	8.56 \pm 0.89	4.86 \pm 0.84	7.90 \pm 0.71	
	3	P.M.	9.65 \pm 0.78	14.14 \pm 0.95	11.59 \pm 0.74	13.67 \pm 0.73	10.48 \pm 0.68	13.35 \pm 0.66	10.07 \pm 0.76	13.50 \pm 0.96	
Protein content of pollen loads (% dry weight)	1	A.M.	22.30 \pm 0.42	21.79 \pm 0.42	22.85 \pm 0.39	22.54 \pm 0.37	22.19 \pm 0.18	22.14 \pm 0.28	22.18 \pm 0.28	22.36 \pm 0.37	
	1	P.M.	22.01 \pm 0.17	22.44 \pm 0.14	22.28 \pm 0.18	22.13 \pm 0.18	22.16 \pm 0.21	22.04 \pm 0.14	21.88 \pm 0.14	22.37 \pm 0.16	
	2	A.M.	22.32 \pm 0.25	22.62 \pm 0.26	21.96 \pm 0.17	22.65 \pm 0.17	22.68 \pm 0.40	22.53 \pm 0.26	21.78 \pm 0.56	22.45 \pm 0.25	
	2	P.M.	22.28 \pm 0.15	22.47 \pm 0.14	22.54 \pm 0.24	22.45 \pm 0.24	21.79 \pm 0.10	22.28 \pm 0.16	22.03 \pm 0.11	22.05 \pm 0.32	
	3	A.M.	21.74 \pm 0.36	21.68 \pm 0.35	22.28 \pm 0.28	22.69 \pm 0.32	22.32 \pm 0.39	22.83 \pm 0.16	21.85 \pm 0.40	22.59 \pm 0.22	
	3	P.M.	22.18 \pm 0.23	22.45 \pm 0.16	22.29 \pm 0.28	22.33 \pm 0.20	22.01 \pm 0.16	22.39 \pm 0.19	22.01 \pm 0.30	22.42 \pm 0.17	

Appendix 2 (continued). Untransformed cell means (\pm SE) for parametric analyses containing forager experience as a model term

Dependent variable analyzed	D a	Time ^b	Quality manipulated treatments				Quantity manipulated treatments			
			High		Low		High		Low	
			Exp. ^c	Inexp. ^d	Exp.	Inexp.	Exp.	Inexp.	Exp.	Inexp.
Gross protein content of pollen loads (mg)	1	A.M.	1.70 \pm 0.19	1.66 \pm 0.21	1.81 \pm 0.28	1.67 \pm 0.11	1.46 \pm 0.13	1.65 \pm 0.11	1.62 \pm 0.23	1.51 \pm 0.16
	1	P.M.	1.89 \pm 0.14	2.59 \pm 0.20	1.78 \pm 0.11	2.33 \pm 0.14	1.86 \pm 0.15	2.06 \pm 0.16	1.84 \pm 0.17	2.46 \pm 0.20
	2	A.M.	1.24 \pm 0.17	1.50 \pm 0.14	1.38 \pm 0.13	1.56 \pm 0.13	1.65 \pm 0.15	1.22 \pm 0.14	1.61 \pm 0.12	1.57 \pm 0.14
	2	P.M.	2.00 \pm 0.24	2.67 \pm 0.12	2.24 \pm 0.17	2.45 \pm 0.19	1.82 \pm 0.13	2.20 \pm 0.17	2.06 \pm 0.13	2.39 \pm 0.11
	3	A.M.	1.64 \pm 0.29	1.65 \pm 0.16	1.44 \pm 0.19	1.66 \pm 0.15	1.59 \pm 0.15	1.65 \pm 0.18	0.89 \pm 0.16	1.49 \pm 0.13
	3	P.M.	1.81 \pm 0.16	2.66 \pm 0.18	2.17 \pm 0.15	2.56 \pm 0.15	1.93 \pm 0.12	2.51 \pm 0.14	1.86 \pm 0.16	2.53 \pm 0.19
Pollen grain size (cross-sectional area, μm^2)	1	A.M.	460.8 \pm 20.6	542.5 \pm 62.9	448.8 \pm 6.5	565.0 \pm 82.9	441.6 \pm 8.7	454.4 \pm 10.6	495.1 \pm 30.2	518.2 \pm 38.7
	1	P.M.	461.1 \pm 17.1	480.9 \pm 17.7	454.1 \pm 15.4	486.1 \pm 18.5	447.7 \pm 6.2	444.1 \pm 5.9	469.2 \pm 20.5	463.1 \pm 10.7
	2	A.M.	443.3 \pm 6.7	521.6 \pm 33.0	475.4 \pm 23.4	507.0 \pm 27.9	449.4 \pm 7.9	459.5 \pm 7.4	521.2 \pm 76.3	466.6 \pm 12.6
	2	P.M.	435.3 \pm 5.5	470.7 \pm 16.5	457.7 \pm 12.6	486.5 \pm 30.9	439.0 \pm 10.1	478.7 \pm 16.7	447.0 \pm 13.6	522.4 \pm 45.8
	3	A.M.	490.1 \pm 47.7	573.6 \pm 37.3	449.6 \pm 6.4	500.0 \pm 39.0	448.5 \pm 9.2	462.7 \pm 9.4	474.3 \pm 38.6	469.5 \pm 10.9
	3	P.M.	442.1 \pm 10.1	496.6 \pm 26.7	462.6 \pm 24.1	492.9 \pm 23.6	434.9 \pm 4.9	476.1 \pm 20.7	469.7 \pm 25.1	468.4 \pm 16.1

Appendix 2 (continued). Untransformed cell means (\pm SE) for parametric analyses containing forager experience as a model term

Dependent variable analyzed	D a Y	Time ^b	Quality manipulated treatments		Quantity manipulated treatments	
			High	Low	High	Low
Floral species collected per colony						
Over treatments:			7.55 \pm 0.69	7.18 \pm 0.54	6.64 \pm 0.45	7.82 \pm 0.54
Over days within treatments:	1		5.36 \pm 0.41	5.64 \pm 0.41	4.73 \pm 0.43	6.00 \pm 0.43
	2		5.36 \pm 0.28	5.73 \pm 0.65	5.18 \pm 0.42	5.64 \pm 0.51
	3		6.09 \pm 0.49	5.55 \pm 0.39	5.36 \pm 0.24	5.27 \pm 0.38
Over time periods within days, treatments:	1	A.M.	3.82 \pm 0.40	4.18 \pm 0.52	3.55 \pm 0.61	4.82 \pm 0.33
	1	P.M.	4.64 \pm 0.47	4.81 \pm 0.35	3.82 \pm 0.40	4.64 \pm 0.41
	2	A.M.	3.45 \pm 0.39	4.36 \pm 0.66	2.91 \pm 0.55	4.27 \pm 0.56
	2	P.M.	4.64 \pm 0.36	4.73 \pm 0.54	4.73 \pm 0.24	4.36 \pm 0.24
	3	A.M.	4.64 \pm 0.61	3.73 \pm 0.45	3.82 \pm 0.42	3.45 \pm 0.39
	3	P.M.	4.09 \pm 0.25	4.91 \pm 0.31	4.55 \pm 0.28	4.18 \pm 0.38

Appendix 2 (continued). Untransformed cell means (\pm SE) for parametric analyses containing forager experience as a model term

Dependent variable analyzed	D a y	Time ^b	Quality manipulated treatments				Quantity manipulated treatments			
			High		Low		High		Low	
			Exp. ^c	Inexp. ^d	Exp.	Inexp.	Exp.	Inexp.	Exp.	Inexp.
Floral species collected per colony	1	A.M.	2.11 \pm 0.39	3.45 \pm 0.37	2.18 \pm 0.50	3.64 \pm 0.43	2.00 \pm 0.30	3.60 \pm 0.62	3.09 \pm 0.25	3.82 \pm 0.35
	1	P.M.	3.27 \pm 0.43	4.00 \pm 0.43	3.55 \pm 0.25	3.91 \pm 0.41	2.82 \pm 0.35	3.18 \pm 0.38	2.80 \pm 0.20	4.00 \pm 0.47
Over forager experience within time periods, day, treatments:	2	A.M.	2.29 \pm 0.29	3.36 \pm 0.36	2.20 \pm 0.39	4.09 \pm 0.67	1.56 \pm 0.18	2.64 \pm 0.49	2.63 \pm 0.53	3.36 \pm 0.31
	2	P.M.	2.50 \pm 0.27	4.45 \pm 0.37	3.09 \pm 0.21	4.18 \pm 0.58	2.82 \pm 0.26	4.00 \pm 0.38	2.91 \pm 0.28	3.73 \pm 0.36
	3	A.M.	2.00 \pm 0.33	4.45 \pm 0.59	2.20 \pm 0.44	3.18 \pm 0.40	2.25 \pm 0.45	3.45 \pm 0.34	1.75 \pm 0.41	3.18 \pm 0.38
	3	P.M.	2.91 \pm 0.21	3.64 \pm 0.31	3.27 \pm 0.36	4.18 \pm 0.30	2.64 \pm 0.24	4.36 \pm 0.31	2.50 \pm 0.43	3.64 \pm 0.36
	3	P.M.	2.91 \pm 0.21	3.64 \pm 0.31	3.27 \pm 0.36	4.18 \pm 0.30	2.64 \pm 0.24	4.36 \pm 0.31	2.50 \pm 0.43	3.64 \pm 0.36
Floral species collected per forager	1	A.M.	1.00 \pm 0.00	1.10 \pm 0.04	1.02 \pm 0.01	1.01 \pm 0.01	1.00 \pm 0.00	1.01 \pm 0.01	1.22 \pm 0.12	1.01 \pm 0.01
	1	P.M.	1.03 \pm 0.02	1.02 \pm 0.01	1.02 \pm 0.01	1.04 \pm 0.02	1.03 \pm 0.02	1.03 \pm 0.02	1.01 \pm 0.01	1.03 \pm 0.02
	2	A.M.	1.05 \pm 0.05	1.01 \pm 0.01	1.01 \pm 0.01	1.03 \pm 0.02	1.00 \pm 0.00	1.01 \pm 0.01	1.13 \pm 0.13	1.01 \pm 0.01
	2	P.M.	1.03 \pm 0.02	1.07 \pm 0.04	1.01 \pm 0.01	1.05 \pm 0.02	1.02 \pm 0.01	1.02 \pm 0.02	1.00 \pm 0.00	1.06 \pm 0.04
	3	A.M.	1.10 \pm 0.06	1.02 \pm 0.01	1.06 \pm 0.04	1.03 \pm 0.02	1.03 \pm 0.02	1.04 \pm 0.02	1.00 \pm 0.00	1.02 \pm 0.01
	3	P.M.	1.03 \pm 0.02	1.01 \pm 0.01	1.02 \pm 0.01	1.03 \pm 0.02	1.02 \pm 0.01	1.05 \pm 0.03	1.02 \pm 0.02	1.05 \pm 0.02

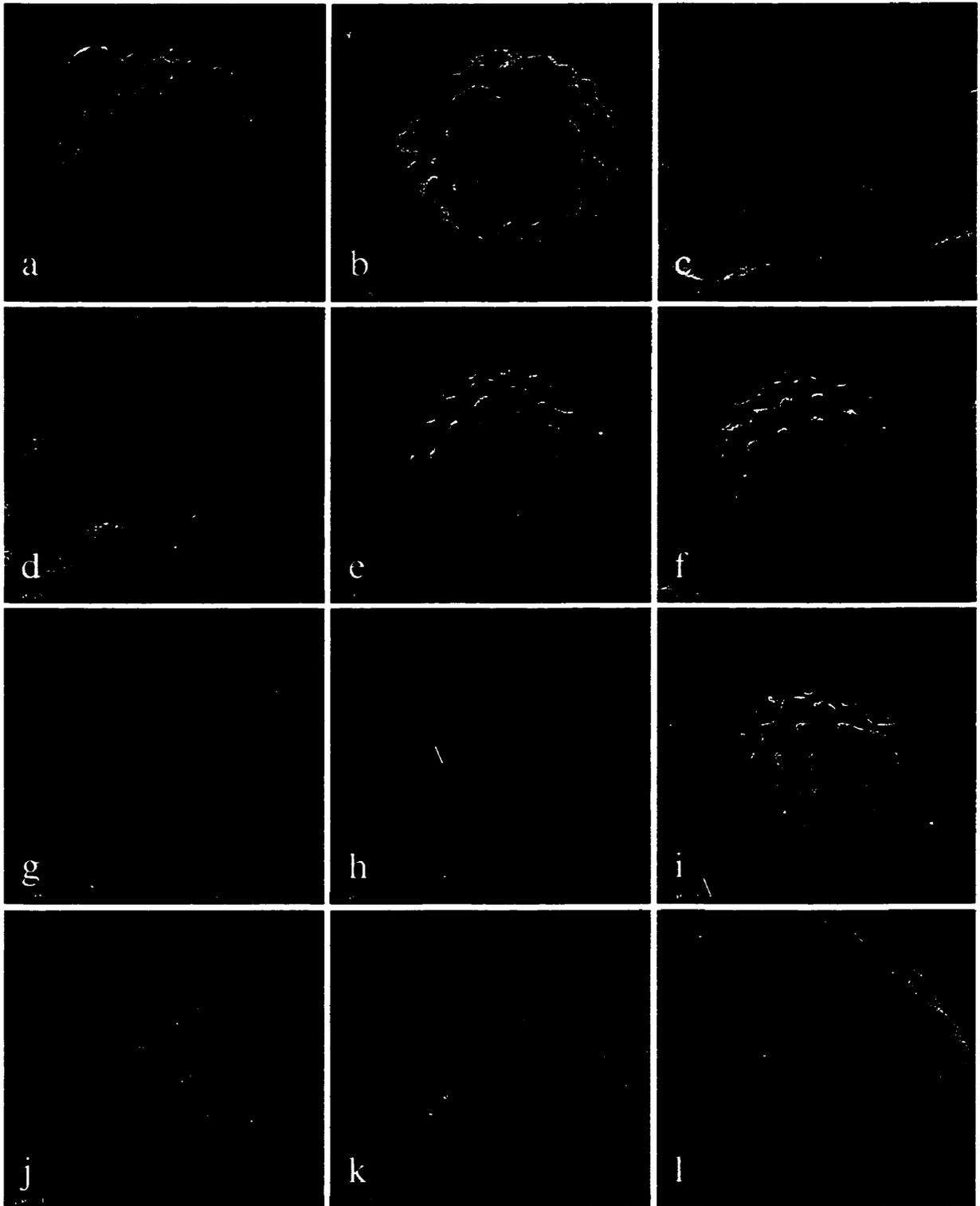
^aDays after colony manipulation.

^bTime of day.

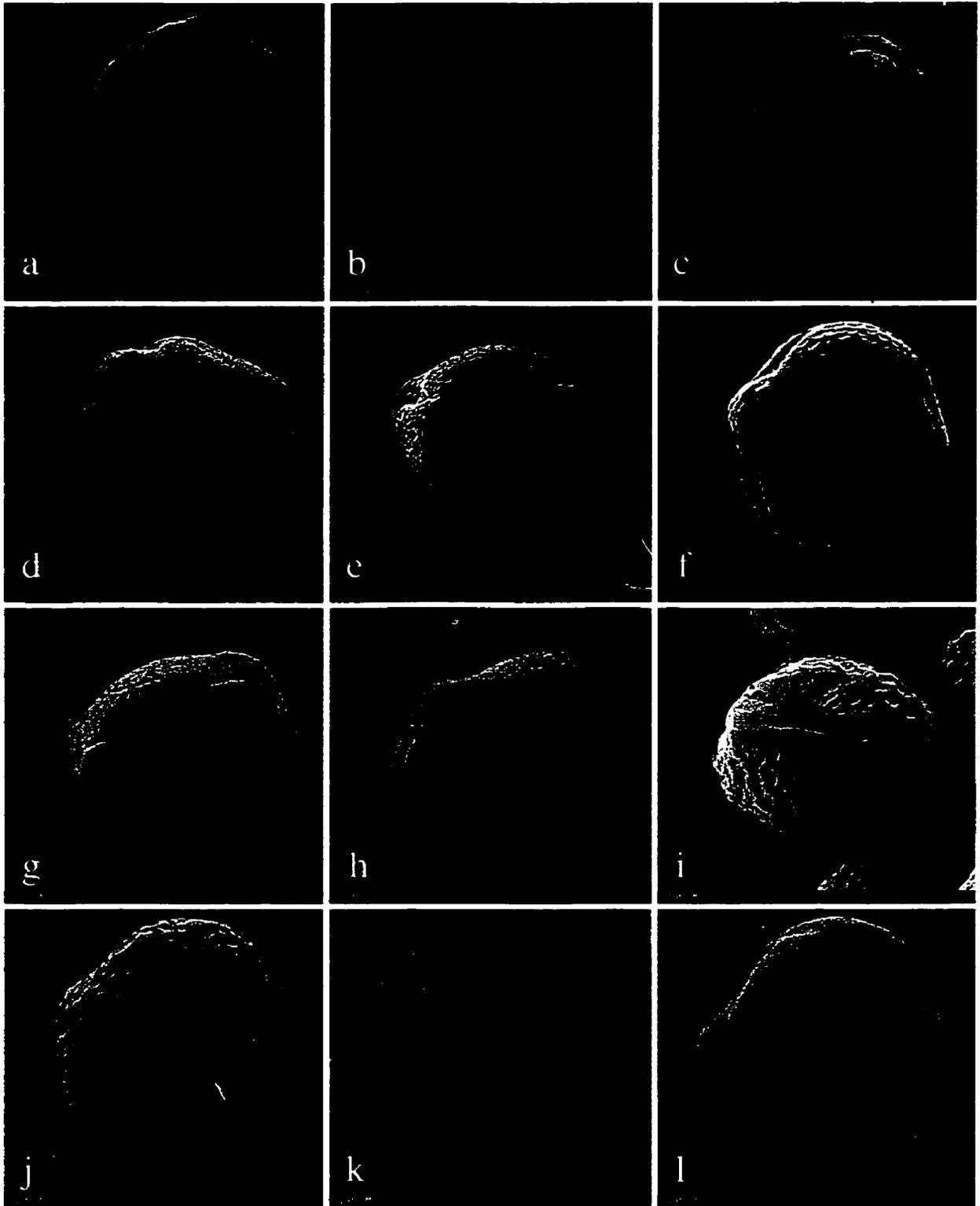
^cExperienced foragers.

^dInexperienced foragers.

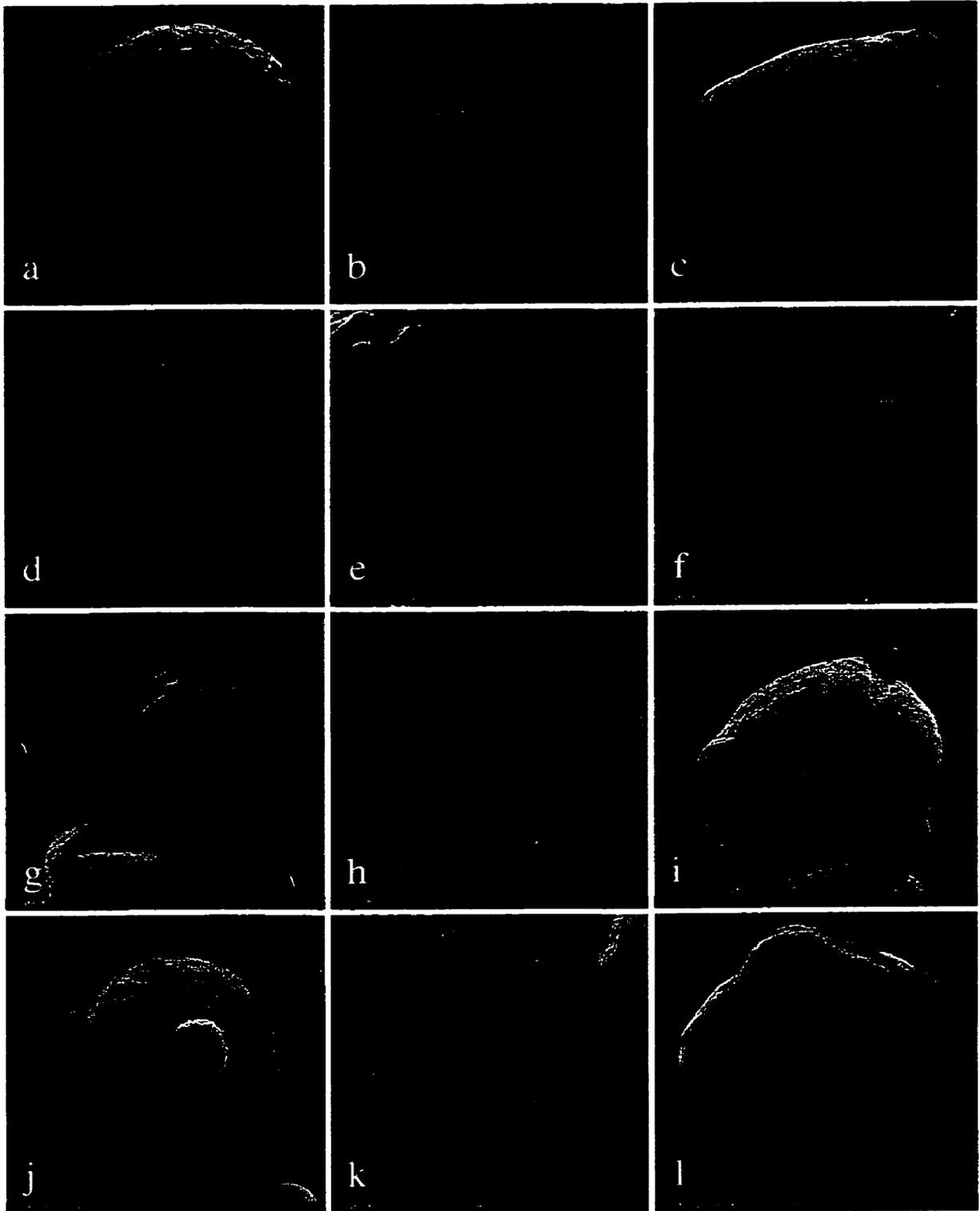
Appendix 3. Plate 1. Scanning electron micrographs of selected pollen species collected by foragers during this study: a-b, *A. lappa* (1400×); c, *A. lappa* (8600×); d, *Aster* spp. #1 (673×); e-f, *Aster* spp. #1 (2800×); g, *Aster* spp. #1 (9000×); h, *Aster* spp. #2 (673×); i-j, *Aster* spp. #2 (2800×); k, *Aster* spp. #2 (9000×); l, *C. arvense* (1400×).



Appendix 3. Plate 2. Scanning electron micrographs of selected pollen species collected by foragers during this study (continued): a, *C. arvense* (1400×); b, *C. arvense* (5820×); c-d, *V. angustifolium* (1800×); e-f, *M. alba* (2800×); g-h, *M. officinalis* (2800×); i-j, *T. hybridum* (2800×); k-l, *T. repens* L. (2800×).



Appendix 3. Plate 3. Scanning electron micrographs of selected pollen species collected by foragers during this study (continued): a, *V. cracca* (2850×); b, *V. cracca* (1400×); c-d, *A. humilis* (2800×); e, *A. humilis* (14000×); f-g, *P. fruticosa* L. ssp. *floribunda* (2800×); h, *P. fruticosa* ssp. *floribunda* (11600×); i-j, *R. acicularis* (1800×); k-l, *R. idaeus* L. var. *strigosus* (2800×).



CHAPTER V

Discrimination and Preferences for Pollen-Based Factors by Foraging Honey Bees

(Apis mellifera L.)

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Discrimination and Preferences for Pollen-Based Factors by Foraging Honey Bees

(Apis mellifera L.)

Abstract

Pollen-based factors influencing the pollen foraging decisions made by honey bees were evaluated using two-choice bioassays. Experiments were conducted under highly controlled conditions, inside a honey bee flight and rearing room. Behaviours related to the choice and collection of pollen by foragers were examined among pollens from six floral species as well as for cellulose powder, soy flour and a commercially-prepared pollen substitute. First, responses of honey bees to the odour produced by pollen (or pollen analogues) and their lipid extracts were examined. Honey bees displayed preferences for all pollens over pollen analogues, with a similar pattern of response exhibited to their extracts. Changes in forager behaviour in response to variations in particle size were also evaluated, using soybean meal that was ground and sifted to achieve a series of particle size classes. Bees preferred particle sizes below 150 μm , but greatest response was exhibited for those particle sizes below 45 μm . The effect of varying protein content on the foraging decisions made by bees were also assayed by mixing soy flour with different proportions of cellulose powder. Foragers, however, were incapable of discriminating protein content. Changes in the response of foragers to different levels of handling time were determined using different sized screens through which bees were forced to crawl to reach an attractive pollen odour source. In these tests, pollen-seeking behaviours were seen to decrease with increases in handling time. When odour was presented simultaneously with other stimuli, it was the primary and overriding

cue used by bees to select pollen. These results suggest that honey bee pollen foragers do not discriminate among food sources based on differences in quality, but instead evaluate factors that may increase their efficiency of collection and recruitment to such a food resource.

Introduction

The survival of a honey bee colony is vitally linked to its ability to collect sufficient quantities of food to rear brood and maintain adults. Pollen is the only protein source naturally available to honey bees, and it also supplies other dietary requirements including lipids, vitamins and minerals. Colonies collect up to 55 kg of pollen per year (Winston 1987), a vast amount considering the average amount collected per foraging trip typically varies between 10 - 20 mg (Maurizio 1953, Winston 1987, Pernal 2000b). Bees collect pollen from a wide variety of floral sources and have distinct preferences for some pollen types over others, as demonstrated in natural settings (Linsley and McSwain 1947, Bohart 1957, Nye and Mackensen 1965, Olsen et al. 1979, Jay and Jay 1984, Free 1993) or by controlled choice experiments (Levin and Bohart 1955, Doull 1966, Wahl 1966, Boch 1982, Schmidt 1982, Boelter and Wilson 1984). Although it is clear that bees possess the ability to discriminate among pollen types, the way in which they utilize pollen-based cues are poorly understood.

Honey bees primarily rely on visual and olfactory stimuli to locate flowers and their rewards (Butler 1951, von Frisch 1967, Menzel et al. 1997, Backhaus 1993). Foragers have innate abilities for discriminating colour and retain certain colour cues more effectively than others (zu Oettingen-Spielberg 1949, von Frisch 1967, Menzel 1990). In

association with alternating nectar rewards, learned colour associations have been widely used to study foraging decisions made by bees (Jones 1978; Waddington and Holden 1979; Wells and Wells 1984, 1986; Giurfa and Núñez 1989; Wells et al. 1992; Banschbach 1994; Giurfa et al. 1994). Also important as visual stimuli are floral shape or form (Free 1970; Anderson 1977a, c; Wehner 1981; Gould 1985; Lamb and Wells 1995), pigmentation patterns (Anderson 1977b, Wehner 1981, Gould 1986a, Lehrer 1991, Petrikin and Wells 1995) and floral symmetry (Free 1970, Møller and Eriksson 1995, Giurfa et al. 1996a, West and Lavery 1998). The presence of nectar guides, stamens, and flower petal microtexture are additional cues used by bees to locate floral rewards when they are in close proximity to flowers, or after they have alighted (Manning 1956, Free 1970, Jones and Buchmann 1974, Kevan 1978, Barth 1985, Kevan and Lane 1985, Lunau 1991).

Although colour is the main stimulus used by bees to locate flowers at a distance, odour is also used in flower selection (DeGrandi-Hoffman 1987; Beker et al. 1989; Pham-Delègue et al. 1989, Masson et al. 1993, Kirchner and Grasser 1998). The use of odour is most important during close-range orientation when bees inspect flowers both before and after alighting (von Frisch 1950, Butler 1951, Manning 1957, Galen and Kevan 1980, Zimmerman 1982, Dobson 1991, Lunau 1991). This inspection allows bees to discriminate between floral species, decide whether or not to land, and may influence their expression of discrete foraging behaviours (McNaughton and Harper 1960; Dobson 1991, 1994; Dobson et al. 1996). Overall floral odour is the result of compounds produced from several structures including the petals, sepals, gynoecium, anthers and pollen (Dobson et al. 1990). However in many insect-pollinated plants, the odour of

pollen is described as being distinct from, and more intense than, the remaining floral odour (von Frisch 1923, von Aufsess 1960, Porsch 1954, 1956, Buchmann 1983). Such observations are further substantiated by qualitative differences in the profiles of volatiles produced by whole-flowers and pollen (Dobson et al. 1987, 1990, 1996; Bergström et al. 1995); within pollen, odour-producing compounds are associated with the oily pollenkitt layer surrounding each grain (Dobson 1988). Honey bees possess the ability to discriminate between the odour of pollen and that of other floral volatiles, and can be trained to collect pollen based its odour alone, even in the absence of supplementary dance information (von Frisch 1923, von Aufsess 1960). Furthermore, the selection of food substances that have little or no nutritional value by bees may be stimulated by the addition of pollen lipid odour components (Taber 1963, Hohmann 1970, Starrat and Boch 1971). Therefore, there is little doubt that pollen odour functions as a distinct and important cue for honey bee foragers.

The nutritional value of pollen, as measured by crude protein content, is another potential cue that honey bee foragers may assess. Although honey bee colonies are known to regulate their collection of pollen to meet their demand for protein (Camazine 1993, Seeley 1995, Pernal 2000b), the question of whether individual foragers can perceive the nutritional value of the pollen they are collecting has never been directly tested. The inability of foragers to assess pollen protein is supported by observational accounts of bees collecting pollen or pollen-like substances with little or no nutritive value (Shaw 1990), as well as choice tests in which foragers were allowed to select among pollens having varying protein contents (Levin and Bohart 1955, Wahl 1966, Schmidt 1982). This apparent lack of discriminatory ability is also consistent with my

previous findings showing that honey bee colonies respond to demands for protein by changing the composition and dynamics of their foraging force, rather than by any changes in the quality of pollen collected by individual foragers (Pernal 2000b).

However, some studies that have analyzed the nutritional value of pollen being collected by foragers, or have observed recruitment dances to different qualities of food, conclude that foragers can assess protein content independently of the colony (Rasheed and Harder 1997a, Waddington et al. 1998) and select pollen species in relation to colony need (Fewell and Winston 1992). Hence, the mechanisms of perception of pollen quality by honey bee foragers are in need of clarification, and may have important ramifications for our understanding of the control of pollen foraging.

The influence that other pollen-based factors may have on the foraging decisions made by *A. mellifera* is even less well known. Theoretically, the size of pollen grains could function as a cue for foragers to elicit pollen-collecting or packing behaviours. Angiosperm pollen grains range in volume over five orders of magnitude (Harder 1998), and their size has been suggested to influence the evolution of pollen gathering hairs in certain bee species (Roberts and Vallespir 1978). The size of grains may affect the ease or efficiency of its collection, or be related to the proportion of certain nutritional factors present (Baker and Baker 1979, Simpson and Neff 1983). The time necessary for a forager to work a flower and extract pollen (“handling time”), may also influence its decision-making process. Foragers incur higher costs as floral architecture becomes more complex (Heinrich 1979; Lavery 1980, 1994a), and the perception of this complexity via the handling time incurred could be used as a criterion for bees to continue pollen-seeking or collection behaviours. Once pollen is being actively collected, a honey bee’s ability to

successfully groom pollen and pack it on her corbiculae can be influenced by its moisture content (van Praagh and Brinkschmidt 1987), the amount of pollenkitt surrounding grains (Stanley and Linskens 1974), the external morphological features of pollen grains (Vaissière and Vinson 1994) and their associated electrostatic charges (Erickson and Buchmann 1983, Chaloner 1986). Finally, factors such as the pH or age of pollen may also be criteria for its acceptability to bees (Stanley and Linskens 1974, Schmidt 1982, Schmidt and Johnson 1984).

There is a dearth of information on the importance of pollen-based cues and handling time for honey bee foragers. Previous investigations that have examined the influence of pollen-based cues on forager behaviour have allowed honey bees to choose from an array of pollen sources, and then have associated properties of the pollens with the preferences exhibited by foragers (Levin and Bohart 1955, Wahl 1966, Boch 1982, Schmidt 1982). Although these attempts have given us some insight into the importance of pollen odour, they are largely confounded by the presence of uncontrolled co-occurring stimuli.

The objective of this study was to evaluate the importance of pollen odour, pollen lipid odour, particle size and protein content as cues for honey bee foragers, and to evaluate the effect of handling time on the pollen collection behaviours elicited by individual foragers. I also determined whether qualitative differences in pollen odour produced by different species of pollen affected forager response. Finally, by presenting discrete, simultaneous combinations of cues and handling times, I attempted to deduce the hierarchy of stimuli used in the decision-making processes of foragers while collecting pollen, and determine competing effects between them.

To accomplish these objectives I manipulated individual pollen-based factors at

several levels. Efforts were made to minimize the effects of co-occurring cues during trials, through the use of artificial substances as pollen surrogates. In addition, all experiments were conducted in a specially-designed flight and rearing room for honey bees (Pernal 2000c) to negate the effect of confounding environmental stimuli and provide identical conditions for all bioassays. Furthermore, the high volume of air exchange present in the bee flight room prevented odour contamination between successive trials.

Materials and Methods

Pollen Collection

Pollen was collected from blooming trees and field crops using honey bee (*Apis mellifera* L.) colonies housed in standard Langstroth hive bodies and fitted with O.A.C. pollen traps (Smith and Adie 1963). Four colonies were placed in each isolated plot of the following species: *Malus domestica* Borkh. (mixed var.) (apple), *Brassica campestris* L. var. 'Goldrush' (oilseed rape), *Phacelia tanacetifolia* L. var. 'Angelia' (phacelia), *Melilotus officinalis* (L.) Pall. var. 'Norgold' (yellow sweetclover), and *Helianthus annuus* L. var. 'Sigco 954' (sunflower). Pine pollen was obtained by collecting male cones from pine trees, *Pinus banksiana* (Lamb.) (jack pine), drying them at 35° C for 1 d, and then shaking the dried cones. *Malus* pollen was collected from the orchards of the Agriculture and Agri-Food Canada Research Centre in Morden, Manitoba. Pollen from field crops was collected at the University of Manitoba Glenlea Research Station or on co-operating producers' farms in southern Manitoba. *Pinus* pollen was collected from the Sandilands Provincial Forest in eastern Manitoba.

The following protocol was observed for the collection and handling of pollen to preserve the integrity of external pollen lipids. Surfaces of O.A.C. pollen traps were rinsed with n-pentane, and the pollen collection trays of the traps were lined with aluminum foil, to ensure that pollen only came into contact with lipid-free surfaces. All laboratory tools and surfaces used for the handling and sorting of pollen were also carefully cleaned to be devoid of lipid and care was taken to avoid contacting pollen by hand. Pollen was separated from non-pollen debris and corbicular loads were sorted on the basis of pollen load colour (Hodges 1984, Kirk 1994). Samples of pollen from each colour cohort were mounted in glycerine jelly (Fægri et al. 1989) and examined under a compound microscope at 400× (Photomicroscope II, Carl Zeiss, Germany) to confirm the identity of pollen species (Crompton and Wojtas 1993). Pollen that was not immediately used for bioassay purposes was prepared for short-term storage by placing it in glass vials (26 by 60 mm, 23 mL). To minimize oxidation and desiccation of pollen during storage, a stream of gaseous nitrogen was discharged into the vials after they were filled with pollen, to displace any air inside. Vials were then immediately sealed with teflon-lined screw-caps and were kept frozen at -30° C.

Pollen Analogues

The pollen analogues used in bioassays were substances that possessed physical properties similar to that of pollen, but did not contain any pollen. These included: Bee-Pro[®] (Mann Lake Supply, Hackensack, MN, USA), a commercially-prepared pollen substitute diet for honey bees; alphacel non-nutritive bulk, a finely ground cellulose product (ICN Biomedicals Canada Ltd., Montreal, PQ, Canada, Catalogue No. 900453);

high protein soybean meal (Archer Daniels Midland Co., Decatur, IL, USA, 48.2 % crude protein, 3.7 % lipid); defatted soy flour (Cenex Harvest States Cooperatives, Mankato, MN, USA, 53.6 % crude protein, 0.93 % lipid) and spruce wood sawdust. Soybean meal and soy flour were extracted with CHCl_3 -MeOH (2:1, v/v) (Folch et al. 1957) at room temperature, to remove any lipids present. Spruce wood sawdust was sieved to exclude all particles greater than 150 μm and less than 75 μm . It was then triple-extracted in hexane and bleached in 6% NaOCl for 1 hr. Afterwards, the sawdust was washed in large volumes of water, dried at room temperature and re-sieved.

Maintenance and Training of Bees

Colonies of honey bees (*A. mellifera*), propagated from New Zealand queens, were maintained at the University of Manitoba apiary in Winnipeg. These served as a pool of available colonies to use in experiments. Bioassays were performed using single colonies situated inside a specially designed honey bee flight and rearing room at the University of Manitoba (Pernal 2000c). The flight room contained an inner cage in which the bees were allowed to fly freely (3.96 m \times 1.91 m \times 2.01 m high), with their colony located at one end and a feeding station at the other. The feeding station consisted of a small rectangular table (76 cm \times 30 cm \times 79 cm high) which provided a platform for a centrally-located sugar syrup feeder; protein feeders were located at each end. Water was provided in a 250 mL dish containing a small sponge, located between the syrup feeder and one of the protein feeders. The syrup feeder, known as a “boardman feeder” (Morse and Flottum 1990), was filled with 2 M sucrose solution. It was comprised of an inverted 1-quart canning jar (0.946 L), having its neck held in a wooden block, with small holes

punched through a metal lid allowing the solution to be dispensed. The protein feeders consisted of the bottoms of 9 cm glass petri dishes filled with pollen substitute, placed on a piece of bright yellow bristol board (15 cm × 15 cm). A larger piece of the same board (21.6 cm × 27.9 cm) had a thick black triangle drawn on it. This was hung below the syrup feeder to serve as an orientation cue for foragers. Protein feeders were provided daily between 0900 and 1600 hrs, and sugar syrup and water were fed *ad libitum*.

A colony that was moved from the apiary to the flight room was reduced to three frames of brood, approximately 6,000 workers, and a queen. The colony was also provided with two empty frames of light-coloured comb for workers to store sugar syrup collected from the feeder. When full, these frames were replaced so space available for egg-laying by the queen was not restricted. Care was taken to ensure that none of the comb placed into the flight room colony contained stored pollen, so that the foraging choices made by honey bees would not be influenced by the type of pollen consumed from the colony's stores. After introduction to the flight room, bees were allowed two weeks to become familiar with their indoor surroundings and train to their sources of food, without the use of artificial odours. This period also allowed for a turnover in the forager population (Winston 1987, Wolf and Schmid-Hempel 1989, Dukas and Visscher 1994, Visscher and Dukas 1997), so that foragers at the beginning of experimental trials would have no foraging experience outside of the flight room. This prevented foragers from making choices among pollen sources after being preconditioning to previously collected flora (Louveaux 1954; Free 1958, 1963).

To ensure that the demand for pollen remained high throughout the experiment, frames containing eggs and young larvae were selected from colonies in the apiary to

supplement brood levels in the experimental colony. I observed that brood rearing occasionally declined during periods of continuous bioassay testing. When brood rearing declined, colonies were supplemented with pollen substitute within the colony as a readily available source of protein for nurse bees to consume. To do this, 500 g of pollen substitute (Bee-Pro[®]) was mixed with sugar syrup until a moist, kneadable texture was obtained, then flattened between wax paper sheets and placed on the top bars of the frames.

For bioassays of whole pollen odour, lipid extract and particle size, individual colonies were maintained in the flight room for several weeks. A colony was replaced after completion of one of the two sets of trials performed for each of the previously-listed factors. For evaluating the effects of protein content, handling time, and the interaction of multiple factors, one colony was used for all sets of trials within a factor, but was replaced between factors.

Common Bioassay Protocol

Flight Room Conditions. The honey bee flight room was a self-contained environmental chamber with its own heating and cooling system. It was programmed to simulate a diurnal cycle, typical of summer conditions in Manitoba. Daytime temperature was maintained at 25.0 ± 0.1 ° C and nighttime temperature was set to 15.0 ± 0.1 ° C. The heating and cooling system moved and exchanged large volumes of air, in a well-diffused circulation, thereby preventing the concentration of odours inside the room. Every minute, 28.6 m^3 of air was circulated, with 7.4 m^3 being exhausted and replaced with fresh air during the same interval. Relative humidity was maintained

between 45-55%.

Each “day” in the flight room consisted of 16 h of light and 8 h of darkness. Artificial crepuscular periods were created by the use of electromechanical timers, each of which cycled one third of the lights on or off, in 30 min increments. Light was supplied by fluorescent lamps that produced light in the ultraviolet (UV) and visible light spectrum. The light available to honey bees in the flight room was measured using a UV actinic radiometer (International Light Inc., Model # IL 730A, Newburyport, MA, USA). The radiometer was used in conjunction with one of two detectors: a visible light detector fitted with filters having absorbance maxima at 450 and 550 nm, or a UV A detector having an absorbance maximum at 360 nm. Measuring irradiance at these wavelengths provided information about the quantity of light actually available to honey bee visual receptors (Autrum and von Zwehl 1964, Menzel and Blakers 1976, Menzel et al. 1986, Menzel and Backhaus 1989). Irradiance in the centre of the flight room, at 1.5 m above the floor, was: $77.9 \mu\text{W}\cdot\text{cm}^{-2}$ (360 nm), $10.4 \mu\text{W}\cdot\text{cm}^{-2}$ (450 nm), and $9.1 \mu\text{W}\cdot\text{cm}^{-2}$ (550 nm).

Experimental Trials. Bioassays were conducted during mid-morning and mid-afternoon periods when colony flight activity was greatest. Protein and sucrose feeders were removed from the flight cage and two bioassay dishes were placed on opposite ends of the feeding station (61 cm apart, on centre), their positions being randomized between trials. Observers were located outside the cage, behind the feeding station. Individual trials were performed for a duration of 20 min, during which time foragers were continuously monitored. For individual bees, only the highest order behaviour from each series of hierarchical behaviours was recorded as it responded to a dish. Trials having 5

or fewer recorded behaviours were disqualified for having insufficient forager activity, and were repeated when colony flight activity was greater. No attempt was made to control the recruitment of additional foragers by those bees having already visited bioassay dishes.

An individual trial consisted of a single, pairwise comparison between factor levels. Factors that were tested included pollen odour and pollen lipid odour, the particle size and protein content of pollen surrogates, and floral handling time. Respective factor levels consisted of different species of pollen, size classes of particles, concentrations of protein or degrees of simulated floral complexity. A set of trials was complete when all possible pairwise comparisons between factor levels had been performed, excluding comparisons containing the same factor levels.

Samples of pollen, pollen analogues and pollen lipid extracts were used for single trials only, and were then discarded. All bioassay apparatus was replaced with clean equipment in between trials. Bioassay dishes and screens were thoroughly washed with soap and water, then rinsed with distilled water and acetone and allowed to dry. They were then rinsed with n-pentane and placed overnight in an oven set at 125° C.

Whole Pollen Odour Bioassay

The behaviour of pollen foragers in response to the odour produced by competing sources of intact pollen was evaluated. Apple, canola, phacelia, yellow sweetclover, sunflower and jack pine pollens were tested, in addition to three pollen analogues: Bee-Pro[®], alpha-cellulose fiber and defatted soy flour. Before using the hand-collected jack pine pollen or pollen analogues in bioassays, a quantity of sugar, which was equivalent to

the amount normally added by bees in the process of collecting pollen (30% sucrose, w/w) (Todd and Bretherick 1942; McLellan 1977; Solberg and Remedios 1980; Schmidt and Buchmann 1986), was added to them. Bee-collected pollen was ground in a mortar and pestle prior to placing it in bioassay dishes. Bioassay dishes were made from round glass crystallizing dishes (70 mm diameter × 50 mm height) (Pyrex® #3140), fitted with screens (Fig. 31). A coarse galvanized metal screen that readily permitted bee entry (9.5 mm openings) was placed on top of the dish, and a screen with smaller openings (3.2 mm) was fixed in position 2.5 cm from the bottom. The upper surface of the lower screen was covered in a double layer of cheesecloth, thereby preventing bees from seeing or touching the 500 mg of pollen in the bottom of the dish. The exterior surface of the bioassay dish was wrapped in black paper.

The following sequence of hierarchical behaviours, from initial to penultimate, was scored during trials: 1. Approaching - Bees flying within 5 cm of the bioassay dish, but not landing; 2. Landing - Bees landing on the upper screen or the exterior surfaces of the dish; 3. Crawling Under - Bees crawling under the upper screen and transferring onto the inside walls of the bioassay dish or the surface of the cheesecloth. Two complete sets of unique pairwise comparisons of the nine pollen species (or analogues), consisting of a total of 72 individual trials, were performed.

Pollen Lipid Extract Bioassay

The behaviour of pollen foragers in response to the odours produced by competing pollen lipid extracts was evaluated. The types of pollen and pollen analogues used, as well as their treatment, were the same as described for the whole pollen odour bioassay.

Samples of pollen or pollen analogue (500 mg) were weighed, extracted in 15 mL of n-pentane and filtered. For bee-collected pollen, samples were also ground in a mortar and pestle prior to being extracted. Extracts were absorbed onto filter paper, and after the solvent had evaporated, were immediately used in bioassays. Individual bioassay dishes were made from the lids of glass petri dishes (10 cm dia × 2 cm deep), with a Whatman® #1 filter paper (9 cm dia) placed in the bottom (Fig. 32) and a coarse galvanized screen positioned on top (9.5 mm openings). Filter papers used in the bioassay were dyed with bright yellow food colouring prior to use, so that any carotenoids present in the extracts would not be visible on them. This prevented the presence of visual cues which could potentially bias the choices of foragers.

The following sequence of hierarchical behaviours, from initial to penultimate, was scored during trials: 1. Approaching - Bees flying within 5 cm of the bioassay dish, but not landing; 2. Landing - Bees landing on the upper screen or the exterior surfaces of the dish; 3. Crawling Under - Bees crawling under the screen and transferring onto the inside walls of the bioassay dish or the surface of the filter paper. Two complete sets of unique pairwise comparisons of the nine pollen (or analogue) extracts, consisting of a total of 72 individual trials, were performed.

Particle Size Bioassay

The behaviour of pollen foragers choosing between different particle size classes of a proteinaceous pollen analogue was evaluated. High protein soybean meal was milled using a laboratory cutting mill (Wiley Mill, Standard Model #3, Thomas Co., Swedesboro, NJ, USA) fitted with a 1 mm screen. The milled soybean meal was then

passed through a series of stackable brass test sieves (20.3 cm dia × 5.1 cm deep, W.S. Tyler, Mentor, OH, USA) having the following pore sizes: 1000, 600, 300, 150, 75, 53, 45 and 38 µm. A final level of sieving was performed using a nylon woven polymer macrofiltration screen (Spectra/Mesh[®], #146510, Spectrum Laboratories Inc., Laguna Hills, CA, USA) having a pore size of 20 µm, which was fitted to a brass test sieve body. Stackable sieves were placed in a test sieve shaker (E.F.L. 1 MK II, Endecotts Ltd., London, UK) and portions of the ground meal were agitated for 30 - 60 min to separate them into the 9 particle size classes (below 1000 µm) delimited by the sieves. For a given particle size class, 25 g portions of soybean meal were placed in the bottom of bioassay dishes for each trial. Bioassay dishes were constructed from round glass crystallizing dishes (70 mm diameter × 50 mm height) (Pyrex[®] #3140) wrapped in black paper (Fig. 33).

The following sequence of hierarchical behaviours, from initial to penultimate, was scored during trials: 1. Approaching - Bees flying within 5 cm of the bioassay dish, but not landing; 2. Landing - Bees landing on the interior surfaces of the dish; 3. Hovering - Bees hovering in flight immediately above the soybean meal, displacing the substrate onto their body using air currents; 4. Scrabbling - Bees actively crawling in the soybean meal and packing it in their corbiculae.

The weight of soybean meal collected was also recorded after each trial, by subtracting the weight of the bioassay dish and its contents at the end of the trial from its initial weight at the beginning of the trial. Care was taken to collect any small quantity of soybean meal that was displaced outside of the bioassay dish by the action of the bees. The relative gain or loss of water vapour by the soybean meal during each trial was

determined by placing an identical set of bioassay dishes within the flight room, immediately outside the flight cage. Relative changes in the water content of the experimental bioassay dishes were corrected against these standards. Two complete sets of unique pairwise comparisons of the nine particle size classes, consisting of a total of 72 individual trials, were performed.

Protein Content Bioassay

The behaviour of pollen foragers choosing between two sources of pollen analogue differing only in crude protein content was evaluated. Different proportions of defatted soy flour and alpha-cellulose fiber were mixed to create a substrate having five different levels of crude protein content: 0, 10, 20, 30 and 40%. Prior to creating these mixtures, the defatted soy flour was sieved and remixed to standardize its particle size classes with that of alpha-cellulose fiber: 59.6% (53 - 75 μm), 25.3% (45 - 53 μm), 15.1% (38 - 45 μm). The general protocol and recording of honey bee behaviour was otherwise identical to the particle size bioassay. The weight of substrate collected during each trial was also recorded, and was standardized for ambient water loss or gain. Three complete sets of unique pairwise comparisons of the five levels of protein content, consisting of a total of 30 individual trials, were performed.

Handling Time Bioassay

The behaviour of pollen foragers choosing between two identical sources of pollen lipid extract that differed only in handling time was evaluated. Bioassay dishes were made from round glass crystallizing dishes (90 mm diameter \times 50 mm height) (Pyrex[®]

#3140) fitted with screens, and contained an attractive pollen lipid extract (Fig. 34).

Odour-containing lipids were extracted from 500 mg of *M. officinalis* pollen and were evaporated onto filter paper as described in the lipid extract bioassay. Handling time was manipulated by fitting two galvanized metal screens to the bioassay dishes, one placed on top, while the other was placed 2.5 cm from the bottom of the dish. Two different types of screens were used, having openings of either 9.5 or 5.1 mm. Bees could readily pass through the 9.5 mm openings, but required considerable effort to pass through the 5.1 mm openings. Low handling time treatments had two screens with 9.5 mm openings per bioassay dish. Intermediate handling time treatments had a 9.5 mm screen on top of the dish with a 5.1 mm screen used as the lower screen. Two 5.1 mm screens were used in dishes with high handling time. The exterior surface of the bioassay dish was wrapped in black paper.

The following sequence of hierarchical behaviours, from initial to penultimate, was scored during trials: 1. Approaching - Bees flying within 5 cm of the bioassay dish, but not landing; 2. Landing - Bees landing on the upper screen or the exterior surfaces of the dish; 3. Crawling Under Screen #1 - Bees crawling under the upper screen and transferring onto the inside walls of the bioassay dish or the upper surface of the lower screen; 4. Crawling Under Screen #2 - Bees crawling under the lower screen and contacting the filter paper. Five complete sets of unique pairwise comparisons of the three levels of handling time, consisting of a total of 15 individual trials, were performed.

Interaction of Multiple Factors Bioassay

The behaviour of bees was evaluated when they were allowed to choose between

dishes having different intensities of simultaneously-presented foraging cues and handling times. Pollen lipid extract, particle size and handling time were varied at either a low and high stimulus intensity while present in the same bioassay dish. Bioassay dishes were made from round glass crystallizing dishes (90 mm diameter × 50 mm height) (Pyrex® #3140) fitted with screens, and having 10 g of cellulose substrate in the bottom (Fig. 35). A high intensity stimulus of lipid extract was created by extracting 500 mg of *M. officinalis* pollen in n-pentane (see lipid extract bioassay), while a low stimulus intensity of odour was provided using a solvent blank. Both types of odour solutions were evaporated onto the cellulose substrate in the bottom of the dish. For small particle sizes (less than 38 µm), sieved alpha-cellulose fiber was used as a substrate but for larger particle sizes (75 - 150 µm), the previously extracted, bleached and sieved spruce wood sawdust was used. Handling time was manipulated using the same screens as described for the handling time bioassay. For treatments with high handling time, two screens having 5.1 mm openings were employed, while for low handling time treatments, both upper and lower screens had 9.5 mm openings. The exteriors of dishes were wrapped in black paper.

For this bioassay, eight possible configurations for an individual bioassay dish were possible depending on the combination of levels (intensities) used for the three factors. Bee behaviour was evaluated for each pairwise permutation of bioassay dish configurations, except those in which the configurations would be identical. Scoring of bee behaviour was performed as described for the handling time bioassay. The weight of substrate collected during each trial was also recorded, and was standardized for ambient water loss or gain. Two complete sets, consisting of 56 individual trials, were performed.

Statistical Analyses

Counts of forager behaviours were log transformed and analyzed by analysis of variance (ANOVA), using a balanced incomplete block design (BIBD) (Montgomery 1991). In addition to the discrete behaviours scored for each bioassay, two derived variables were analyzed: one which was the sum of all behaviours, and another which was the sum of all behaviours except approaches (post approaching behaviours). Treatments corresponded to the different factor levels tested in each bioassay (e.g. types of pollen, particle sizes, % protein content). However, for the interaction of multiple factors bioassay, treatments corresponded to each discrete combination of the three simultaneously presented factor levels. For this analysis lipid extract, particle size and handling time were coded as individual model terms and analyzed as separate factor levels. For all analyses, experimental trials were considered to be blocks with the number of treatments per block being fixed at two. The number of replicates was equal to the number of times that a particular factor level was tested in a complete set of bioassay trials. A variable representing each set of trials (SET) was also included in the analysis, thereby subdividing blocks into separate groups. Pre-planned comparisons between pollen species and pollen analogues were performed using single df contrasts.

In addition to the main BIBD analyses, a preliminary set of analyses was conducted on the dependant variable representing the sum of all behaviours scored. For this set of analyses, an additional independent model term (TIME) was added, which coded for the time of day during which trials were performed (morning or afternoon). These preliminary analyses allowed us to ascertain whether any differences in the response of foragers existed between the times of day during which trials were conducted.

In addition to each discrete behaviour being analyzed by univariate ANOVA, the complete hierarchical sequence of behaviours in a bioassay was analyzed by multivariate analysis of variance (MANOVA) (PROC GLM, SAS Institute 1989b). The MANOVA procedure permitted an analysis of treatment effects taking into account any correlations among the dependent variables, and also permitted direct comparison of differences among dependent variables. Treatment effects were examined by performing linear contrasts, or by a comparison of least squares means (LSMs), these values being adjusted for the total activity in the blocks in which a treatment was present. A matrix of LSMs probabilities [t -test, H_0 : $LSMean(I) = LSMean(j)$] was produced by invoking the LSMEANS statement with the PDIFF option (PROC GLM, SAS Institute 1989b). After applying a Bonferroni correction to these probabilities, multiple comparisons were manually derived.

For bioassays of particle size, protein content and the interaction of multiple factors, the weight of substrate collected per forager was estimated by dividing the amount of substrate collected by bees at the end of a trial by the number of bees whose penultimate behaviours were not approaches. Untransformed data were analyzed using a BIBD.

The behavioural responses of honey bees to a given treatment were further analyzed to determine whether their relative magnitude was influenced by the presence or absence of a competing treatment. Because the bioassays only allowed bees to make choices between two different factor levels at one time, it was not always clear how the number of choices made to one stimulus were affected by the presence or absence of a competing stimulus. Furthermore, it was not clear how the responses of bees to one stimulus might vary, based on a change in the composition or number of competing stimuli. In order to

address these questions, a competing effects model was employed (Raghavarao and Wiley 1986, Rathnam 1989). Independent variables in the model included TREAT (treatment) and TREAT*COMPTMT, the interaction of a treatment and its competing treatment during a trial. Univariate ANOVA was performed using log-transformed data, on bees scored as approaching bioassay dishes and on the sum of all behaviours scored per bioassay (PROC MIXED, SAS Institute 1989b). To determine the relative effect of individual competitors on treatments, tests of simple effects were generated for the TREAT*COMPTMT term by invoking the LSMs statement with the SLICE option (PROC MIXED, Littell et al. 1996). Probabilities associated with the differences between LSMs were adjusted using a Bonferroni correction.

Results

Whole Pollen Odour Bioassay

Over all behaviours scored, univariate analyses clearly indicated that the behaviour of foragers was significantly affected by the type of pollen or pollen analogue presented (Table 12). For total behaviours, pollen sources (apple, canola, jack pine, phacelia, sunflower and yellow sweetclover) had significantly higher responses than non-pollen sources (Bee-Pro[®], cellulose fiber and soy flour) ($F = 204.90$; $df = 1, 64$; $P < 0.0001$) (Fig. 36). Similar patterns were seen among treatments for the other behaviours scored. The response to treatment for total behaviours was independent of the time of day during which bioassays were performed ($F = 2.53$; $df = 1, 64$; $P = \text{NS}$).

Multivariate analysis further verified that the type of pollen or analogue presented directly influenced the frequency of behaviours observed (Wilks' $\lambda = 0.109$; $F = 4.36$; df

= 40, 264.3; $P < 0.0001$). Comparisons of the mean response levels of treatments among penultimate behaviours scored showed that equal numbers of bees approached bioassay dishes as landed on them ($F = 0.65$; $df = 8, 64$; $P = \text{NS}$), and that these numbers were greater than the number of foragers crawling under the upper screen ($F = 2.35$; $df = 8, 64$; $P = 0.028$).

Using the competing effects model, significant overall treatment effects for total behaviours ($F = 2.89$; $df = 8, 72$; $P = 0.008$) and for those foragers whose penultimate behaviour was approaching ($F = 0.65$; $df = 8, 72$; $P = 0.016$) were found. However, no competing effects were discovered among treatments for total behaviours ($F = 0.39$; $df = 63, 72$; $P = \text{NS}$), or for those bees only approaching bioassay dishes ($F = 0.55$; $df = 63, 72$; $P = \text{NS}$).

Pollen Lipid Extract Bioassay

Pollen lipid extracts also had a significant effect on foraging behaviour (Table 12). For total behaviours, foragers responded in greater frequency to extracts made from naturally occurring pollen species than from extracts derived from non-pollen sources ($F = 68.76$; $df = 1, 64$; $P < 0.0001$); similar patterns were also observed for the remaining behaviours (Fig. 37). Total foraging response was independent of the time of day during which bioassays were performed ($F = 3.85$; $df = 1, 64$; $P = \text{NS}$).

Multivariate analysis also showed that the type of extract present in trials affected the frequency of forager response (Wilks' $\lambda = 0.262$; $F = 2.38$; $df = 40, 264.3$; $P < 0.0001$). Comparisons of the mean response levels of treatments among penultimate behaviours scored showed that similar numbers of bees approached bioassay dishes as

landed on them ($F=0.91$; $df=8, 64$; $P=NS$). Furthermore, the number of foragers crawling under the screen (placed on top of the bioassay dish) was similar to the number of bees approaching ($F=1.61$; $df=8, 64$; $P=0.028$), or landing on ($F=1.28$; $df=8, 64$; $P=0.028$) dishes.

From the competing effects model, no overall treatment effect was evident for total behaviours ($F=1.97$; $df=8, 72$; $P=NS$) or for those foragers whose penultimate behaviour was approaching ($F=1.68$; $df=8, 72$; $P=NS$). Similarly, the term in the model representing competing effects was not significant for total behaviours ($F=0.51$; $df=63, 72$; $P=NS$), or approaches ($F=0.67$; $df=63, 72$; $P=NS$). However, examination of these effects by individual treatments revealed that the number of bees approaching bioassay dishes containing soy flour extract was influenced by competition from other treatments (Table 13). In particular, the number of approaches to soy flour extract was decreased when a competing treatment containing apple pollen or Bee-Pro[®] extract was present, relative to the increased response seen for the remaining, more favourable competitors.

Particle Size Bioassay

Foragers responded to variations in particle size by increasing foraging activity as particle size decreased (Fig. 38). The number of approaches, landings or scrabbings made to treatments did not differ between particles of different sizes, but reflected changes in the number of bees hovering to collect substrate and in the sum total of behaviours scored (Table 12). The most attractive particle size classes ranged from < 20 to 38 (particles < 45 μm), while lowest response was seen for particles > 300 μm .

Foraging behaviour was independent of the time of day during which bioassays were performed ($F = 2.26$; $df = 1, 64$; $P = \text{NS}$).

Multivariate analysis provided further confirmation that variation in particle size affected the frequency of forager response (Wilks' $\lambda = 0.214$; $F = 2.26$; $df = 48, 294.4$; $P < 0.0001$). Comparisons of the mean response levels of treatments among penultimate behaviours scored showed that the numbers of bees approaching bioassay dishes was similar to those landing on them ($F = 1.90$; $df = 8, 64$; $P = \text{NS}$), and that there was no difference between the numbers of bees hovering above the substrate and those actively scabbling in it ($F = 1.56$; $df = 8, 64$; $P = \text{NS}$). In addition, the frequency of bees approaching or landing on bioassay dishes was significantly greater than those bees exhibiting hovering or scabbling behaviours ($F = 5.00$; $df = 8, 64$; $P < 0.0001$).

The weight of soybean meal collected per forager varied among particle size classes ($F = 2.36$; $df = 8, 64$; $P = 0.027$) (Fig. 39). Although similar amounts of substrate were collected among most treatment levels, larger amounts of soybean meal per forager were collected for particle classes $53 \mu\text{m}$ and below than for particle classes $75 \mu\text{m}$ and above (26.84 ± 1.50 vs 16.86 ± 1.73) (LSM \pm SE).

The competing effects model indicated a significant overall treatment effect for total behaviours ($F = 6.97$; $df = 8, 72$; $P < 0.0001$), but not for those foragers approaching bioassay dishes ($F = 0.90$; $df = 8, 72$; $P = \text{NS}$). Overall, no competing effects occurred among total behaviours ($F = 0.51$; $df = 63, 72$; $P = \text{NS}$) or approaches ($F = 0.59$; $df = 63, 72$; $P = \text{NS}$), however multiple comparisons did reveal differences between discrete pairs of treatments with competing treatments. For example, the number of foragers approaching dishes containing particles in the $45 \mu\text{m}$ size range was increased by the

presence of competing treatments containing particles in the 75, 20, <20, 38 and 53 μm size classes, but was decreased by the presence of a competing treatment containing particles equal to or greater than 600 μm (Table 14). In addition, foraging for particles in the 45 μm size class increased when a competing treatment containing particles in the 75 μm size class were present, but decreased when particles in the 150 μm size class were present (Table 15). Foraging for particles in the 300 μm size class was increased by the presence of a competitor having particles in the 20 μm size class, but was lower in response to the presence of particles in 75 μm size class.

Protein Content Bioassay

The behaviour of pollen foragers was not affected by the protein content of the soy flour they were collecting (Fig. 40). Variables representing the sum total of all behaviours or all post approaching behaviours, were also independent of treatment effects (Table 12). Foraging for pollen analogues of different protein content was also unaffected of the time of day during which bioassays were performed ($F = 3.95$; $df = 1, 26$; $P = \text{NS}$).

Multivariate analysis also showed that the frequency of foraging behaviours was insensitive to the crude protein content of the substrate being presented to bees (Wilks' $\lambda = 0.292$, $F = 1.31$; $df = 24, 74.5$; $P = \text{NS}$). Comparisons of the mean response levels of treatments among penultimate behaviours scored indicated that equal numbers of bees approached dishes as landed on them ($F = 0.45$; $df = 4, 26$; $P = \text{NS}$) and that similar numbers of foragers landed on dishes as hovered in them ($F = 1.53$; $df = 4, 26$; $P = \text{NS}$). The number of foragers that hovered to collect soy flour also did not differ from the

number of bees actively scrabbling for soy flour in the bottom of dishes ($F = 0.66$; $df = 4, 26$; $P = \text{NS}$).

The weight of soy flour collected per forager did not vary with protein level ($F = 0.44$; $df = 4, 26$; $P = \text{NS}$). Overall, the mean amount of soy flour collected per forager was 17.85 ± 0.34 mg (LSM \bullet SE).

Analysis based on the competing effects model showed a lack of treatment effect for total behaviours ($F = 0.76$; $df = 4, 40$; $P = \text{NS}$) and for those foragers only approaching dishes ($F = 0.54$; $df = 4, 40$; $P = \text{NS}$). No competing effects were seen among treatments for either total behaviours ($F = 0.71$; $df = 15, 40$; $P = \text{NS}$), or among those bees whose penultimate behaviour was approaching ($F = 0.85$; $df = 15, 40$; $P = \text{NS}$).

Handling Time Bioassay

The behaviour of pollen foragers was considerably influenced by the degree of handling time necessary for them to contact an attractive pollen lipid. Bees increased their frequency of all foraging behaviours as the degree of handling time associated with bioassay dishes decreased (Fig. 41). Univariate analyses showed that bees crawling under the upper and lower screens of the bioassays dishes had a greater influence on treatment effects than the number of bees approaching or landing on them (Table 12). Foraging behaviour was independent of the time of day during which bioassays were performed ($F = 3.10$; $df = 1, 13$; $P = \text{NS}$).

Using multivariate analysis, handling time was again shown to significantly influence forger behaviour (Wilks' $\lambda = 0.014$; $F = 9.81$; $df = 12, 16$; $P < 0.0001$).

Comparisons of the mean response levels of treatments among penultimate behaviours

scored showed that the frequency of bees approaching and landing on bioassay dishes was similar ($F = 0.64$; $df = 2, 13$; $P = \text{NS}$). However, the frequency of bees crawling under screen 1 was greater than of bees crawling under screen 2 ($F = 23.23$; $df = 2, 13$; $P < 0.0001$), and the number of foragers either approaching or landing on dishes was greater than the number crawling under both screens ($F = 12.46$; $df = 2, 13$; $P < 0.001$).

The competing effects model indicated a significant overall treatment effect for total behaviours ($F = 3.91$; $df = 2, 24$; $P = 0.034$), but not for foragers only approaching dishes ($F = 0.01$; $df = 2, 24$; $P = \text{NS}$). The term in the model representing competing effects was not significant for total behaviours ($F = 0.22$; $df = 3, 24$; $P = \text{NS}$) or for bees whose penultimate behaviour was approaching ($F = 0.83$; $df = 3, 24$; $P = \text{NS}$).

Interaction of Multiple Factors Bioassay

When presented simultaneously, the three factors that elicited greatest forager response in previous bioassays (pollen lipid extract, particle size and handling time) had noticeably different influences on bee behaviour. Of the three factors, pollen lipid extract had a much greater influence on foraging decisions than particle size or handling time (Fig. 42). The frequency of behaviours scored at bioassay dishes was affected by pollen lipid extract and handling time (Table 16). Although the presence of pollen lipid extract caused consistent increases in all behaviours compared with its absence, a similar pattern was not evident for low and high levels of handling time. High handling time increased the number of bees whose penultimate behaviour was approaching or landing, and low handling increased the numbers of foragers crawling beneath each of the two screens. Hence, low handling time increased behaviours associated with processes of seeking or

collecting pollen, while high handling time decreased such behaviours. For variables representing total behaviours or all post approaching behaviours, only the presence of pollen lipid extract caused significant increases in foraging behaviour. Total behaviours were found to be independent of time of day ($F = 1.92$; $df = 1, 72$; $P = \text{NS}$).

Based on the type behaviours of examined, two prominent interaction terms were identified from the previously analyzed models. There was a significant interaction between particle size and handling time for those foragers whose penultimate behaviour was either approaching or landing on bioassay dishes ($F = 6.70$; $df = 1, 86$; $P = 0.011$). The response of foragers to handling time differed with different particle sizes. High handling time increased the number of approaches and landings relative to low handling time, only when particle size was small (Fig. 43). There was also an interaction between pollen lipid extract and handling time for bees crawling under the lower screens of bioassay dishes ($F = 4.11$; $df = 1, 86$; $P = 0.046$) (Fig. 44). Bees responded to the presence of pollen lipid extract by increasing their foraging effort in relation to treatments with no pollen lipid, particularly when handling time was low. When this effect was evaluated over total behaviours, no interaction existed ($F = 0.51$; $df = 1, 86$; $P = \text{NS}$).

Multivariate analysis of variance for the each of three stimuli used indicated significant treatment effects for pollen lipid extract (Wilks' $\lambda = 0.367$; $F = 23.24$; $df = 6, 81$; $P < 0.0001$) and handling time (Wilks' $\lambda = 0.638$; $F = 7.67$; $df = 6, 81$; $P < 0.0001$), but not for particle size (Wilks' $\lambda = 0.969$; $F = 0.43$; $df = 6, 81$; $P = \text{NS}$). Comparisons of the mean response levels of treatments among behaviour types scored showed that there were differences in the number of foragers approaching dishes versus landing on them in response to lipid extract treatments ($F = 40.82$; $df = 1, 86$; $P < 0.0001$), but no differences

were found for particle size ($F = 0.06$; $df = 1, 86$; $P = \text{NS}$) or handling time treatments ($F = 3.49$; $df = 1, 86$; $P = \text{NS}$). The number of foragers crawling beneath the upper versus lower screen did not differ in lipid extract ($F = 1.94$; $df = 1, 86$; $P = \text{NS}$), particle size ($F = 0.16$; $df = 1, 86$; $P = \text{NS}$) or handling time ($F = 3.71$; $df = 1, 86$; $P = \text{NS}$) treatments. However, comparison of the number of bees either approaching or landing on dishes with those crawling under screens differed in lipid extract ($F = 15.05$; $df = 1, 86$; $P = 0.0002$) and handling time ($F = 26.31$; $df = 1, 86$; $P < 0.0001$) treatments, but not in particle size treatments ($F = 0.01$; $df = 1, 86$; $P = \text{NS}$).

Over all treatments, the amount of alpha-cellulose fiber collected per forager was relatively small (1.84 ± 0.25 mg) (LSM \pm SE), however this average reflects the influence of unattractive treatments in which little if any substrate was collected. The amount of cellulose fiber collected per forager did not change in response to the particle size treatment ($F = 2.42$; $df = 1, 86$; $P = \text{NS}$), but bees collected more when handling time was low than when it was high ($F = 38.97$; $df = 1, 86$; $P < 0.0001$) and also collected greater quantities when lipid extract was present than when it was absent ($F = 10.28$; $df = 1, 86$; $P < 0.0001$). A significant interaction between lipid extract and handling time was also detected ($F = 14.75$; $df = 1, 86$; $P = 0.0003$); considerably more cellulose was collected when extract was present and handling time was low, than in other treatment combinations (Fig. 45).

From the competing effects model, significant overall treatment effects were found for total behaviours ($F = 34.94$; $df = 7, 56$; $P < 0.0001$) and foragers approaching bioassay dishes ($F = 19.81$; $df = 7, 56$; $P < 0.0001$). The term in the model representing competing effects was not significant for total behaviours ($F = 1.14$; $df = 48, 56$; $P = \text{NS}$), or for the

frequency of approaches ($F = 1.07$; $df = 48, 56$; $P = NS$). However, examination of these effects on an individual treatment basis illustrates the role that certain stimuli play in the attraction of foragers and their elicitation of behaviours.

Competing effects were found for bees approaching bioassay dishes that did not exhibit higher order behaviours (Table 17). It is evident from these data that pollen lipids provided the most important cue stimulating foragers to approach bioassay dishes. Competing effects were only seen for treatments without lipid extract; when lipid extract was present in any treatment, no other competing treatment affected the frequency of responses to it. Pollen lipid extract was also present in most of the favourable competitors and served to increase the frequency of approaches to a given treatment, rather than decrease them. This unambiguously shows that the presence of lipid odour in a competing treatment served to increase the activity of bee visitation to a neighbouring dish without pollen odour. Handling time appeared to play a secondary role to that of lipid odour, as favourable competing treatments were predominantly ones in which handling time was low. Particle size exerted no discernable role in eliciting approaches.

Trends among unfavourable competing treatments are less clear. When unfavourable competing treatments contained pollen lipid extract and had high handling time, the frequency of responses to treatments appears to be lessened. Compared with treatments with low handling time, those with high handling time had more foragers that only approached dishes and had fewer bees that continued in the behavioural sequence towards substrate collection (Fig. 42). Hence, the larger relative number of approaches to a high handling time competitor could exert a significant competing effect. For non-favourable competitors without lipid extract, the decrease in activity caused by a lack of

odour stimulus is more likely to account for the relative decrease in activity to the treatment than the competition caused by low handling time.

Based on analysis of total behaviours, significant competing effects were seen for two treatments (Table 18). Similar to the approach data, competing effects were not seen for treatments containing pollen lipid extract. For the first treatment (lipid extract absent, large particle size, high handling time), the difference between its favourable and unfavourable competing treatments is only the presence of pollen lipid extract, which is present in the favourable competitor. Again, the presence of pollen lipid odour served to increase the activity of foragers for both the treatment and competitor, resulting in more foragers completing part, or all, of the behavioural sequence leading to substrate collection. For the remaining treatment having significant competing effects (lipid extract absent, small particle size, low handling time), the influence of pollen-based factors was more ambiguous. This treatment's favourable competitors contain opposing treatment levels. The first favourable competing treatment (lipid extract present, large particle size, low handling time) would have been highly attractive to foragers and likely served to increase the foraging activity to both bioassay dishes. The remaining favourable competitor (lipid extract absent, particle size small, handling time high) would be highly unattractive in terms of lipid odour and handling time and may have increased the relative behaviours scored to the treatment by attracting few foragers to itself. The unfavourable competitor for this treatment (lipid extract present, particle size small, handling time high) is similar to the previously described favourable competitor, except that it contains lipid extract. Its effect on the frequency of total behaviours was opposite, and functioned to preferentially attract foragers to itself to the detriment of the treatment.

Discussion

My findings demonstrate the influence of several pollen-based factors on honey bee foragers. The experiments in this study were novel in evaluating the relative importance of individual cues and handling time by direct experimental manipulation, under conditions that were unconfounded by environmental influences. I showed that the behaviour of individual foragers is highly influenced by the odour of pollen, the size of food particles and the handling time necessary for collection. Choices made by individual foragers are not, however, affected by the protein content of a food resource. When factors are presented simultaneously, foragers respond to them in a hierarchical fashion, with pollen odour being dominant over handling time or particle size. This study was also unique in examining the competing effects between simultaneously-presented foraging stimuli. These analyses revealed that the intensity of foraging behaviours elicited to a weakly scented food source can be greatly increased or decreased by the presence of a competing food source containing pollen lipid odour, irrespective of other factors present.

My results clearly indicate that choices made by individual pollen foragers are influenced by the odour of pollen, and that similar responses can be elicited by pollen lipid extracts. Studies that sequentially extracted external and internal pollen lipids (Evans et al. 1987, 1988, 1990; Dobson 1988) would suggest that the extraction procedure used in this study is likely to have selectively removed lipids from the outer oily layer of pollen grains, known as pollenkitt (Knoll 1930, Pankow 1957). In angiosperms, pollenkitt facilitates the dispersal of pollen grains by adhering them to insects (Hesse 1981, 1984) and is also the site from which pollen odours originate

(Dobson et al. 1987; Dobson 1989). I found that the absence of pollenkit in *P. banksiana* did not preclude odour production. The extract of *P. banksiana* pollen was qualitatively different to our senses from the odours produced by the angiosperm species. Although the total lipid content of *Pinus* spp. pollen (1 - 3 %) (Ching and Ching 1962; Andrikopoulos et al. 1985) is considerably lower than that of most angiosperm pollen (1 - 20%) (Stanley and Linskens 1974), the lipid profile of both groups is comprised of compounds sharing similar neutral lipid classes. It is therefore possible that lipid-based compounds may also contribute to the production of odour in jack pine pollen.

Several studies have shown that pollen lipids influence honey bee behaviour. Within the hive, the consumption of sugar paste, pollen supplements and artificial diets is increased by the addition of the lipid soluble fraction from pollen (Robinson and Nation 1968, Doull and Standifer 1970, Doull 1974). Schmidt (1985) and Schmidt et al. (1989) also found phagostimulants for several species of pollen from extracts prepared with a wide range of solvent polarities. They concluded that consumption was induced by the cumulative effects of numerous, rather than a few specific, pollen-based compounds.

Pollen odour also plays a role in stimulating pollen collection by foragers outside the hive. My data show that pollen lipid volatiles not only attract honey bees, but also increase their probability of landing and performing pollen-collecting behaviours. Louveaux (1959) and Hügel (1962) showed that bees are attracted to extracts of pollen containing phytosterols or steroids. Taber (1963) demonstrated that pollen extracts contained substances that initiate the corbicula packing response. After these extracts were made, bees would not collect the pollen, even though it still contained over 97% of its total dry weight, including most nutritive substances. Lepage and Boch (1968) and

Hopkins et al. (1969) isolated a free fatty acid from clover pollen which functioned as a foraging attractant for honey bees in a flight room. When this compound was added to cellulose powder, it elicited intense visitation by foragers. Increased collection and dancing behaviour for cellulose powder also occurs after the addition of alder and hazel pollen extracts (Hohmann 1970).

The production of pollen lipid odour by plants imparts several advantages to them including increased fitness via enhanced flower constancy, greater pollen export, and increased probability of pollen reaching receptive stigmas (Stanton et al. 1986, Galen 1992). However, use of pollen odour by honey bees also confers advantages on them through increased efficiency of individual and colony-level pollen foraging. Foragers quickly learn to orient to a floral species based on the odour of its pollen (von Frisch 1923, von Aufsess 1960), and if offered a choice, tend to select among pollens in relation to the intensity their odour (Levin and Bohart 1955). Foragers usually collect pollen from the same floral patch as long as it remains available (Free 1963), thereby improving their efficiency over successive trips by reducing the time spent searching for flowers and by eliminating the time wasted switching between different handling techniques (von Frisch 1953). Because odours are learned by honey bees more rapidly than colours and evoke stronger discrimination between flowers (Menzel 1985), foragers incur less cost and commit fewer errors when locating or returning to a new floral source if odour is present. For example, a single association of an odour with a food reward induces constancy in over 90% of workers (Koltermann 1969), while the same association with colour requires five exposures to achieve similar fidelity (Menzel and Erber 1978). Improvements in individual foraging efficiency may also result from the quantitative use of pollen odour in

assessing the presence or abundance of pollen within individual flowers (Dobson et al. 1996). Preferences of honey bees for nectarless flowers (*Papaver rhoeas* L., *Eschscholtzia* spp., *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson) indicate that landing behaviours are stimulated by the quantity of pollen available (Ribbands 1949, Goodwin and Steven 1993). Such preferences appear to be insensitive to previous visitation by bees, inferring that repellent odour markings (Giurfa 1993, Giurfa et al. 1994) do not exist for pollen collection or are inferior to pollen odour (Ribbands 1949). The quantitative evaluation of pollen volatiles by foragers would be of great adaptive advantage for bees working flowers where pollen is not readily visible and pollination-induced changes in floral structure are not apparent (Gori 1983). In such flowers, bees could locate and decide to collect pollen on the basis of its availability, without expending time and energy manipulating the floral structure to search for pollen. Finally, pollen odour may also improve the foraging efficiency of honey bees discriminating between male-fertile and male-sterile flowers of the same species, where visible differences in floral morphology are not apparent (Goodwin and Steven 1993).

Although pollen odour allows individual foragers to optimize their collection of pollen among flowers, it also influences the foraging efficiency of the colony as a whole. At the end of a foraging trip, workers use pollen odour to communicate the presence of an attractive food source to “unemployed” foragers. Odours are quickly perceived by workers as they antennate and lick pollen from the corbiculae of a returning forager, often even before her recruitment dance begins (von Frisch 1967). Odour can stimulate experienced foragers to return to a previously visited floral patch, even in the absence of dance information (Johnson and Wenner 1966, Wells and Wenner 1971). The use of

odour in recruiting inexperienced foragers is paramount. Recruits often require several flights from the colony before successfully locating a food source (Gould et al. 1970, Friesen 1973, Seeley 1995) and may fail to find a food source without odour cues (Wenner et al. 1969, Wells and Wenner 1971, Friesen 1973). The ability of a forager to recruit nestmates and the success of those recruits in locating a food source are enhanced with pollens possessing a strong odour (von Frisch 1923). In my experiments, it is unlikely that the recruitment of foragers using pollen odour cues affected the interpretation of results, as no differences in preference were seen among pollen species or their extracts. Bees also were prevented from contacting the pollen in the whole pollen odour bioassay, thereby decreasing the probability that odour-containing compounds would be transferred onto their bodies. Furthermore, pollen was not available to be collected as a reward in the whole pollen odour or pollen lipid odour bioassays, thereby precluding any learned association of a pollen reward with an odour source (Menzel 1993). Any effects of odour-based recruitment would also be minimized because of the randomization of treatments between trials.

Although my results are limited to a single concentration of pollen volatiles, the similarity of worker response among all species tested suggests that honey bees may not possess innate preferences for the odour of some pollens over others. Studies of scent perception in honey bees have demonstrated the ability of foragers to discriminate among floral odour components, usually after a period of preconditioning, but provide little evidence for innate olfactory preferences used in host-plant selection (Ribbands 1955; Waller et al. 1973, 1974; Henning et al. 1992; Masson et al. 1993; Pham-Delègue et al. 1989, 1993, 1997; Blight et al. 1997; Le Métayer et al. 1997). My results show that

foragers respond to the odour of nutritionally deficient pine pollen (Pernal and Currie 2000) as intensively as other species, thereby insinuating that honey bees lack adaptively-based discrimination based on odour. Although anemophilous species may comprise a significant portion of the total pollen consumed by some colonies (O'Neal and Waller 1984), honey bees typically collect little pine pollen. This may be a result of its unpalatability to bees (Pernal and Currie 2000), its nutritional unsuitability for normal development and longevity (Svoboda 1940, de Groot 1953), or because it lacks pollenkitt which would facilitate the adherence of pollen grains for transport. Because wind-pollinated plants have no requirement to attract a zoophilous pollinator, the presence of distinctive pollen odours in many species (Porsch 1956, von Aufsses 1960) suggests that odours have evolved to serve other purposes, such as defense of the male gametophyte against pathogens and pollen-feeding animals (Dobson et al. 1996). The hypothesis that honey bees possess an innate ability to discriminate against deleterious floral species, such as *P. banksiana*, is not supported by my data. Instead, it appears more probable that foragers learn to associate any readily-perceived pollen odour with the location of a food source (Waller et al. 1973, Pham-Delègue et al. 1986, Menzel 1993), with the odour subsequently serving as a primary cue for recruitment. Although this strategy may establish a foraging constancy to a nutritionally poor pollen source, the polylectic foraging nature of honey bee colonies may serve to ameliorate such maladaptive behaviour. In addition, colonies regulate their foraging to compensate for deficiencies in the quality of their stored pollen reserve (Pernal 2000b), further minimizing the effects of collecting nutritionally inferior pollen.

In my bioassays, honey bees increased the frequency of their collection behaviours

as particle sizes of soybean meal decreased (particularly for particles less than 45 μm), and removed greater quantities of substrate from dishes when particles were less than 75 μm . In nature, pollen size varies considerably, with the majority of anemophilous species ranging from 17 - 58 μm in diameter and zoophilous species ranging from 5 - 200 μm (Wodehouse 1935). Ninety-seven percent of bee-collected species have dimensions between 10 and 100 μm , with an overall mean of 34 μm (Roberts and Vallespir 1978). These values tend to correspond with collection behaviours of bees foraging on soybean meal particles. Although the greatest frequencies of pollen-collecting behaviours occurred when particle size was less than 45 μm , bees did forage for particles up to and within the 150 - 300 μm size class. For particles 300 μm and greater, the frequency of behaviours decreased, suggesting that visual or tactile cues offered by this size of particle were sufficient to reduce further collection attempts by foragers. Although honey bees are capable of collecting very large pollen grains, such as *Cucurbita pepo* L. (Cucurbitaceae) (134.6 μm) (Vaissière and Vinson 1994), I seldom observed successful corbiculae packing with particles between 150 to 300 μm , and never witnessed it for larger particles. The mass of soybean meal reported as being collected by bees within and above the 150 μm size class must be considered in light of these observations. The data for these size categories probably reflect the mass of unrecoverable substrate physically displaced from dishes or lost in flight by foragers, during their unsuccessful, and occasionally prolonged attempts to collect, groom and pack these particles.

Although I observed an upper limit to the particle size which a bee could successfully collect and pack, it is of interest to examine whether preferences below this limit could have any adaptive significance. Several reasons could account for such

choices. For example, preference for larger grains may be related to their ease of collection by specialized morphological adaptations, such as branched hairs and combs (Thorp 1979) and could be better suited to behavioural adaptations such as in-flight grooming (Michener et al. 1978). Because the nutrient content of pollen grains is largely confined to the protoplasm (Peng et al. 1985), the choice of larger pollen grains, which have a lower surface area to volume ratio, may also provide bees with greater food value (Simpson and Neff 1983).

Baker and Baker (1979) suggested that bees select small pollen types because they are lipid-rich and starchless ($\bar{x} = 36.7 \mu\text{m}$) compared with larger starch-containing grains ($\bar{x} = 65.6 \mu\text{m}$). Smaller sizes of grains could also be preferentially collected and consumed because they are filtered from the crop more efficiently by the action of the proventriculus (Bailey 1952). In my study, the greatest frequencies of pollen collection behaviours were seen for particles less than $45 \mu\text{m}$. For these very small and light particles, foragers achieved greatest collection success by hovering over the surface of the bioassay dishes and collecting airborne particles that clung to their hairs, and then groomed them off and packed them into their corbiculae. This technique of pollen loading has also been reported by Parker (1926) for bees foraging on catkins and spikes of anemophilous species like *Zea mays* L. For larger particles that were able to be successfully packed on bees' corbiculae ($45 - 150 \mu\text{m}$), the weight of substrate removed from dishes was similar, suggesting that scrabbling may have been used more effectively than hovering for their collection. Although bees exhibited greater frequencies of post approaching behaviours for the smaller-sized particles, their choices were not influenced by lipid content because all lipids were extracted from the soybean meal. Preferences for

very small particles could be an indication of selection toward increased efficiency of digestion, however forager behaviours did not substantially increase for the smallest of the particle size classes. Bailey (1952) showed increased digestive efficiency only for particles as small as *Nosema apis* Zander spores (3 by 6 μm), in a comparison against unspecified types of pollen (16-36 μm). It is also important to consider that factors other than the physical dimensions of a particle can affect its suitability for collection and packing. For example, the presence and abundance of oily pollenkitt on the surface of grains aids bees in forming pollen pellets (Stanley and Linskens 1974). The surface morphology of the pollen grain is also influential, with highly echinate (spiny) species physically interfering with the aggregation of grains while being groomed and packed into the corbiculae (Vaissière and Vinson 1994). Therefore, evidence from this and other studies suggests that the choice of pollen by foragers is not influenced by the criterion of size alone, but may be more affected by the interaction of pollen size with other factors that affect its ease of handling and collection (Buchmann and Shipman 1990, Vaissière and Vinson 1994, Harder 1998).

This study has been unique in examining the role of pollen quality in the foraging choices made by individual bees, using an experimental design that decoupled protein content from other pollen-based cues. Over a wide range of levels, individual foragers showed no preference or aversion for the protein content of a soy flour-cellulose mixture they were collecting. This suggests that foragers do not evaluate pollen quality or, that they lack tarsal or antennal chemoreceptors for detecting free amino acids, peptides soluble enzymes or other indicators of dietary protein (Bernays and Chapman 1994). Although honey bees add small quantities of regurgitated nectar from their proboscis to

pollen during its collection and packing, they do not consume pollen while foraging (Hodges 1984). Furthermore, amino acids and proteins are largely restricted to the intine of pollen grains (Stanley and Linskens 1974), making them inaccessible to bees until digestion has taken place in the midgut (Peng et al. 1985). Although it is possible that foragers may detect compounds associated with pollen quality using glossal or other oral receptors, honey bees do not appear to use this information to adjust their individual foraging strategies.

My results showing that foragers do not choose pollen in relation to its protein content are consistent with many documented accounts of bees collecting pollen or pollen-like substances with little or no nutritive value (Root and Root 1935, Haydak and Tanquary 1943, Morse 1975, Fægri and van der Pijl 1979, Johannsmeir 1981, Shaw 1990) and their lack of regulation of pollen consumption in relation to its crude protein content (Pernal and Currie 2000). Experimental choice tests also support the view that individual foragers do not possess the ability to assess protein content. Levin and Bohart (1955), using six pollen sources situated at outdoor feeding stations, showed that none of the pollen characteristics evaluated (reflectivity, colour, percent total sugar and crude protein content) showed any association with the attractiveness of pollen to foragers. Wahl (1966) demonstrated that bees given a choice of forage collected worthless substances in addition to pollen substitutes having some nutritive value. Wahl concluded that bees lacked the ability to discriminate protein content, based on the tendency of foragers to abandon worthless substances at the same rate as pollen substitutes, when attractive pollen was made available. Schmidt (1982) also examined pollen-collecting preferences of bees in a greenhouse by presenting them with a choice of eight different

species placed in beakers. Although the selection of pollen species were influenced by their odour, no conclusive relationship between protein content and foraging preferences was evident. The lack of protein discrimination among foragers is consistent with my previous findings showing that responses to variation in pollen quality occur at the level of the colony, rather than by decisions made by individual foragers (Pernal 2000b). I have found that colonies respond to variations in the quality of pollen returned by foragers by adjusting the proportion of naive foragers in the foraging population, resulting in changes in the overall quantity and, potentially, quality of pollen being collected. Colony-level discrimination of protein intake is also consistent with a feedback mechanism employing trophallactic exchange between nestmates (Crailsheim 1991, 1992, 1998), whereby foragers assess and respond to the colony's need for pollen in relation to the amount of protein they are fed by nurse bees (Camazine 1993, Camazine et al. 1998). My conclusions do not support the theory proposed by Fewell and Winston (1992) which states that individual foragers select high nitrogen (protein) content pollen when colony demand for pollen is high. Waddington et al. (1998) also suggested that foragers could discriminate pollen quality, based on their characterizations of dance rate and probability of dancing by foragers that were collecting mixed pollens or a 1:1 mixture of mixed pollens and cellulose powder. My findings, however, emphasize that the potential differences in recruitment shown in Waddington et al. (1998) were confounded by the lack of standardization for pollen odour concentrations between treatments and by other factors affecting the efficiency of pollen collection.

Bumble bee (*Bombus* spp.) foragers appear to be sensitive to the availability of pollen and the costs associated with collecting it, and forage in a manner that maximizes

their site-specific collection efficiency (Rasheed and Harder 1997a, b). Rasheed and Harder (1997a) suggest that pollen-collecting bumble bees are sensitive to interspecific variation in pollen protein content within a site, but not at the intrafloral level. However, further investigations are required to clarify the influence of factors affecting bumble bee foraging choices, as the pollen-collection criteria evaluated in Rasheed and Harder (1997a) were inconsistent in explaining the collection preferences of bees among experimental study sites. Currently, there is no evidence to suggest that bumble bees can directly or indirectly discriminate pollen protein content, and some floral-based cues that were not examined in the previous study could also have affected pollen choice. Decisions made by pollen-collecting bumble bees, moreover, cannot necessarily be extrapolated to the more highly social environment of honey bees, where colony-level feedback is integral to modulating individual forager behaviour (Seeley 1995).

From the handling time bioassay it was shown that simulated increases in floral complexity, created using different sized screens, decreased frequencies of pollen-seeking behaviours by bees. Pollen lipid odour served as a reliable stimulus to attract foragers to bioassay dishes, with the number of bees approaching and landing being almost identical over different levels of handling complexity. Although I did not record individual handling times, my general observations confirmed that the number of behaviours scored per bee was proportional to the length of time a bee spent in a bioassay dish, and was inversely associated with the effort required to cross the screens. The levels of handling time used in my bioassay also represent changes in floral handling costs and are similar to the nectar foraging concept of “access time”, or the time required for a forager to locate a nectary after landing on a flower (Gegear and Lavery 1995). It is important to

emphasize, however, that my use of the term “handling time” differs from other studies that interpret it as a function of the amount of reward being received by a forager at a flower (e.g. Chittka et al. 1997).

In order for pollen collection to conform to concepts of optimality, honey bees are expected to be influenced by floral handling costs and be driven by the need to maximize collection efficiency (Cheverton et al. 1985). However, these relationships have been poorly studied in either artificial or natural settings. Handling times are known to increase for honey bees as floral architecture becomes more complex (Buchmann and Shipman 1990). For example, bees foraging on the large simple flowers of saguaro (*Carnegiea gigantea* (Engelm.) Britt. and Rose) harvest pollen at a rate similar to that for collecting from an open dish. When working complex flowers having hidden poricidal anthers, bees harvest pollen at much less than one hundredth that rate (Buchmann 1983, Buchmann and Shipman 1990). The response of bees to changes in handling costs in this study was similar to those reported for nectar-collecting bumble bees (*Bombus* spp.), which work morphologically simple flowers quickly, and incur higher handling and learning times as floral architecture becomes more complex (Heinrich 1979; Lavery 1980, 1994a). In addition, many of my honey bees working bioassay dishes with high handling costs exhibited a behaviour consistent with the ‘giving-up response’ of bumble bees (Lavery 1994a), in which foragers departed the dish before crossing the lower screen and contacting the source of attractive pollen lipids. Strickler (1979) compared the solitary oligolectic bee *Hoplitis anthocopoides* (Schenck) (Megachilidae) to several polylectic species and showed that reductions in handling time actually increase the amount of pollen collected per unit foraging time, and thereby the potential reproductive

output of the species. Pollen foraging efficiency is also maximized by the solitary bee *Ptiloglossa arizonensis* Timberlake (Colletidae) and the social *Bombus sonorus* (Say) that adjust their handling times in response to intrafloral changes in the amount of pollen harvested (Buchmann and Cane 1989). Because honey bees clearly respond to changes in handling costs, it is likely that foragers evaluate or alter handling time to optimize their pollen collecting efficiency.

The effect of handling time on foraging decisions made by nectar-foraging honey bees and bumble bees has been well investigated, and these results pose interesting questions that relate to the effects of handling time on pollen foragers. For honey bees, increases in handling time lead to decreases in the directionality of flight to succeeding flowers, apparently because bees may “forget” their arrival direction on a flower with increasing time (Schmid-Hempel 1984). However, directionality was not assessed in my bioassays as arrays of choice dishes were not used; only two were present at any one time. Hence, when a bee left a previously-visited dish, only one unvisited dish remained, free from any directional competition. The influence of directional biases would be detected as negative competing effects in high handling time treatments, however none were detected. A second factor related to handling time that may influence subsequent floral choices made by bees are the costs associated with switching between different floral morphologies. In natural settings, pollinators often restrict their visits to flowers of only one species, even when they are intermixed with those of other species (Free 1963). The most widely accepted explanation for such floral constancy was proposed by Darwin (1883), who suggested that pollinators forage more efficiently if they continue foraging on flowers that they have recently learned how to work. After a reexamination of

Darwin's original idea, Waser (1983) suggested the reason for this is associated with the memory constraints of individual pollinators, as the learning of a second flower-handling skill is suggested to interfere with a pollinator's ability to recall a previously-learned technique. As such, pollinators must relearn specific-flowering handling techniques after every switch to a different plant species, resulting in a temporary increase in floral handling times and thereby decreasing the number of flowers visited per unit time. However, recent evidence suggests that interference effects incurred by bees switching between similar or different levels of floral complexity are unlikely to account for floral constancy (Woodward and Laverty 1992, Laverty 1994b, Gegear and Laverty 1995). Instead, floral constancy may be influenced more by cues involved in perceptual conditioning (odour, colour, size) (Wilson and Stine 1996), which were carefully standardized among treatments.

The results from the bioassay examining the interactive effects of pollen lipid odour, particle size and handling time provide us with further insight into the relative importance of these factors for pollen collection by honey bees. My results show that pollen odour is the overriding cue used by foragers, as bees exhibited increased frequencies of behaviours whenever odour was present, even when handling time and particle size were not optimal. Greater numbers of pollen-seeking behaviours occurred when handling time was low, but this factor was secondary to pollen odour; the size of substrate had little effect on the behaviour of foragers. The relative importance of these factors was also reflected in the amount of substrate removed from dishes by bees. Greatest quantities were collected when pollen lipid odour was present, particularly if handling time was low.

The predominant influence of pollen odour suggests that flowers may have evolved

to produce greater concentrations of odours to maintain a constancy with pollinators in spite of the decreased attractiveness caused by higher handling times. The average amount of substrate collected per forager in the interaction of multiple factors bioassay was considerably reduced compared with the protein and particle size bioassays, because little or no substrate was collected when lipid odour was absent. In addition, the design of the bioassay dishes did not permit bees to hover effectively beneath the lower screen, which reduced their collection efficiency. Moreover, the actions of bees crawling out of bioassay dishes, particularly through the narrow-meshed screens of the high handling time treatments, occasionally dislodged substrate from their bodies. Hence, estimates of the amount of pollen collected per forager in high handling time treatments may be low.

My data imply that when bees land on flowers, a hierarchical sequence of factors is used by foragers to decide whether to collect pollen. Honey bees appear to be capable of using a combination of sensory abilities to enhance their pollen collection. In species such as carpenter bees (*Xylocopa micans* Lepeletier), a hierarchy of information is used to learn the location of nectar-bearing flowers in vertical inflorescences, whereby the flower colour takes precedence over spatial information (Orth and Waddington 1997). For honey bees, the recognition of repellent scent marks on nectar-depleted flowers overrides the use of visual stimuli when the colour of rewarding and non-rewarding flowers is similar (Giufra et al. 1994). The use of pollen odour as a primary cue for *A. mellifera* to evaluate whether to engage in pollen-collecting behaviours is supported by its ability to discern olfactory cues better than other types of stimuli. For example, changes in odour are known to evoke stronger discrimination by honey bees than changes in flower pattern or shape (Manning 1957), and odour is more important in conditioning foraging

preferences than colour, form or time of day (von Frisch 1967, Koltermann 1969). If the biochemical basis for such pollen-based olfactory cues were elucidated, they might provide a mechanism for selectively conditioning honey bees to pollinate agricultural crops not normally attractive to bees (Jay 1986; Free 1958, 1993). This seems to be especially promising given that pollen odour plays such a pivotal role in the attraction of pollen foraging honey bees.

In conclusion, I have manipulated specific pollen-based factors, under carefully controlled conditions, to examine whether they are evaluated by foraging honey bees. I have determined that honey bees are most sensitive to the odour of pollen, but also have preferences for the size of particles they collect. Honey bees are further influenced by the handling costs incurred while attempting to collect a pollen reward, and on an individual level, appear unable to evaluate the protein content of the resource they are collecting. When presented simultaneously, pollen odour is the cue that predominates, with handling time being secondary and particle size exerting little influence for collection. The presence of pollen odour increases foraging at a food source and is dominant enough to override co-occurring factors that decrease pollen collection behaviours, such as high handling time. Furthermore, pollen odour affects the competitive ability of flowers to attract bees. Flowers with attractive pollen odours can decrease visitation to a weakly odoured heterospecific neighbour by selectively attracting foragers away from it, or increase its neighbour's visitation rate by attracting more bees to the forage site. My findings also suggest that honey bees do not discriminate among food sources based on differences in quality, but instead evaluate factors that may increase their efficiency of collection and recruitment to such a food resource. In nature, several other factors

including the age, moisture content, colour, pH and physical configuration of pollen grains may influence the choices made by foragers (Stanley and Linskens 1974). Further examination of these stimuli may help us to understand the intricate nature of pollen choice in *A. mellifera*.

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Table 12. Univariate ANOVA statistics for behaviours analyzed by BIBD models for bioassays of whole pollen odour, pollen lipid extract, particle size, protein content and handling time

Bioassay	Behaviour	N ^l	F	df	P
Whole Pollen Odour	Approaching	1078	13.70	8, 64	< 0.0001
	Landing	402	2.67	8, 64	0.0134
	Crawling	206	6.98	8, 64	< 0.0001
	Total Behaviours	1686	28.43	8, 64	< 0.0001
	Post Approaching ^a	608	4.01	8, 64	0.0007
Pollen Lipid Extract	Approaching	906	3.81	8, 64	0.0010
	Landing	709	2.76	8, 64	0.0111
	Crawling	450	3.46	8, 64	0.0022
	Total Behaviours	2065	10.49	8, 64	0.0001
	Post Approaching	1159	3.75	8, 64	0.0012
Particle Size	Approaching	931	0.89	8, 64	NS
	Landing	1290	2.01	8, 64	NS
	Hovering	413	10.51	8, 64	< 0.0001
	Scrabbling	263	1.57	8, 64	NS
	Total Behaviours	2897	6.37	8, 64	< 0.0001
	Post Approaching	1966	6.85	8, 64	< 0.0001
Protein	Approaching	646	0.95	4, 26	NS
	Landing	582	0.37	4, 26	NS
	Hovering	224	1.34	4, 26	NS
	Scrabbling	110	0.51	4, 26	NS

	Total Behaviours	1552	0.51	4, 26	NS
	Post Approaching	906	0.12	4, 26	NS
Handling Time	Approaching	242	1.54	2, 13	NS
	Landing	292	0.21	2, 13	NS
	Crawling Under 1 ^b	189	53.13	2, 13	< 0.0001
	Crawling Under 2 ^c	103	58.19	2, 13	< 0.0001
	Total Behaviours	826	54.09	2, 13	< 0.0001
	Post Approaching	584	51.45	2, 13	< 0.0001

¹Untransformed sum of occurrences of a behaviour, over all trials.

^aSum of all behaviours performed subsequent to approaching.

^bBees crawling through upper screen of bioassay dish.

^cBees crawling through lower screen of bioassay dish.

NS, Non-Significant

Table 13. Competing effects of pollen lipid extracts for numbers of bees approaching bioassay dishes (log-transformed

LSMs)

Extract treatment	Competing treatment effects								Competing treatment effect ^a	Treatment as competitor ^b
	Favourable				Non-Favourable					
Apple	Cellulose 0.98	Bee-Pro 0.86	Jack Pine 0.85	Soy Flour 0.81	YSClover 0.81	Phacelia 0.66	Sunflower 0.59	Canola 0.41	NS	NS
Canola	Sunflower 0.97	Cellulose 0.90	YSClover 0.89	Soy Flour 0.75	Bee-Pro 0.66	Phacelia 0.66	Jack Pine 0.60	Apple 0.60	NS	NS
Jack Pine	Phacelia 1.12	Cellulose 0.90	Bee-Pro 0.86	Soy Flour 0.86	Canola 0.82	Sunflower 0.79	Apple 0.76	YSClover 0.72	NS	NS
Phacelia	Jack Pine 0.98	Cellulose 0.93	Bee-Pro 0.91	Apple 0.83	Sunflower 0.81	Soy Flour 0.73	Canola 0.71	YSClover 0.61	NS	NS
Sunflower	Cellulose 0.88	Soy Flour 0.86	Jack Pine 0.78	Apple 0.71	Canola 0.70	Bee-Pro 0.66	Phacelia 0.54	YSClover 0.41	NS	NS
YSClover	Bee-Pro 0.97	Jack Pine 0.88	Canola 0.88	Soy Flour 0.86	Cellulose 0.83	Sunflower 0.70	YSClover 0.64	Phacelia 0.59	NS	NS
Bee-Pro	Soy Flour 0.93	Cellulose 0.87	Phacelia 0.85	YSClover 0.82	Sunflower 0.81	Canola 0.70	Apple 0.47	Jack Pine 0.41	NS	NS
Cellulose	Soy Flour 0.82	Apple 0.81	Cellulose 0.79	Canola 0.61	Phacelia 0.52	Bee-Pro 0.50	Jack Pine 0.45	Sunflower 0.41	NS	NS
Soy Flour	YSClover 0.81a	Sunflower 0.75a	Cellulose 0.73a	Canola 0.70a	Jack Pine 0.64a	Phacelia 0.60a	Bee-Pro -0.15b	Apple -0.25b	0.010	NS

Within each row, numbers represent approaches scored to the specified treatment in the presence of a single competing treatment. Favourable competitors increased the number of responses to the specified treatment, and unfavourable competitors decreased responses. A competitor on the far right side of the table attracted bees away from the specified treatment to itself, but a competing treatment on the far left side of the table increased the number of responses to the specified treatment by increasing the general level of attraction to both treatments. Competing treatments in the middle of the table exerted little effect on the number of bees approaching the specified treatment. L.S.Ms within the same row followed by the same letter are not significantly different (*t*-test, $\alpha = 0.05$, Bonferroni correction applied to within-row comparisons).

^aOmnibus comparison of responses to treatment, over all competing treatments (*F*-test, $\alpha = 0.05$).

^bOmnibus comparison of responses to all other treatments when specified treatment acts as the competitor (*F*-test, $\alpha = 0.05$).

YSClover, Yellow Sweetclover; NS, Non-significant.

Table 14. Competing effects of particle size classes for numbers of bees approaching bioassay dishes (log-transformed LSMs)

Particle size class treatment ^a	Competing treatment effects										Competing treatment effect ^b	Treatment as competitor ^c
	Favourable					Non-Favourable						
<20	300	38	53	150	75	20	600	45	NS	NS		
	0.98	0.96	0.91	0.85	0.85	0.85	0.79	0.75				
20	150	600	300	<20	38	45	75	53	NS	NS		
	1.00	0.96	0.93	0.90	0.85	0.82	0.75	0.75				
38	<20	53	45	75	20	600	150	NS	NS	NS		
	0.93	0.91	0.85	0.85	0.78	0.60	0.52				0.47	
45	75	20	<20	38	53	150	300	0.020	0.020	NS		
	1.00a	0.85a	0.85a	0.79a	0.78a	0.59ab	-0.020b					
53	<20	38	20	45	300	600	150	NS	NS	NS		
	0.88	0.79	0.71	0.70	0.64	0.60	0.59				0.50	
75	<20	600	20	45	150	38	300	NS	NS	NS		
	0.91	0.88	0.85	0.79	0.76	0.70	0.60				0.45	
150	<20	53	300	20	45	75	38	NS	NS	NS		
	0.98	0.85	0.84	0.81	0.75	0.71	0.67				0.56	
300	20	150	<20	45	38	600	75	NS	NS	NS		
	0.98	0.90	0.88	0.81	0.66	0.64	0.47				0.45	
600	20	<20	53	45	75	300	150	NS	NS	NS		
	0.93	0.88	0.81	0.79	0.70	0.70	0.55				0.45	

Within each row, numbers represent approaches scored to the specified treatment in the presence of a single competing treatment.

Favourable competitors increased the number of responses to the specified treatment, and unfavourable competitors decreased

responses. A competitor on the far right side of the table attracted bees away from the specified treatment to itself, but a competing treatment on the far left side of the table increased the number of responses to the specified treatment by increasing the general level of attraction to both treatments. Competing treatments in the middle of the table exerted little effect on the number of bees approaching the specified treatment. LSMs within the same row followed by the same letter are not significantly different (t -test, $\alpha = 0.05$, Bonferroni correction applied to within-row comparisons).

^aExcept for particles < 20 μm , the number designating each particle size class represents the lower threshold for that size range.

Particles within a given class were upper-bound by the threshold of the next larger particle size class. The upper limit for the 600 μm size class was 1000 μm .

^bOmnibus comparison of responses to treatment, over all competing treatments (F -test, $\alpha = 0.05$).

^cOmnibus comparison of responses to all other treatments when specified treatment acts as the competitor (F -test, $\alpha = 0.05$).

YSClover, Yellow Sweetclover; NS, Non-significant.

Table 15. Competing effects of particle size classes for total behaviours (log-transformed LSMs)

Particle size class treatment ^a	Competing treatment effects												Competing treatment effect ^b	Treatment as competitor ^c
	Favourable						Non-Favourable							
<20	20	53	150	75	300	38	45	600	NS	NS	600	1.41		
	1.54	1.54	1.51	1.49	1.49	1.47	1.45	1.41						
20	150	<20	300	38	600	75	53	45	NS	NS	45	1.38		
	1.58	1.53	1.50	1.46	1.43	1.39	1.39	1.38						
38	20	<20	75	53	45	150	300	600	NS	NS	600	1.22		
	1.44	1.44	1.43	1.40	1.34	1.30	1.29	1.22						
45	75	20	53	<20	300	38	600	150	NS	NS	150	0.91b		
	1.41a	1.33ab	1.27ab	1.24ab	1.15ab	1.10ab	1.04ab	0.91b						
53	<20	38	20	150	600	45	75	300	NS	NS	300	0.98		
	1.41	1.39	1.27	1.24	1.16	1.02	1.01	0.98						
75	<20	45	20	38	53	150	600	300	NS	NS	300	1.05		
	1.38	1.26	1.24	1.22	1.14	1.13	1.09	1.05						
150	<20	20	38	53	300	75	45	600	NS	NS	600	1.08		
	1.43	1.38	1.26	1.22	1.22	1.16	1.08	1.08						
300	20	<20	150	45	38	53	600	75	NS	NS	75	0.81b		
	1.31a	1.21ab	1.19ab	1.08ab	1.05ab	1.01ab	0.98ab	0.81b						
600	<20	20	53	300	150	45	38	75	NS	NS	75	0.88		
	1.22	1.15	1.07	1.01	1.00	0.98	0.93	0.88						

Within each row, numbers represent the sum of all behaviours scored to the specified treatment in the presence of a single competing treatment. Favourable competitors increased the number of responses to the specified treatment, and unfavourable competitors decreased responses. A competitor on the far right side of the table elicited more behaviours from bees, to the detriment of the specified treatment. However, a competing treatment on the far left side of the table increased the number of responses to the specified treatment by increasing the general level of activity to both treatments. Competing treatments in the middle of the table exerted little effect on the total number of behaviours scored to the specified treatment. LSMs within the same row followed by the same letter are not significantly different (*t*-test, $\alpha = 0.05$, Bonferroni correction applied to within-row comparisons).

^aExcept for particles <20 μm , the number designating each particle size class represents the lower threshold for that size range.

Particles within a given class were upper-bound by the threshold of the next larger particle size class. The upper limit for the 600 μm size class was 1000 μm .

^bOmnibus comparison of responses to treatment, over all competing treatments (*F*-test, $\alpha = 0.05$).

^cOmnibus comparison of responses to all other treatments when specified treatment acts as the competitor (*F*-test, $\alpha = 0.05$).

YSClover, Yellow Sweetclover; NS, Non-significant.

Table 16. Univariate ANOVA statistics for main treatment effects from BIBD models, by each behaviour scored for the interaction of multiple factors bioassay

Behaviour	N ^a	Treatment	F	df	P
Approaching	1055	Lipid Extract	69.94	1, 86	< 0.0001
		Particle Size	0.43	1, 86	NS
		Handling Time	5.13	1, 86	0.0261
Landing	577	Lipid Extract	105.80	1, 86	< 0.0001
		Particle Size	0.34	1, 86	NS
		Handling Time	8.75	1, 86	0.0040
Crawling	219	Lipid Extract	28.25	1, 86	< 0.0001
		Particle Size	0.02	1, 86	NS
Under 1 ^b		Handling Time	5.16	1, 86	0.0256
Crawling	144	Lipid Extract	44.06	1, 86	< 0.0001
		Particle Size	0.09	1, 86	NS
Under 2 ^c		Handling Time	18.18	1, 86	< 0.0001
Total	1995	Lipid Extract	122.60	1, 86	< 0.0001
		Particle Size	0.16	1, 86	NS
		Handling Time	0.76	1, 86	NS
Post	940	Lipid Extract	106.34	1, 86	< 0.0001
		Particle Size	0.03	1, 86	NS
Approaching ^d		Handling Time	0.01	1, 86	NS

^aUntransformed sum of occurrences of a behaviour, over all trials.

^bBees crawling through upper screen of bioassay dish.

^cBees crawling through lower screen of bioassay dish.

^dSum of all behaviours performed subsequent to approaching.

NS, Non-Significant

Table 17. Competing effects of multiple factors for numbers of bees approaching bioassay dishes (log-transformed LSMs)

Treatment			Competing treatment effects							Competing treatment effect ^a	Treatment as competitor ^b
Lipid extract	Particle size	Handling time	Favourable				Non-favourable				
Absent	Small	Low	P:L:Lo 0.91a	P:L:H 0.87ab	A:S:H 0.85ab	A:L:H 0.75abc	P:S:Lo 0.70abc	A:L:Lo 0.66bc	P:S:H 0.55c	0.017	0.0045
Absent	Small	High	P:S:Lo 1.02a	A:L:Lo 0.93ab	P:L:Lo 0.89ab	P:L:H 0.88ab	A:S:Lo 0.87ab	A:L:H 0.85ab	P:S:H 0.73b	NS	0.0031
Absent	Large	Low	P:L:Lo 0.96a	P:S:Lo 0.93a	P:L:H 0.91ab	A:S:H 0.88ab	P:S:H 0.85ab	A:L:H 0.83ab	A:S:Lo 0.70b	NS	<0.0001
Absent	Large	High	P:S:Lo 0.98a	P:L:Lo 0.91ab	P:L:H 0.91ab	P:S:H 0.88ab	A:S:H 0.78ab	A:L:Lo 0.78ab	A:S:Lo 0.73b	NS	0.0028
Present	Small	Low	A:L:Lo 1.08	P:L:Lo 1.06	P:S:H 1.06	A:S:H 1.04	A:S:Lo 1.03	P:L:H 1.00	A:L:H 1.00	NS	0.027
Present	Small	High	A:S:H 1.10	A:L:Lo 1.09	P:L:H 1.08	P:S:Lo 1.04	P:L:H 1.02	A:L:H 1.02	A:S:Lo 0.98	NS	<0.0001
Present	Large	Low	P:L:H 1.16	A:L:Lo 1.13	A:L:H 1.12	A:S:H 1.11	A:S:Lo 1.04	P:S:H 1.04	P:S:Lo 0.98	NS	NS
Present	Large	High	A:S:H 1.15	A:L:H 1.13	A:L:Lo 1.12	P:L:Lo 1.11	P:S:H 1.06	P:S:Lo 1.05	A:S:Lo 1.00	NS	NS

Within each row, numbers represent approaches scored to the specified treatment in the presence of a single competing treatment. Favourable competitors increased the number of responses to the specified treatment, and unfavourable competitors decreased responses. A competitor on the

far right side of the table attracted bees away from the specified treatment to itself, but a competing treatment on the far left side of the table increased the number of responses to the specified treatment by increasing the general level of attraction to both treatments. Competing treatments in the middle of the table exerted little effect on the number of bees approaching the specified treatment. LSMs within the same row followed by the same letter are not significantly different (*t*-test, $\alpha = 0.05$, Bonferroni correction applied to within-row comparisons).

^aOmnibus comparison of responses to treatment, over all competing treatments (*F*-test, $\alpha = 0.05$).

^bOmnibus comparison of responses to all other treatments when specified treatment acts as the competitor (*F*-test, $\alpha = 0.05$).

NS, Non-significant; A, Lipid extract absent; P, Lipid extract present; S, Small particle size; L, Large particle size; Lo, Low handling time; H, High handling time.

Table 18. Competing effects of multiple factors for total behaviours (log-transformed LSMs)

Treatment		Competing treatment effects										Competing treatment effect ^d	Treatment as competitor ^b
Lipid extract	Particle size	Handling time	Favourable					Non-favourable					
Absent	Small	Low	A:S:H 1.10a	P:L:Lo 1.10a	P:L:H 1.06ab	A:L:H 0.96abc	P:S:Lo 0.93abc	A:L:Lo 0.83bc	P:S:H 0.70c			0.022	< 0.0001
Absent	Small	High	P:S:Lo 1.23a	P:L:H 1.08ab	A:L:Lo 1.06ab	P:L:Lo 1.06ab	A:S:Lo 1.04ab	P:S:H 0.97b	A:L:H 0.95b			NS	< 0.0001
Absent	Large	Low	P:L:Lo 1.12	A:S:H 1.06	P:S:Lo 1.06	P:L:H 1.02	P:S:H 1.02	A:L:H 0.97	A:S:Lo 0.93			NS	< 0.0001
Absent	Large	High	P:S:Lo 1.26a	P:L:Lo 1.08abc	P:L:H 1.06abc	P:S:H 1.00bc	A:L:Lo 0.93bc	A:S:H 0.88bc	A:S:Lo 0.83c			0.029	< 0.0001
Present	Small	Low	A:S:H 1.53	P:S:H 1.46	P:S:Lo 1.45	P:L:Lo 1.39	P:L:H 1.36	A:S:Lo 1.33	A:L:H 1.29			NS	0.0038
Present	Small	High	P:S:Lo 1.40	A:L:Lo 1.37	A:L:H 1.36	A:S:H 1.33	P:L:Lo 1.33	P:L:H 1.28	A:S:Lo 1.16			NS	0.0001
Present	Large	Low	A:L:H 1.47	A:L:Lo 1.47	P:L:Lo 1.47	A:S:H 1.42	A:S:Lo 1.39	P:S:H 1.38	P:S:Lo 1.37			NS	0.0087
Present	Large	High	A:S:H 1.45	A:L:Lo 1.44	P:L:Lo 1.44	A:L:Lo 1.42	P:S:Lo 1.35	A:S:Lo 1.31	P:S:H 1.30			NS	0.0031

Within each row, numbers represent the sum of all behaviours scored to the specified treatment in the presence of a single competing treatment.

Favourable competitors increased the number of responses to the specified treatment, and unfavourable competitors decreased responses. A

competitor on the far right side of the table elicited more behaviours from bees, to the detriment of the specified treatment. However, a

competing treatment on the far left side of the table increased the number of responses to the specified treatment by increasing the general level of activity to both treatments. Competing treatments in the middle of the table exerted little effect on the total number of behaviours scored to the specified treatment. LSMs within the same row followed by the same letter are not significantly different (*t*-test, $\alpha = 0.05$, Bonferroni correction applied to within-row comparisons).

^aOmnibus comparison of responses to treatment, over all competing treatments (*F*-test, $\alpha = 0.05$).

^bOmnibus comparison of responses to all other treatments when specified treatment acts as the competitor (*F*-test, $\alpha = 0.05$).

NS, Non-significant; A, Lipid extract absent; P, Lipid extract present; S, Small particle size; L, Large particle size; Lo, Low handling time; H, High handling time.

Figure 31. Dish used for whole pollen odour bioassay, showing position of screens containing 9.5 and 3.2 mm openings. Glass dishes were wrapped in black paper and contained 500 mg of pollen or pollen analogue.

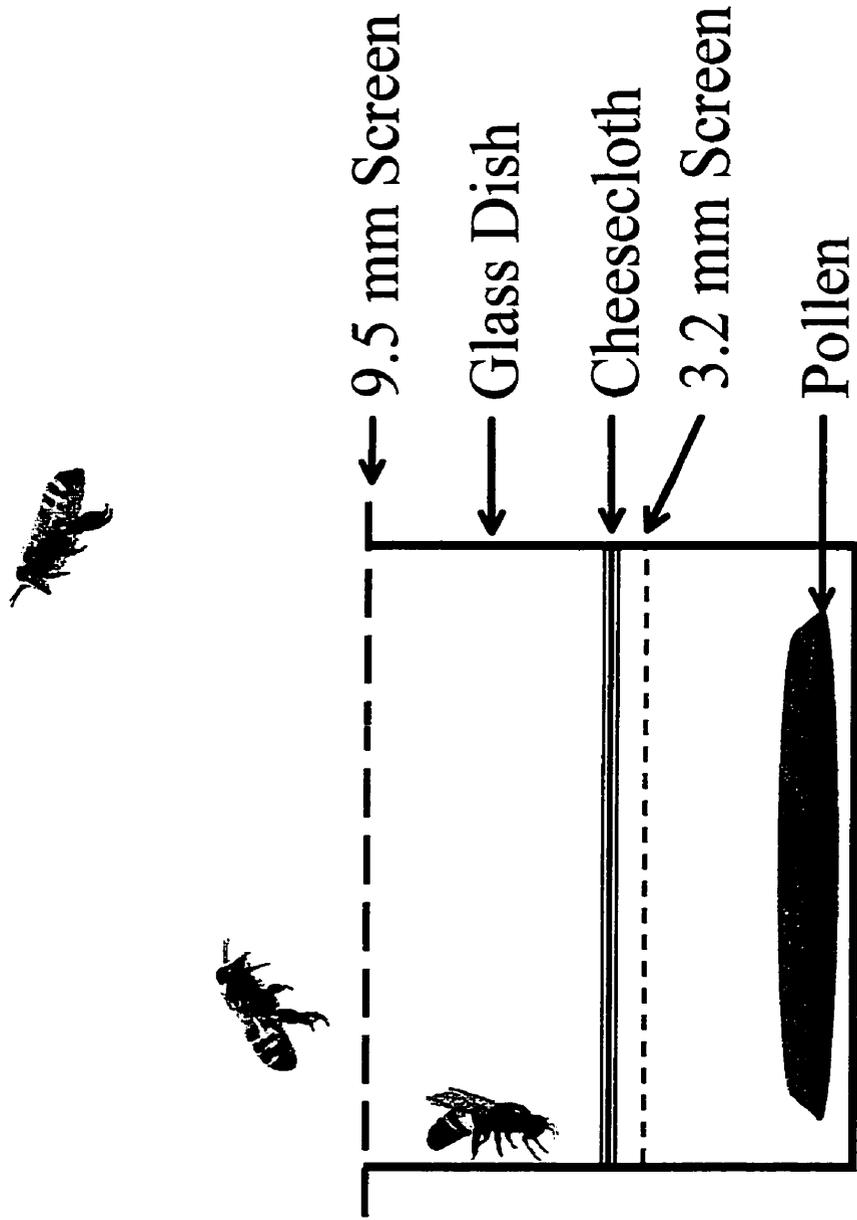


Fig. 32. Petri dish used for lipid extract bioassay, containing filter paper and a screen with 9.5 mm openings. Five hundred mg samples of pollen were extracted in 15 mL of n-pentane.

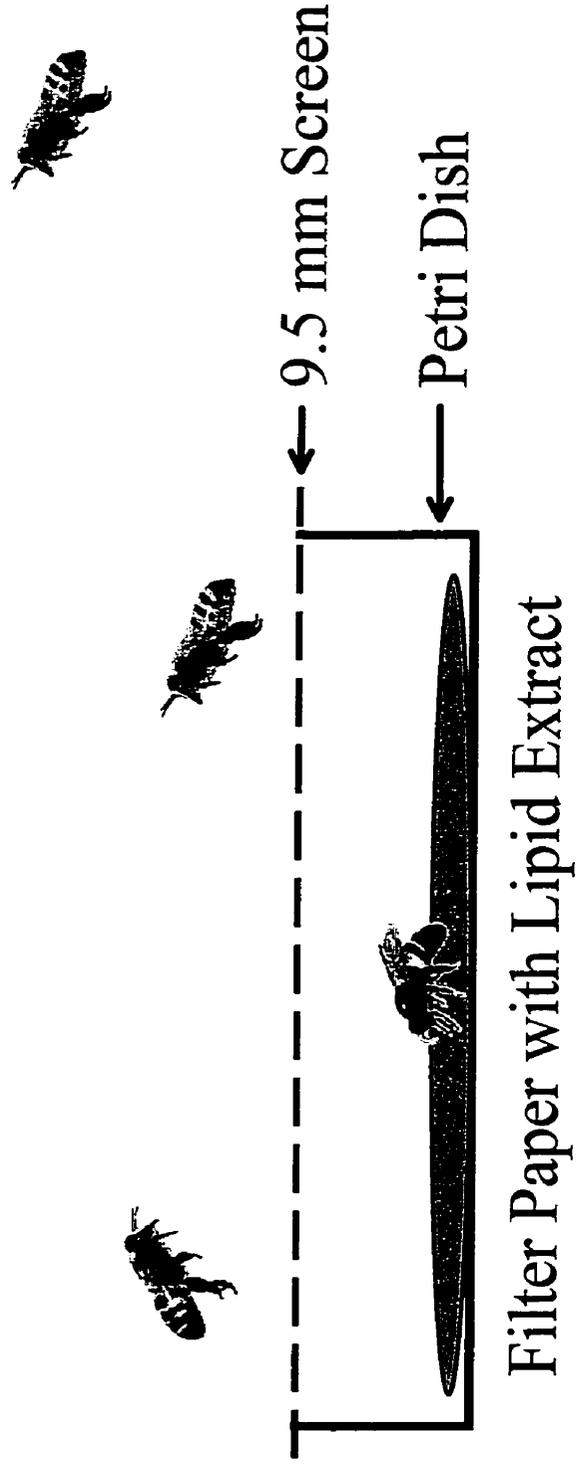


Fig. 33. Bioassay dish used for particle size and protein bioassays. Dishes were wrapped in black paper and contained 25 g of soybean meal (particle size bioassay) or a 25 g mixture of defatted soy flour and alpha-cellulose fiber (protein bioassay).

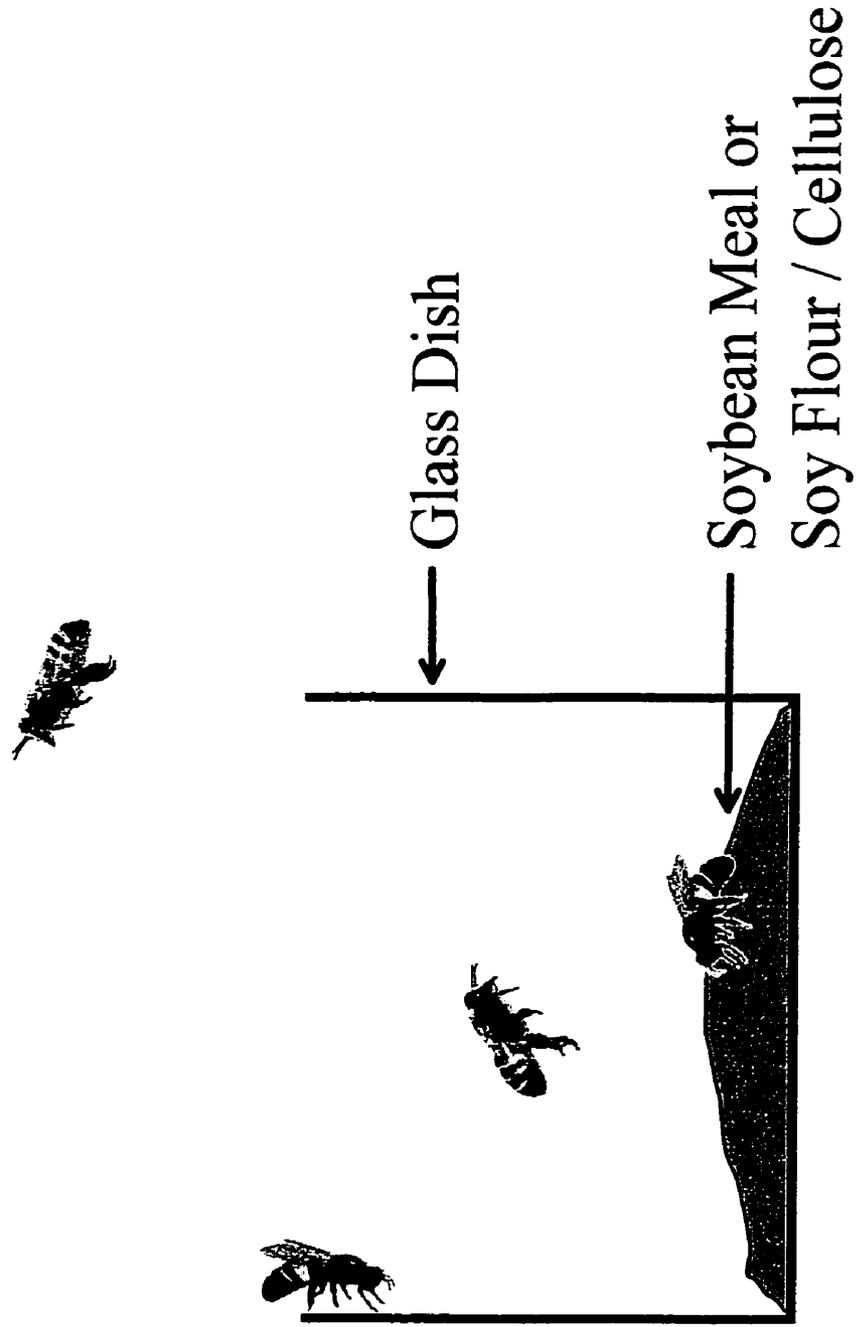


Fig. 34. Bioassay dish used for handling time bioassay. Depending on the level of handling time tested, different combinations of screens having 5.1 and 9.5 mm openings were used (screen #1 and #2). The filter paper in all trials contained pollen lipids from *M. officinalis*. Dishes were wrapped in black paper.

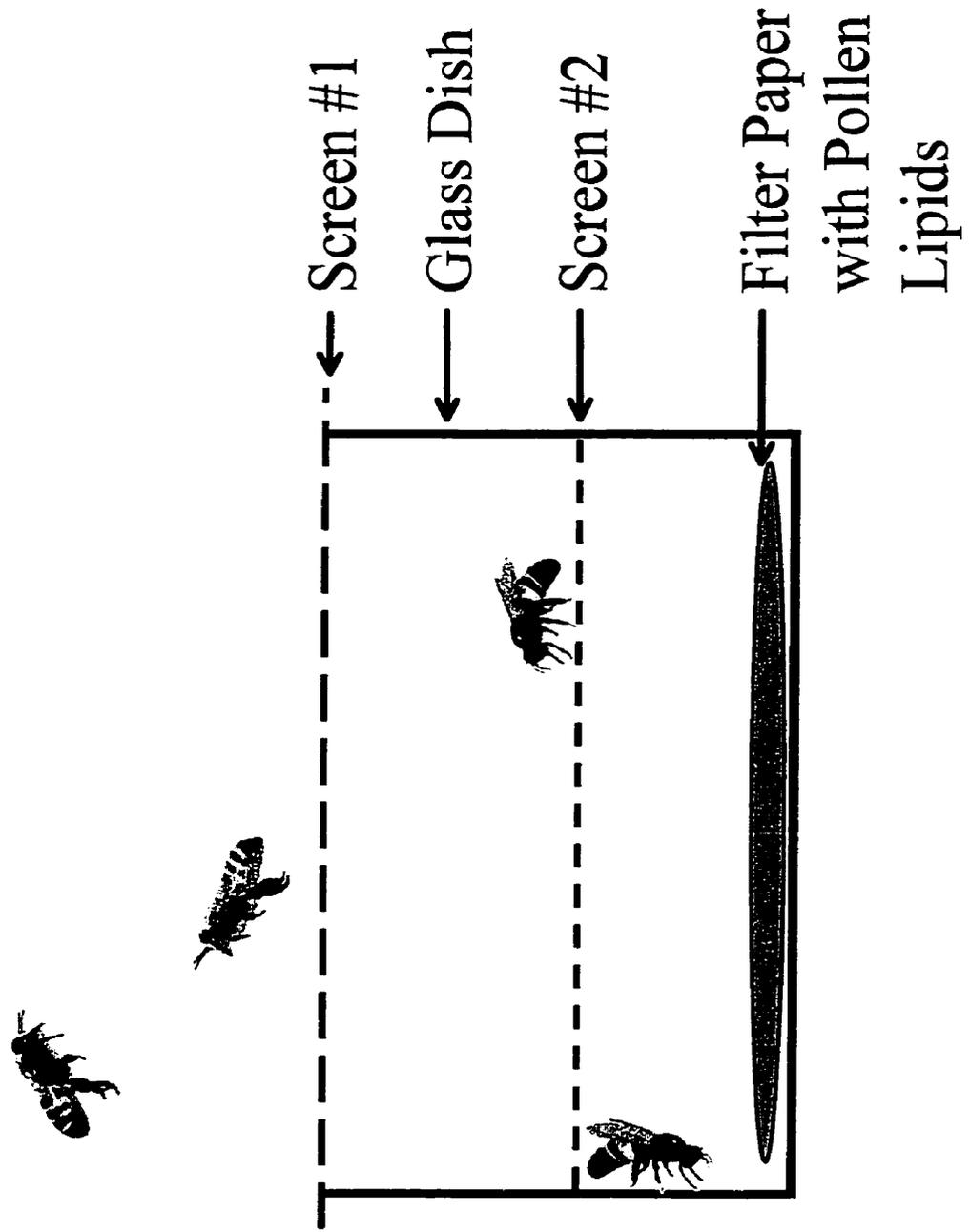


Fig. 35. Bioassay dish used to test a combination of pollen-based factors, presented simultaneously to honey bees. Pollen lipid extract, particle size and handling time were varied in different combinations of low and high stimulus intensities. For individual dishes, screens #1 and #2 identical, having either 5.1 mm (high handling time) or 9.5 mm (low handling time) openings. Cellulose substrate used in the dish (10 g) was either alphacel non-nutritive bulk [small particle sizes (<38 μm)] or bleached spruce wood sawdust [large particle sizes (75 - 150 μm)]. Pollen lipid extract consisted of an n-pentane extract of *M. officinalis* and, if no odour was required, a solvent blank was used. Dishes were wrapped in black paper.

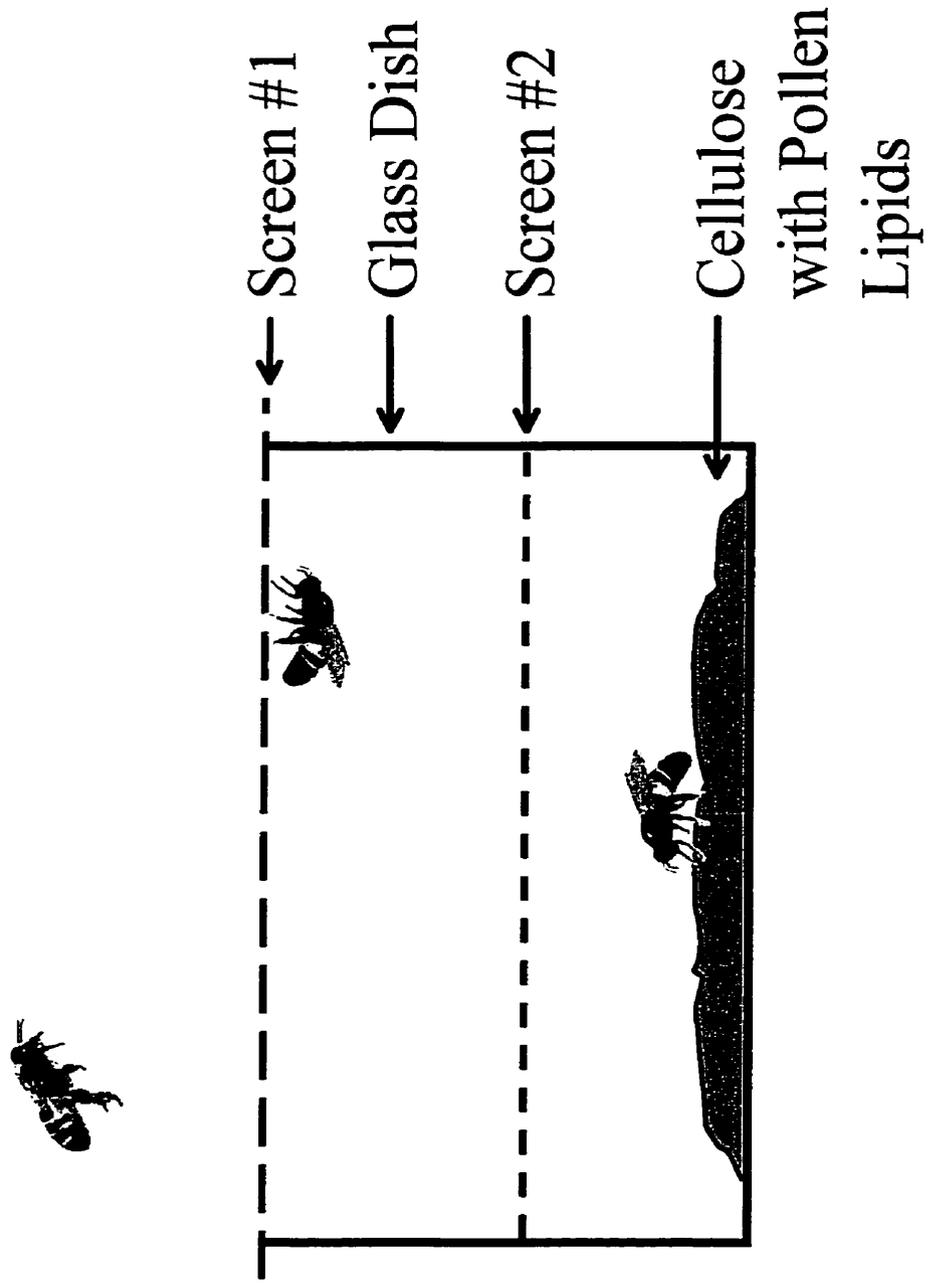


Fig. 36. Behaviours of foragers scored to pollen and pollen analogues in whole pollen odour bioassay. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not different from each other, for the sum of all behaviours per bar (*t*-test, $\alpha = 0.05$, Bonferroni correction) (YSClover, Yellow Sweetclover).

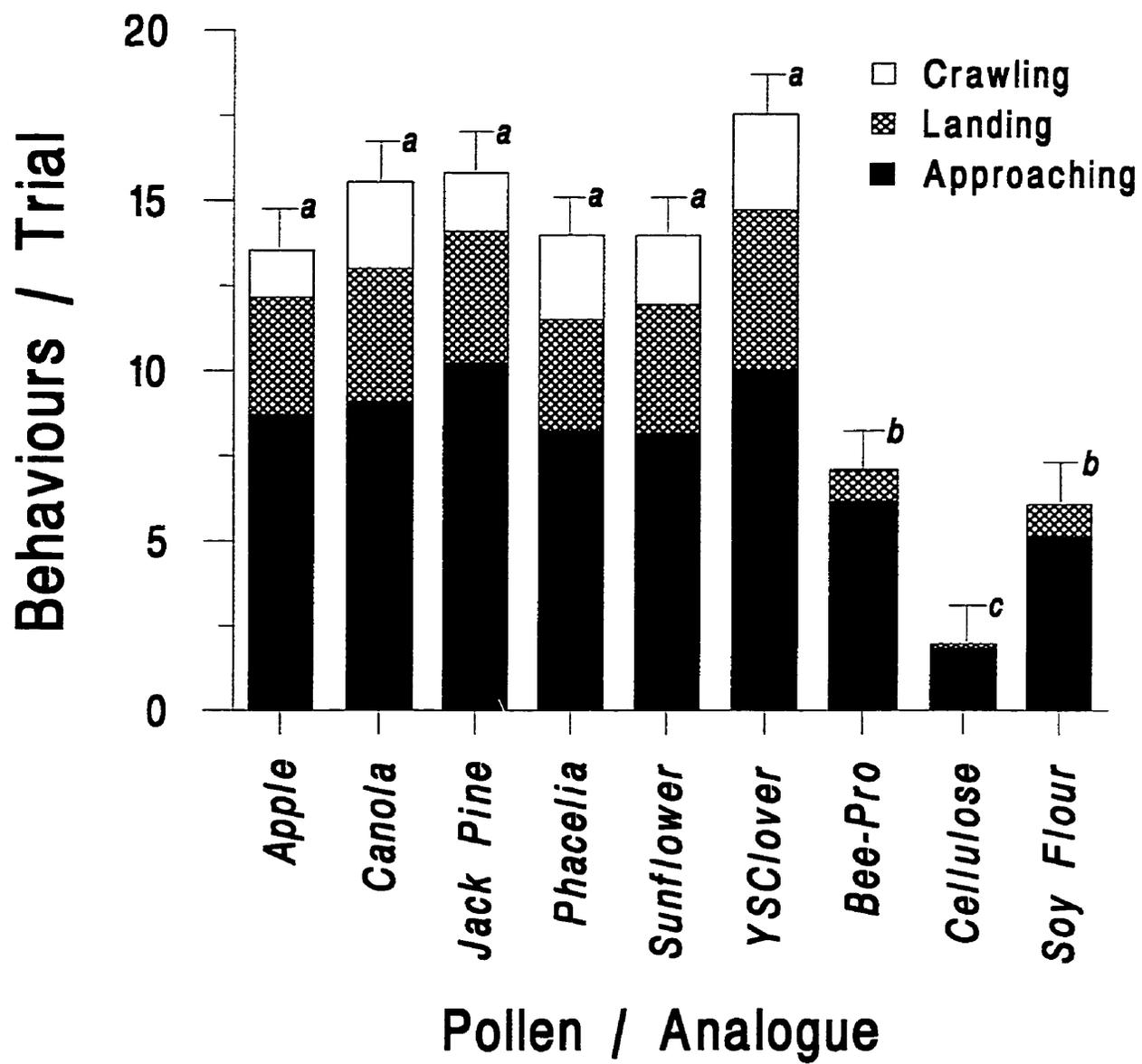


Fig. 37. Behaviours of foragers scored to pollen and pollen analogues in pollen lipid extract bioassay. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not different from each other, for the sum of all behaviours per bar (*t*-test, $\alpha = 0.05$, Bonferroni correction) (YSClover, Yellow Sweetclover).

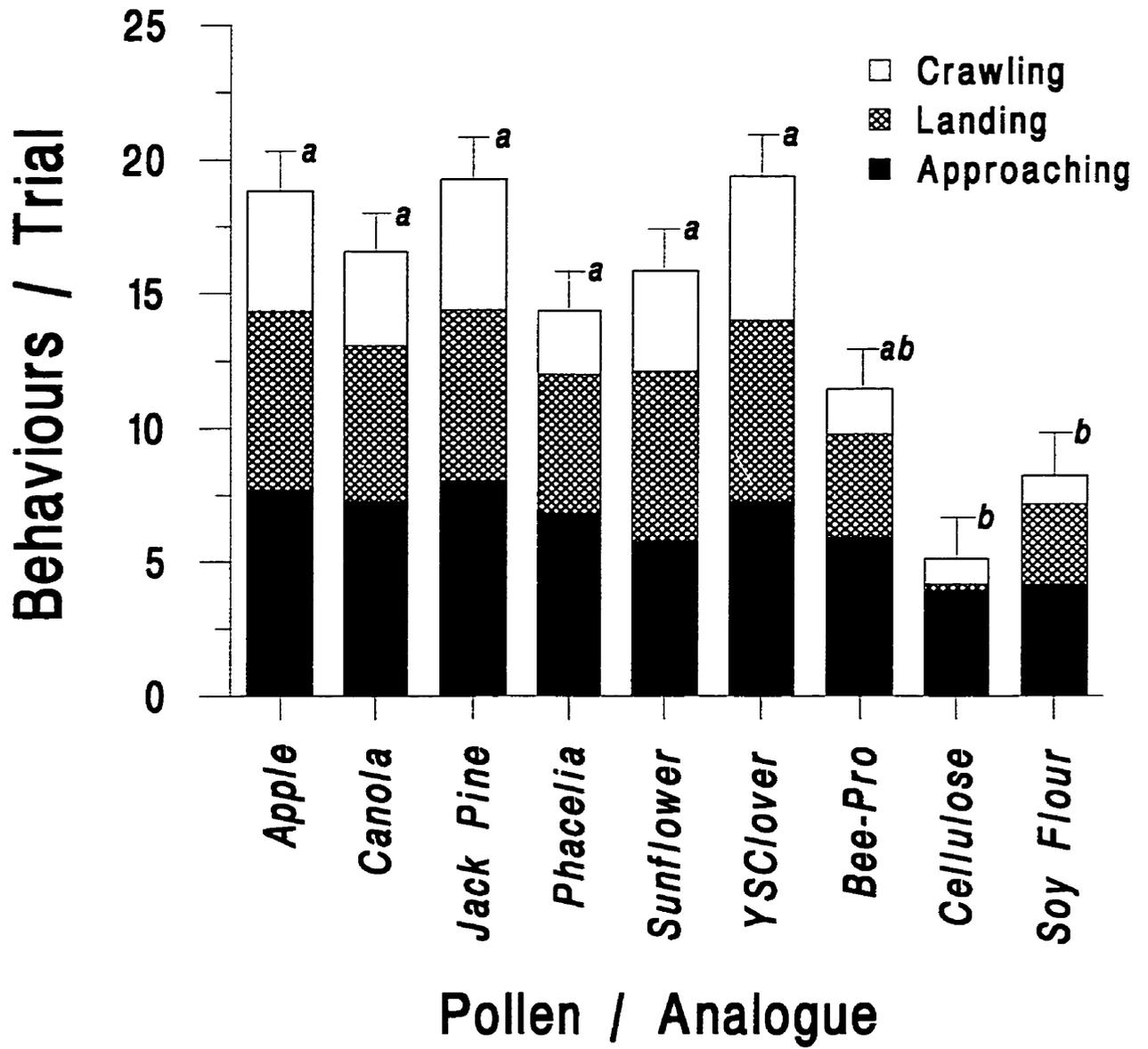


Fig. 38. Behaviours of foragers scored to different size ranges of soybean meal in particle size bioassay. Except for particles $< 20 \mu\text{m}$, the number designating each particle size class represents the lower threshold for that size range. Particles within a given class were upper-bound by the threshold of the next larger particle size class; the upper limit for the $600 \mu\text{m}$ size class was $1000 \mu\text{m}$. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not significantly different from each other, for the sum behaviours per bar (t -test, $\alpha = 0.05$, Bonferroni correction).

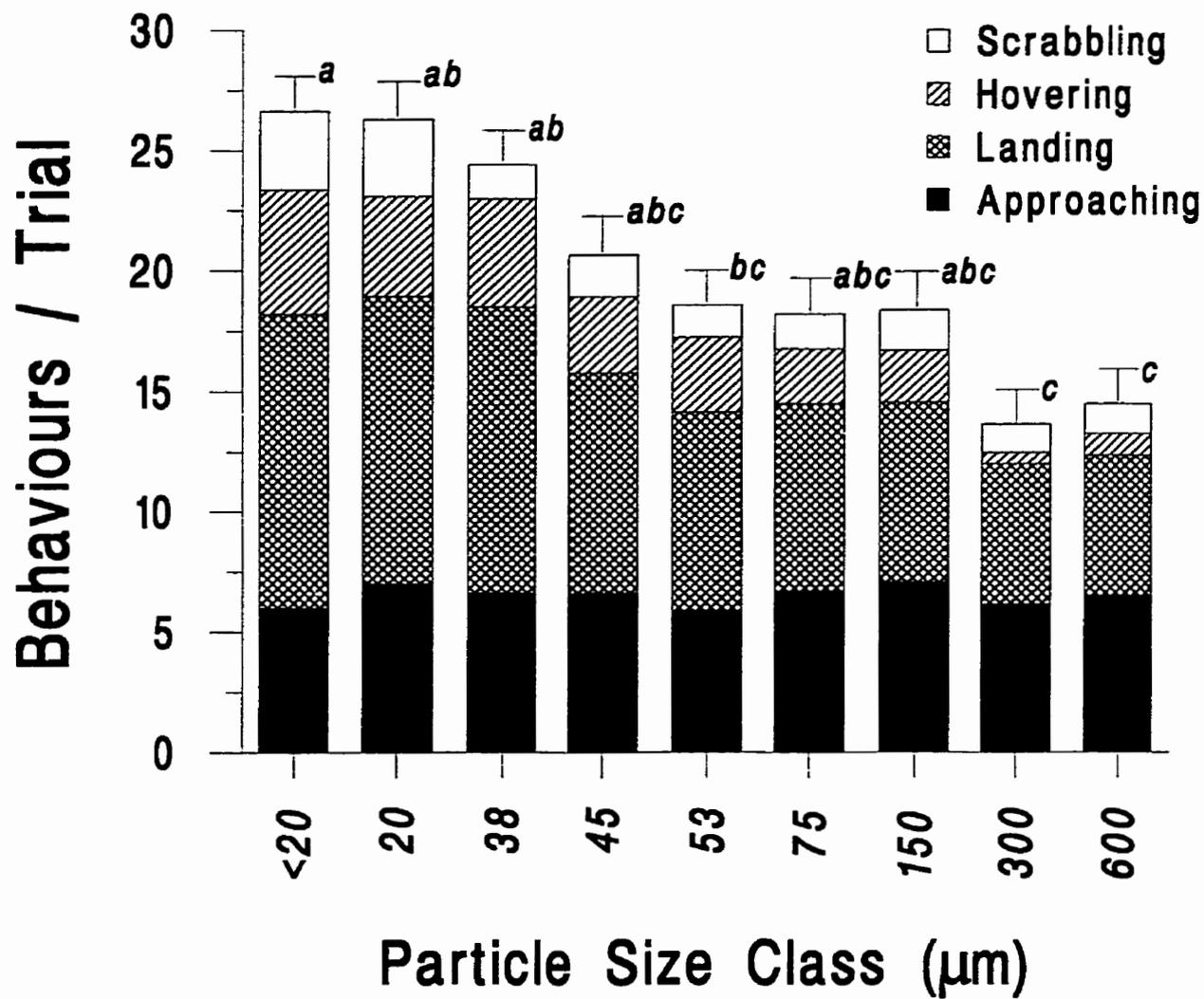


Fig. 39. Weight of soybean meal collected per forager for size ranges used in particle size bioassay. Except for particles $< 20 \mu\text{m}$, the number designating each particle size class represents the lower threshold for that size range. Particles within a given class were upper-bound by the threshold of the next larger particle size class; the upper limit for the $600 \mu\text{m}$ size class was $1000 \mu\text{m}$. Data are LSMs and vertical lines denote standard errors. Treatments with the same letter are not significantly different from each other (t -test, $\alpha = 0.05$, Bonferroni correction).

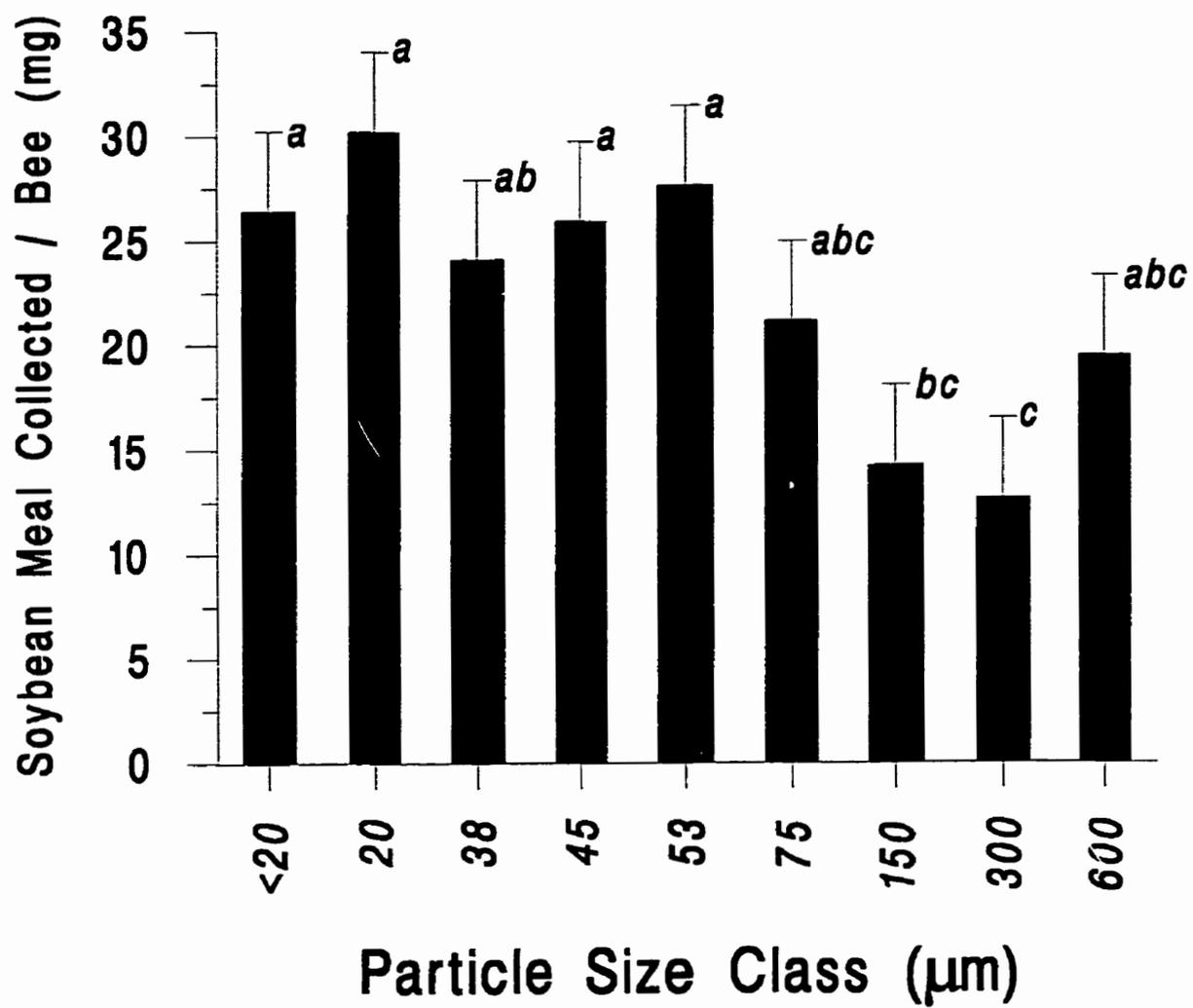


Fig. 40. Behaviours of foragers scored to soy flours with different levels of crude protein (w/w), in protein content bioassay. Data are untransformed LSMs and vertical lines denote standard errors for the sum of all behaviours per bar.

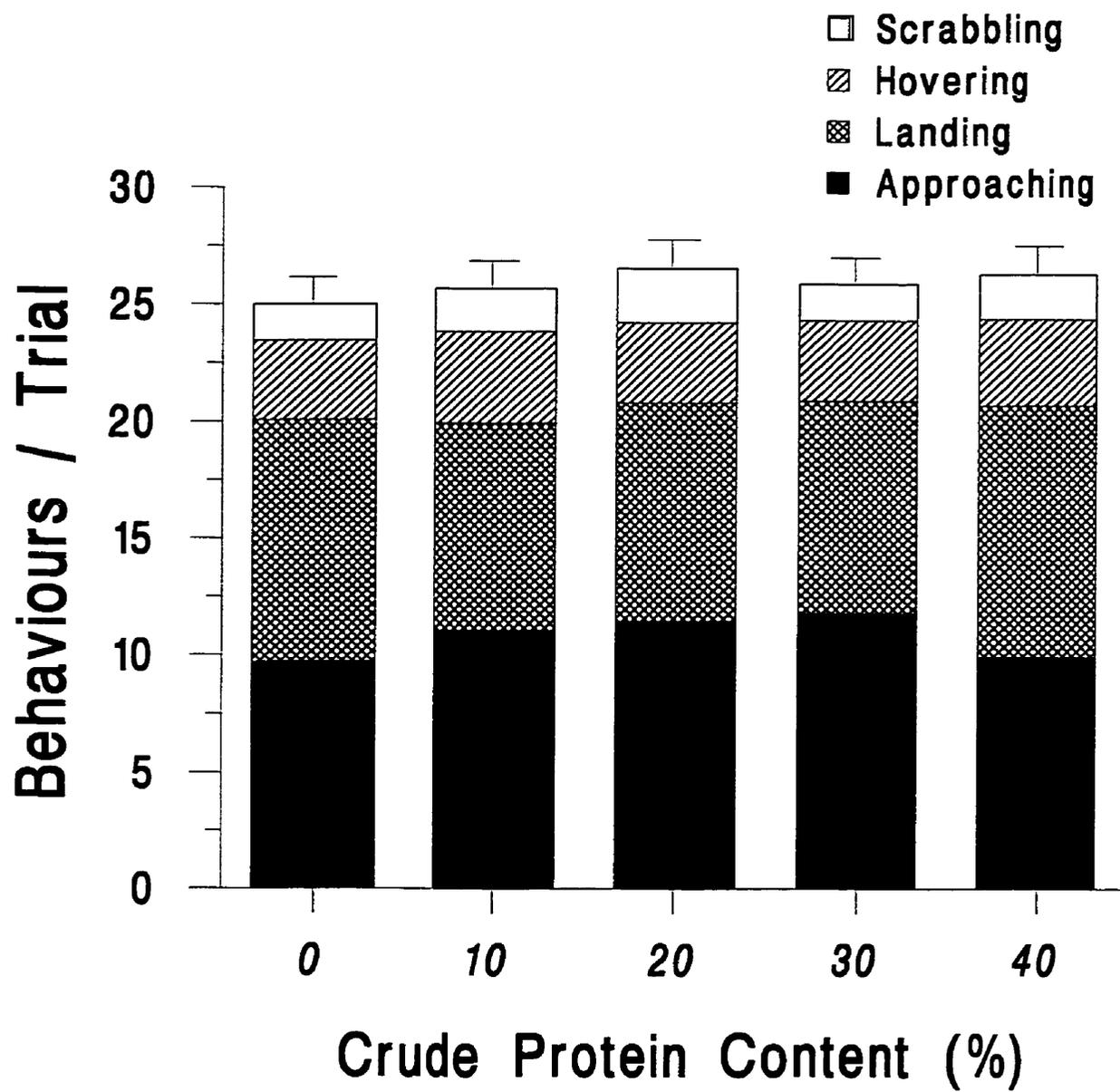


Fig. 41. Behaviours of foragers scored to bioassay dishes having different levels of handling time. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not different from each other, for the sum of all behaviours per bar (t -test, $\alpha = 0.05$, Bonferroni correction) (Under 1, bees crawling through upper screen of bioassay dish; Under 2, bees crawling through lower screen of bioassay dish).

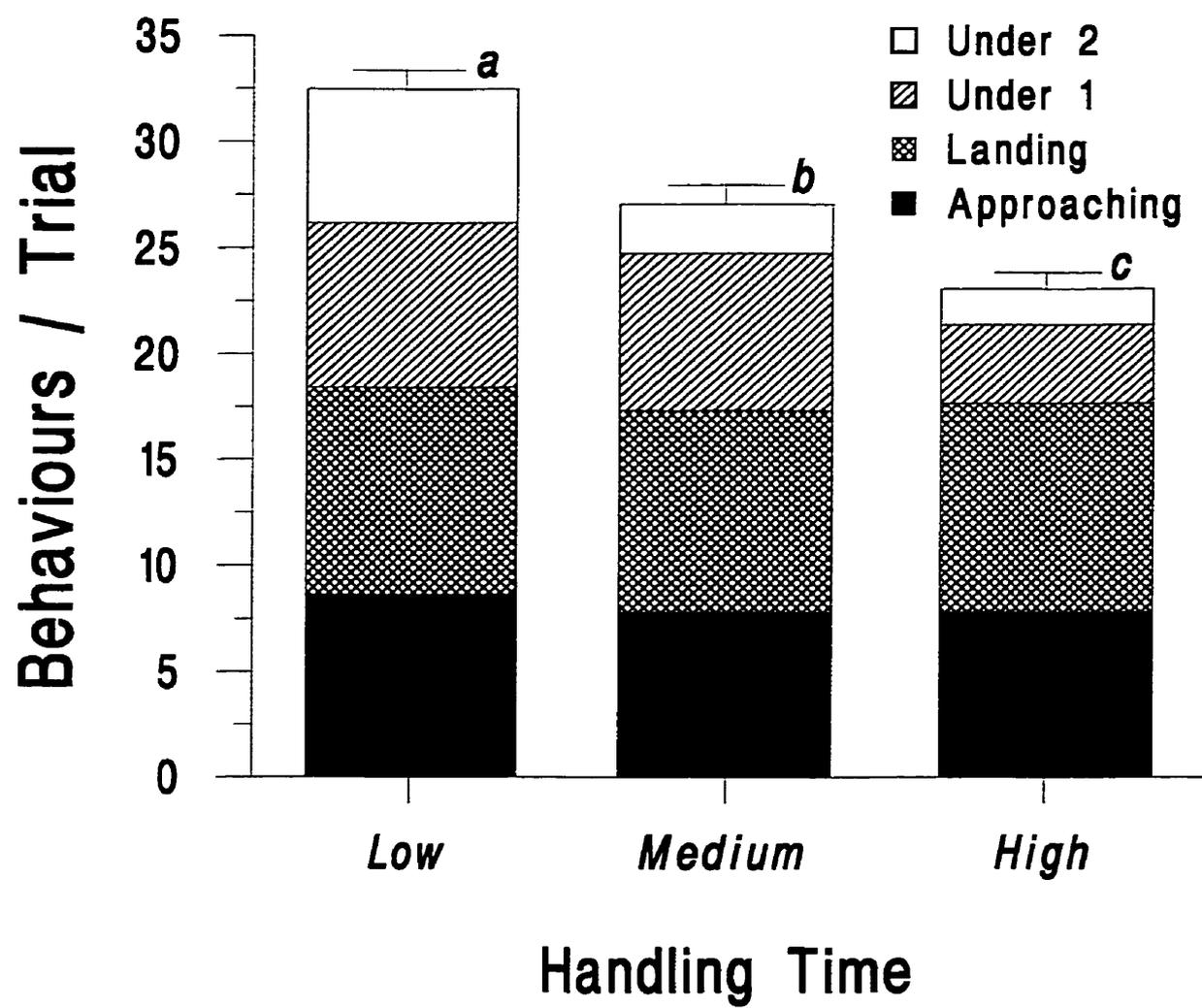


Fig. 42. Effect of simultaneous manipulation of three factors (presence of pollen lipid extract, size of particle and handling time), on worker foraging behaviour in the interaction of multiple factors bioassay. Graphs show the main effects for each of the three treatments. For detailed explanation of treatments levels, see Figure 35. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not different from each other, for the sum of all behaviours per bar (t -test, $\alpha = 0.05$, Bonferroni correction) (Under 1, bees crawling through upper screen of bioassay dish; Under 2, bees crawling through lower screen of bioassay dish).

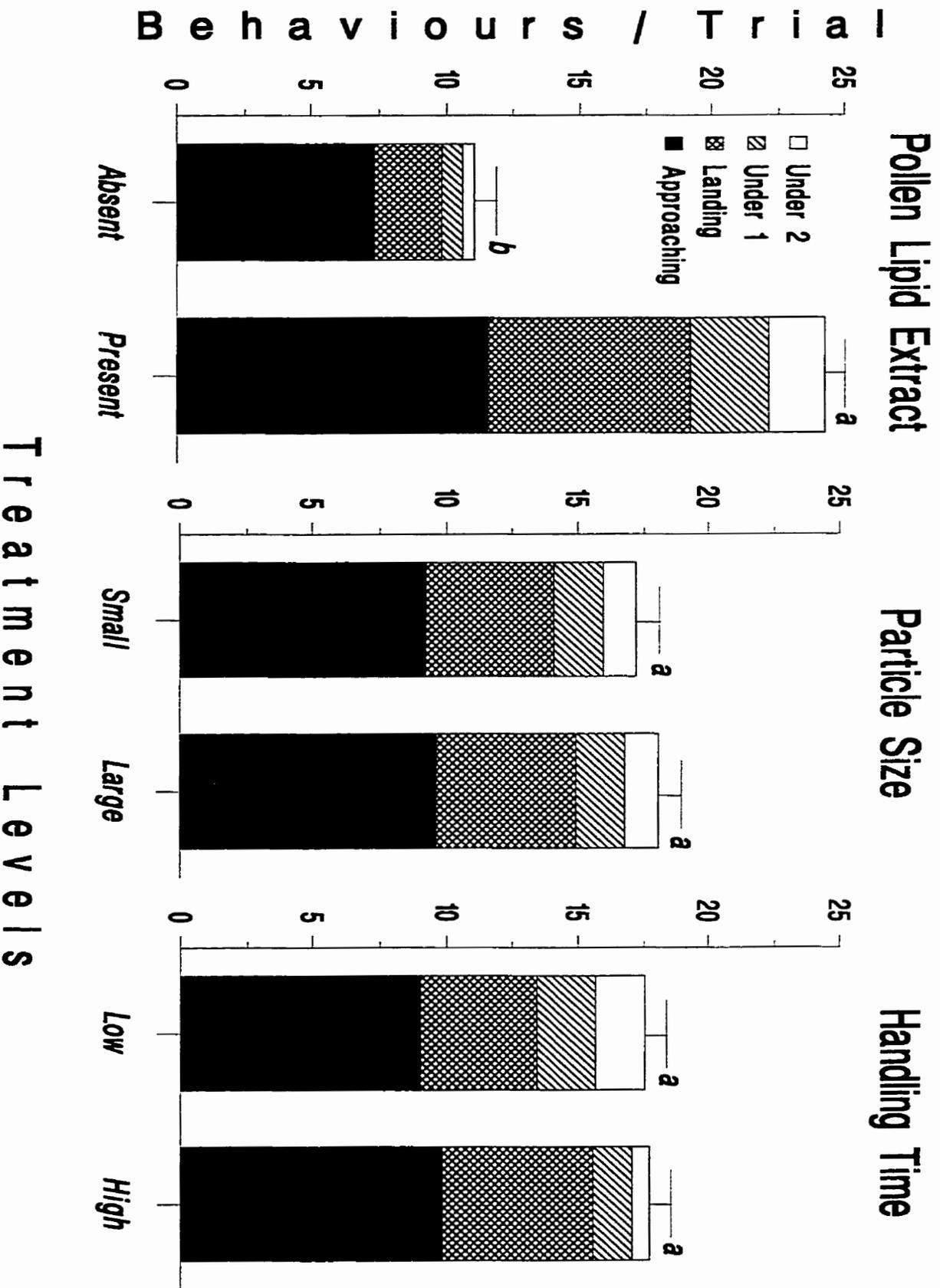


Fig. 43. Interactive effects of handling time and particle size on the number of bees approaching and landing on dishes during the interaction of multiple factors bioassay. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not different from each other (t -test, $\alpha = 0.05$, Bonferroni correction).

Approaches and Landings / Trial

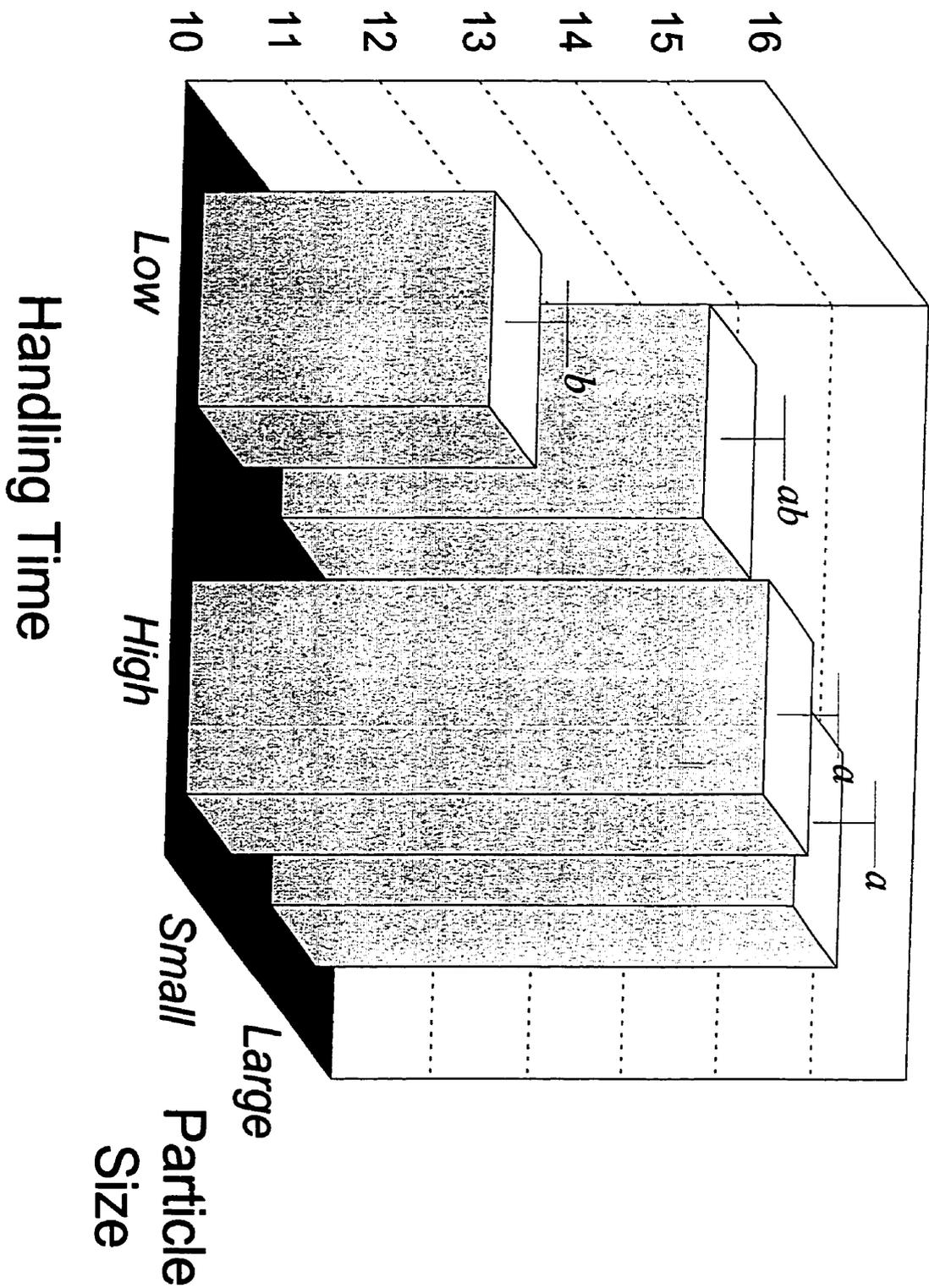


Fig. 44. Interactive effects of pollen lipid extract and handling time on the number of bees crawling under the lower screen (screen #2) of dishes during the interaction of multiple factors bioassay. Data are untransformed LSMs and vertical lines denote standard errors. Treatments with the same letter are not different from each other (*t*-test, $\alpha = 0.05$, Bonferroni correction).

Bees Under Screen #2 / Trial

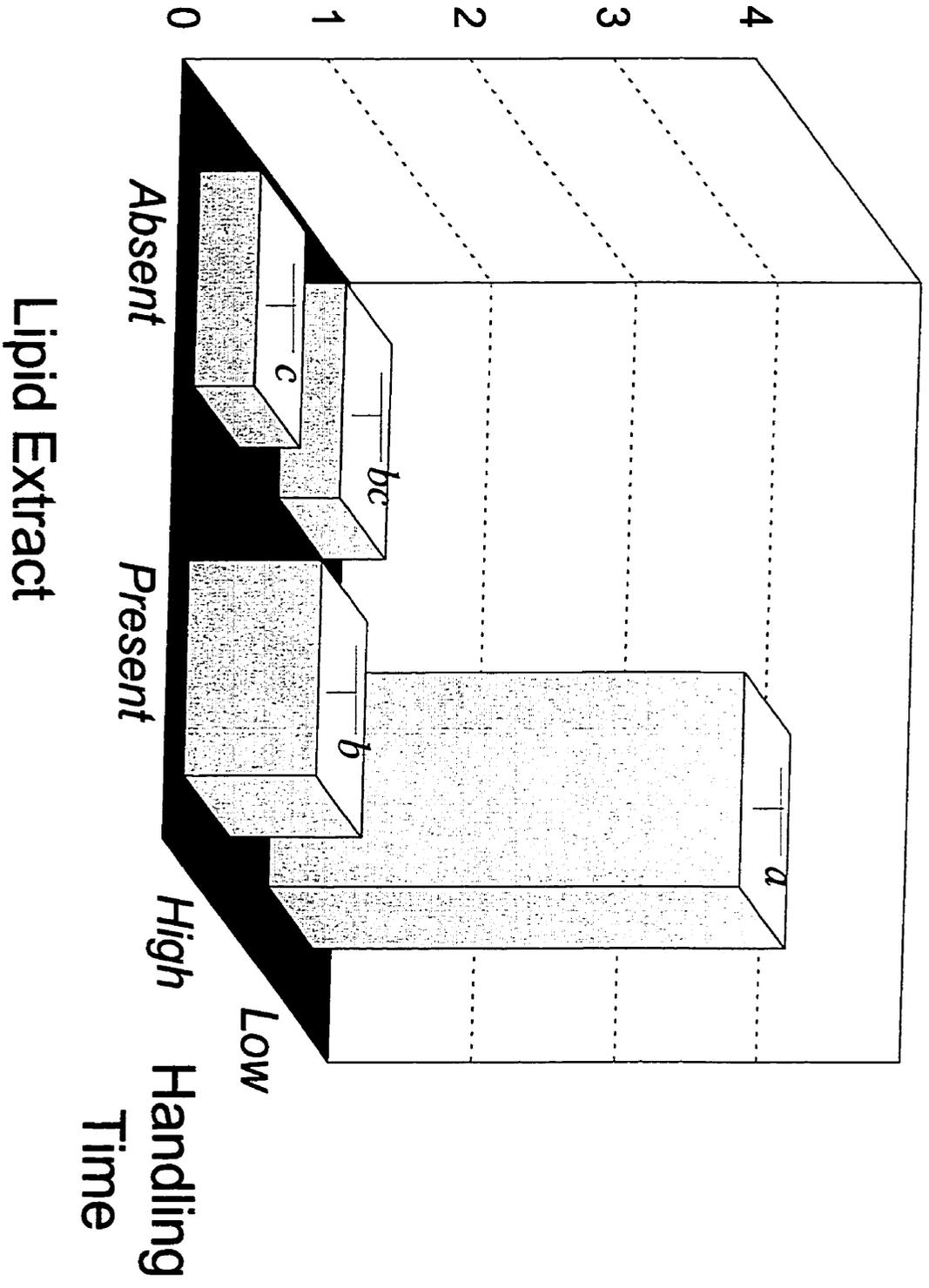
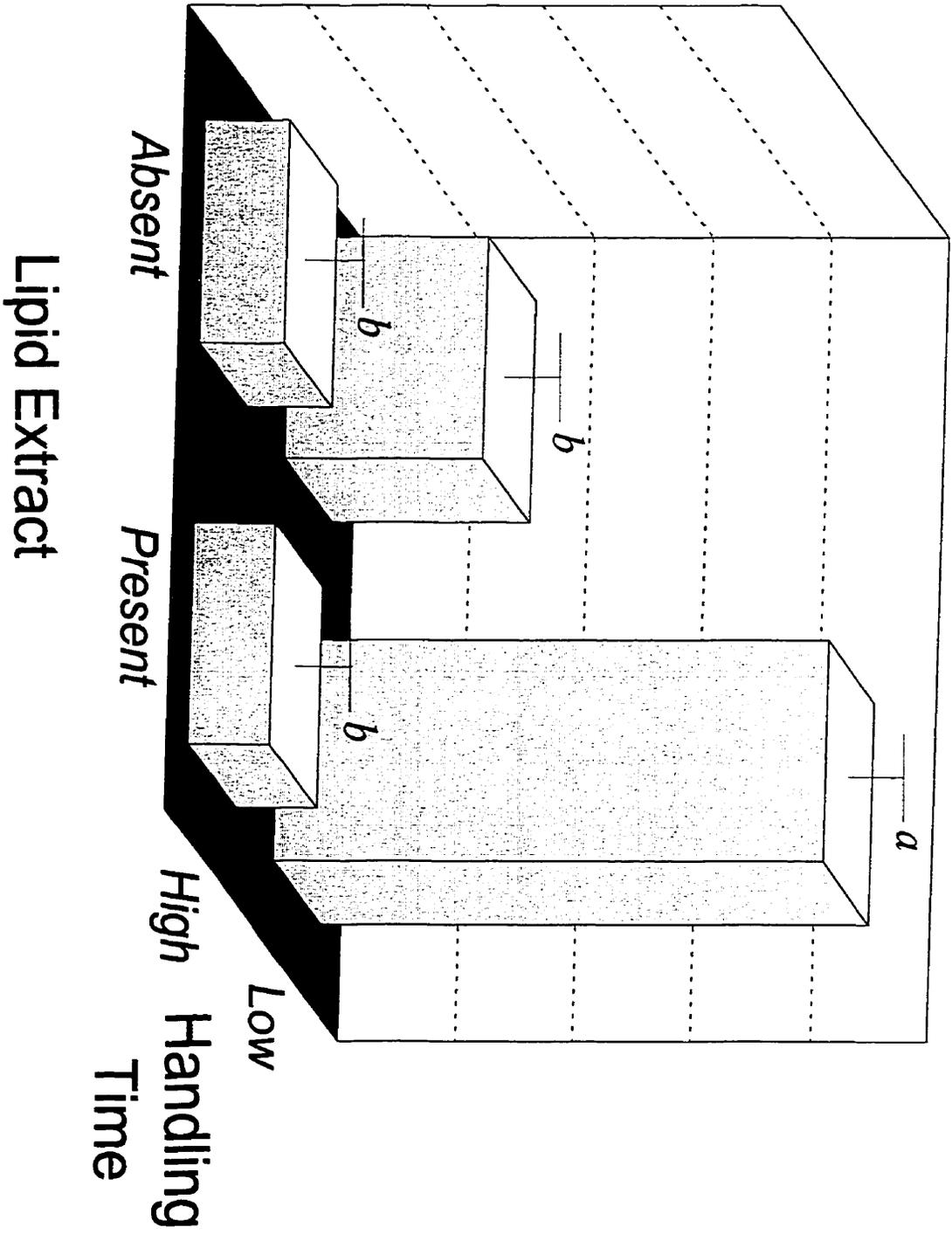


Fig. 45. Interactive effects of pollen lipid extract and handling time on the weight of cellulose collected per forager during the interaction of multiple factors bioassay. Data are LSMs and vertical lines denote standard errors. Treatments with the same letter are not significantly different from each other (*t*-test, $\alpha = 0.05$, Bonferroni correction).

Cellulose Collected / Bee (mg)

0 1 2 3 4 5



CHAPTER VI

An Improved Flight and Rearing Room Design for Honey Bees

(Apis mellifera L.)

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An Improved Flight and Rearing Room Design for Honey Bees

(Apis mellifera L.)

Abstract

A detailed technical description of a flight and rearing room for bees is provided, highlighting improvements made since its first installation (Jay 1964b) relative to other existing facilities. The primary innovation was the development of a draft-free air handling system that is capable of circulating large volumes of air with high rates of fresh air exchange and continuous electrostatic cleaning. This has led to a dramatic improvement in the quality of air recirculated in the flight room, and has prevented the recurrence of asthmatic symptoms to bee-produced aeroallergens in researchers. Other specific improvements include the incorporation of high frequency fluorescent lamp ballasts and the choice of lamp types that provide a greater proportion of long wavelength energy. Improvements in control system technology have also permitted more precise regulation of environmental conditions and the maintenance of a simulated diurnal cycle. Honey bees foraged in a manner similar to outdoor conditions, and were free of behaviours associated with design problems seen in earlier flight rooms. Observations on bee behaviour and colony performance are provided, and the utility of studying chemically-based foraging attractants indoors is discussed.

Introduction

Insect rearing and flight rooms confer many advantages to researchers interested in the study of behaviour. In temperate climates, these facilities extend the length of the

field season and may allow experiments to be continued throughout winter months. Some insects can be reared year-round, so that large-scale production of experimental subjects can be achieved under conditions that are optimal for growth and development and that preclude disease (Jackson 1960, King and Leppla 1984). Furthermore, tightly-regulated environmental conditions allow researchers to minimize the effects of stimuli that would otherwise confound the same experiment performed outdoors. Such controlled environments thereby allow the scrutiny of insect behaviours in response to precisely manipulated variables, and are ideal for studying the effects of specific stimuli on the foraging choices made by honey bees.

The advantages of keeping honey bees (*Apis mellifera* L.) indoors have long been realized, resulting in repeated attempts at improving rearing conditions. Priiutskii (1927) was one of the first to successfully maintain captive populations of overwintering bees, with his techniques later adopted by Drabaty (1931) and Chauvin (1953). However, all early attempts to rear bees under artificial conditions encountered serious limitations. A major shortcoming was the lack of normal foraging behaviour; bees walked to feeding stations instead of flying, and had difficulty in orienting back to their own colony. Foragers typically flew into and around light fixtures, often until they dropped to the floor in exhaustion. Furthermore, the frequency with which foragers defecated outside the hive was low, leading to the early onset of disease. As a result, most colonies kept in captivity ceased brood rearing after only a few weeks, or rapidly succumbed to infectious agents.

The first modern bee flight room that simulated summer conditions was designed by Renner (1955) at the University of Munich. It featured several innovations including complete temperature and humidity control and light supplied by fluorescent tubes.

Renner kept a colony of bees alive for almost two years, with foragers actively flying and collecting food. This success stimulated the construction of several similar facilities (Smith 1961, Nye 1962, Jay 1964b) in which bee colonies were not only maintained, but also achieved modest increases in size. However, limitations in the design of these facilities also became evident. Notably, foragers failed to collect sufficient quantities of pollen for colony survival, necessitating within-hive feeding (Renner 1955). Bees also had difficulty orienting under artificial light, resulting in 'trapping' at light sources (Verheijen 1958) that prevented large numbers of bees from returning to their colony (Nelson and Jay 1967a). Furthermore, environmental control systems in these rooms were crude, cumbersome and prone to frequent failure (Nelson 1966).

The present generation of flight rooms have stemmed from the seminal work of van Praagh and Velthuis, at the University of Utrecht. Van Praagh and Velthuis realized that the lighting conditions used in earlier flight rooms did not meet the spectral demands of the honey bee compound eye (particularly in the UV region), nor did they account for the frequency of light ripple that could be perceived (van Praagh and Velthuis 1971, van Praagh 1972). Lighting conditions were improved through careful selection of UV-producing fluorescent tubes, and by having different banks of light fixtures wired to separate electrical phases to produce a light flicker frequency above the 300 Hz detected by bees (Autrum and Stöcker 1950, Ruck 1958, van Praagh 1976). In addition, light fixtures were positioned to produce indirect, well-scattered light, thereby eliminating trapping seen in earlier flight rooms (van Praagh 1972). These lighting techniques, in whole or in part, have been adopted in the construction of subsequent flight and rearing rooms (Engels 1977, Ruttner 1977, Vesely 1977, Doualt 1978, Pflumm et al. 1978,

Czoppelt et al. 1980, Rothe and Nachtigall 1980, Engels and Engels 1987, Nakamura et al. 1996, Poppy and Williams 1999).

A largely neglected aspect of honey bee flight room design has been air handling. Flight rooms have typically suffered from low rates of air exchange and airflow has often been directed from point sources, creating drafty and irregular air circulation. In addition, cleaning of recirculated air has never been attempted beyond the use of large particle size filters. Inadequate or drafty airflows and insufficient air exchange present many problems for studying bee behaviour. Aspects of bee flight may change in relation to the continuity and directionality of air circulation in a room, and low rates of air exchange do not readily disperse odours, precluding the testing of successive olfactory cues. Moreover, poor circulation and low rates of air exchange are conditions coincident with the development of serious respiratory allergies in researchers who have spent long periods of time in bee flight rooms (S. C. Jay, personal communication). Similar forms of asthma have been experimentally induced in animals by contaminating room air with cockroach allergen (Kang et al. 1995, 1996), and immunochemical sensitivity to airborne particles from honey bees and other insects has been shown to occur in humans (Ostrom et al. 1986, Kino et al. 1987, Helm et al. 1993).

The design of a fully integrated flight and rearing room for honey bees is provided, based on a facility completed at the University of Manitoba in the fall of 1994. This plan features the incorporation of a high capacity air handling system that permits draft-free circulation of air, with high rates of air exchange. I evaluated improvements in lighting conditions by measuring irradiance throughout the room in the regions of the spectrum corresponding to the peak sensitivity of honey bee visual receptors. I also provide a

comparison of the light energy available for honey bee vision to that perceived for humans, and discuss issues concerning the balance of energy produced at different wavelengths for bee vision. Preliminary observations on bee behaviour are provided, and the suitability of using such a controlled environment for studying foraging behaviour is discussed.

Materials and Methods

Gross Room Features

The interior dimensions of the flight room (Figs. 46, 47) were 5.17 by 2.72 by 2.61 m high, with an exterior door opening 2.13 by 0.91 m. The walls and roof were metal, built over cedar structural members and insulated with fibreglass insulation. The floor was comprised of cedar joists with plywood sheeting, and was covered in a waterproof corlon flooring material, graded toward a central drain. The self-contained heating and cooling system was located in a crawl space above the ceiling. Inside the flight room, banks of fluorescent lights were suspended from the ceiling and mounted on the walls. Interior to the light fixtures, a large cage was used to confine the bees. Outside the cage, a small sink and counter was located at the South end of the room.

Lighting

Seven banks of light fixtures were arranged parallel to the long axis of the room, facing the walls and ceiling to provide indirect light (Figs. 48, 49). The walls, ceiling and reflectors of lighting fixtures were lined with aluminum foil (dull side facing outward) to increase the amount of radiant energy that was reflected and scattered. Three light banks,

each composed of four fixtures, were suspended from the ceiling, two banks of four fixtures were mounted on the West wall and two banks of three fixtures were located on the East wall. Each lighting fixture contained two T12 fluorescent tubes (3.8 cm dia by 122 cm long) for a total of 52 tubes in the room. Two types of 40 W fluorescent tubes (Philips Lighting, Scarborough, ON) were used: Philips black light (F40/BL), primarily emitting long wavelength UV radiation (λ_{\max} 365 nm); and Philips colortone 75 (F40/C75, 7500 K), which emitted light in equal proportions across the human visible spectrum. Individual light fixtures contained one of each type of tube, their relative positions alternating between successive fixtures within a bank. Each lighting fixture was powered by a 120 V high efficiency electronic lamp ballast (Cat. # E240SR120G01, Powerlighting Products, El Paso, TX) that had an output lamp frequency (flicker frequency) in excess of 20 kHz. Three electromechanical timers, each controlling different banks of lights (Figs. 48, 49), were used to create artificial crepuscular periods. Light banks labelled 1, 2 and 3 were successively illuminated in half-hour increments at sunrise, and in reverse order for sunset. Day length was set to 16 h.

The amount of radiant energy in the flight room was measured using a UV actinic radiometer (Model # IL 730A, International Light, Newburyport, MA). The radiometer was used in conjunction with one of two detectors: a visible light detector fitted with one of two band-pass filters (λ_{\max} 450 nm, half bandwidth 32 nm; λ_{\max} 550 nm, half bandwidth 40 nm), or a UVA detector (λ_{\max} 360 nm, half bandwidth 51 nm). Measuring irradiance at these wavelengths provided information about the quantity of light available to honey bee visual receptors (Autrum and von Zwehl 1964, Menzel and Blakers 1976, Menzel et al. 1986, Menzel and Backhaus 1989). Illuminance was also measured using a

standard light meter (Control Company, Friendship, TX). Measurements were taken along the East-West centre line of the room and in two additional transects positioned 1 m North and South of it. Within each transect, four readings (0.60 m apart) were taken at heights of 0.5, 1.5 and 1.9 m above the floor. Irradiance values were corrected for the relative response of the detectors at different absorption maxima, and for the transmission factor of each filter. Illuminance readings were multiplied by a factor of 0.98 to correct for the visible light spectra produced by fluorescent lamps.

Heating, Cooling and Airflow

The heating and cooling system was entirely self-contained and located above the ceiling of the room (Figs. 50, 51). An air-conditioning evaporator coil (M) regulated temperature by alternately heating and cooling the airflow. Electric solenoids controlled the source freon (R-12) flowing through the coil; cold liquid freon was used for cooling, and hot return gas was used for heating. Freon was circulated by a 3.5 kW (1 ton) compressor with a water-cooled condenser, located two floors below. Additional heating capacity was provided by a 2 kW, 220 V electric heating element (J), which normally remained on at all times. This device was manually disabled if very low temperatures were to be maintained.

A blower (K) powered by a 187 W (0.25 hp) 120 V electric motor (L), circulated air through two ducts (I) which traversed the ceiling to the East and West walls of the room. The airflow from each duct (I) was then split toward the North and South, and diffused through perforated spiral ducting (20.3 cm dia, ends capped) (G) at a rate of $28.6 \text{ m}^3 \cdot \text{min}^{-1}$. Air returned to the heating and cooling system via a large return duct (R) located

on the North wall of the room. After the returning air had moved above the level of the ceiling, a portion was exhausted ($7.4 \text{ m}^3 \cdot \text{min}^{-1}$), with an equivalent volume of fresh air being added through a damper-controlled intake (P). Fresh air was provided by the building's air conditioning system, which supplied air into the crawl space above the flight room at $8.5 \text{ m}^3 \cdot \text{min}^{-1}$. Airborne particulates were removed by an electronic air cleaner (O) (Model F50E, Honeywell, Scarborough, ON) before air passed through the heating/cooling coil (M).

All heating and cooling system components above the ceiling of the flight room were internally insulated with 2.5 cm fibreglass insulation to reduce noise and thermal loss; ceiling-traversing ducts (I) were externally insulated with 2.5 cm fibreglass duct insulation with reinforced foil/kraft (FRK) vapour retarder facing.

Humidity

Relative humidity was regulated using two humidifiers (B) (Figs. 50, 51) (Model 707 SM, Sovereign, Richmond Hill, ON), mounted on the North and South walls of the flight room. Humidifiers were of centrifugal design, using a small electric motor to aspirate water into a fine mist without heating. Both were supplied with water from a reservoir (A) located above the roof of the flight room, which was connected to the building's reverse osmosis water system (D). The reservoir employed a float-activated check valve that permitted water to enter the tank only when it decreased below a preset level; should a failure occur, an overflow drain prevented spillage. Relative humidity was maintained at 45 - 55%, a level previously determined to be suitable for colony brood rearing (Büdel 1948, van Praagh 1975).

Control Systems

Environmental conditions were programmed using several electronic devices contained within a wall-mounted control panel (U) (Fig. 51). Two microprocessor-based auto-tuning controllers (Series 985, Watlow Controls, Winona, MN) regulated heating and cooling by activating solenoids on the liquid freon and hot gas discharge lines. Each controller could be independently programmed to maintain a desired room air temperature ($\pm 0.5^{\circ}\text{C}$), which was continuously monitored using thermocouples (T) (Fig. 50). Operation of the heating and cooling system was performed by a single controller, that switched between devices using an electromechanical timer. Controllers were programmed to simulate a diurnal cycle, typical of summer conditions in Manitoba, whereby daytime temperature was 25°C and nighttime was 15.0°C . High and low temperature alarms could be also programmed to desired thresholds; if air temperature exceeded these set points, the heating, cooling and lighting systems would automatically be shut off and an audible alarm would sound. The electromechanical timers controlling lighting were synchronized so that daytime temperature coincided with the first set of light banks being turned on at sunrise, and nighttime temperature coincided with total darkness. Humidity was also maintained under automatic control, with continuous humidity monitoring provided by an electronic sensor (T). A thermohygrograph (V) was used to provide a 7-d record of environmental conditions in the flight room.

Flight Cage and Feeding Station

A flight cage (3.96 by 1.91 by 2.01 m high) was constructed inside the room to confine bees and prevent them from contacting lighting fixtures or defecating on

reflective surfaces (Figs. 52, 53). It was fabricated from construction grade spruce lumber, with 3.8 by 3.8 cm stock used for the walls (G) and 1.9 by 3.8 cm stock used for the roof. The cage was built as individual component panels, whose vertices were reinforced with wooden gussets (H). Wooden components were painted white and the outside of each panel was covered with fibreglass screening (I) ($4.8 \text{ meshes} \cdot \text{cm}^{-1}$), affixed with staples. Panels were bolted to each other to facilitate their disassembly for future flight room maintenance. A vestibule (0.74 by 0.86 by 2.01 m high) with two zippered canvas doors (A, B) was located on the South end of the cage to prevent bees from escaping as people entered or exited. The floor within the flight cage was covered with polyethylene sheeting.

Inside the flight cage, a honey bee colony (J) was situated at the North end and a feeding station was placed at the South end. The feeding station consisted of a small rectangular table (0.76 by 0.30 by 0.79 m high) which provided a platform for a sugar syrup feeder (C), a water dish (D), and two protein feeders (E). The syrup feeder, known as a “boardman feeder” (Morse and Flottum 1990), was comprised of an inverted 1-quart canning jar (0.946 L) having its neck held in a wooden block; small holes penetrating through a metal lid allowed bees access to the solution. Water was provided in a 250 mL dish containing a small sponge, and the protein was fed to bees using 9 cm glass petri dishes placed on 15 cm squares of bright yellow bristol board. On a larger piece of the same material (21.6 by 27.9 cm), a thick black triangle was drawn, which was hung below the syrup feeder as an orientation cue (F).

Colony Maintenance

A colony that was moved into the flight room from the university apiary was reduced to three full-depth Langstroth frames of brood, approximately 6 000 workers, and a laying queen. My experiments examined honey bee behaviours in response to pollen-based foraging cues (Pernal 2000a), therefore no pollen and only a small amount of honey was placed into these colonies to ensure that demand for food, and hence foraging activity, would be high. Colonies were also provided with two empty frames of light-coloured comb for workers to store sugar syrup collected from the feeder. At the feeding station, bees were offered a solution of 2 M sucrose, water and a pollen substitute diet (Bee-Pro[®], Mann Lake Supply, Hackensack, MN) for protein. Sugar syrup and water were offered continuously, and pollen substitute was provided between 0900 and 1600 h. Sanitation was provided by daily sweeping of dried faeces and dead bees, and replacement of the polyethylene sheet lining the floor approximately every two weeks. Periodic washing of the flight cage was also necessary to remove the accumulated faeces. Daily measurements of food consumption and weekly estimates of the area of sealed brood, unsealed brood and eggs were measured, during my initial attempts to keep bees in captivity, and prior to the use of colonies in foraging experiments. General observations on colony performance and foraging behaviour were also recorded.

Results and Discussion

Effect of Lighting

The spectral quality and irradiance of light in the flight and rearing room allowed bees to fly and forage as if they were kept outdoors. My observations showed that

workers did not exhibit trapping, become disoriented, fly into walls or ceiling of the flight cage, or exhibit any other behaviour that could be interpreted as a response to an unsuitable visual environment. In order to describe the ambient lighting conditions produced by the type and placement of fluorescent tubes used, levels of irradiance and illuminance were determined at twelve locations in the centre of the room. By using these measures of flux density, the light energy available for honey bee and human vision could be compared, and evaluated against values from previous studies. As noted by van Praagh and Velthuis (1971), the photometric term illuminance (lux) is an inappropriate measure of the light energy available for honey bee vision because it is scaled to the photopic response of the human eye, sensitive to electromagnetic radiation between 380 - 770 nm (λ_{\max} 555 nm) (Murdoch 1985). To quantify the energy available for bee vision, irradiance was measured in those portions of the spectrum corresponding to the absorption maxima of honey bee photoreceptors (350, 440, 540 nm) (Menzel and Blakers 1976). These measurements also permitted a determination of the relative distribution of energy among receptor types. Van Praagh (1972) employed a similar technique to determine the light energy available to bees in his flight room, but chose to measure radiance ($\text{W} \cdot \text{m}^{-2} \cdot \text{sr}^{-1}$) instead of irradiance ($\text{W} \cdot \text{m}^{-2}$), which is typically used as the dose rate in photobiology. Although radiance is a radiometric term, it describes the rate of energy leaving a source per solid angle (the “brightness” of the source), and is often used for comparing among different types of light sources (Sloney and Wolbarsht 1980). Irradiance, however, describes the radiant energy arriving from a continuous source on a real surface at given point in space, such as the photon flux falling on a bee’s ommatidium as it flies through a given location within the flight room. Therefore,

irradiance is a more appropriate term for describing the visual energy available to a honey bee forager in the flight room, and is the most biologically meaningful term to compare different types of visual environments.

Irradiance and illuminance in the centre of the flight room are listed in Table 19. Both measures of flux density increased at positions closer to the ceiling-hung fixtures. The proportion of UV light was approximately 10× greater than that of blue or green wavelengths, however the greater breadth of the UVA detector response (51 nm at half bandwidth) compared with the band pass filters (blue = 32 nm, green = 40 nm) may have resulted in a slight overestimation of its contribution to bee vision. To determine how well artificial lighting resembled natural conditions, the relative proportions of UVA: blue: green energy present in daylight were calculated (7500 K, Henderson 1977) for the same wavelengths measured in the flight room (at half bandwidth). The proportions of daylight energy are 1: 1.2 :1.3, indicating that the amount of UVA in the flight room was approximately 10× greater than in a natural environment, and that the ratio of blue to green was approximately correct. Providing sufficient UV energy for honey bees is critical for normal orientation and behaviour because UV photoreceptors control or heavily influence such processes as phototaxis, learning inhibition, the detection of polarized light and the threshold response for visual energy detection (reviewed by Menzel 1979). Previous flight room work has shown that high proportions of UV energy are necessary for bees to fly normally, and that replacement of UV-producing lamps with those emitting longer wavelengths causes decreased flight activity (van Praagh 1972). The high proportion of UV energy in the flight room may have also affected the colour vision capability of the bees, as the unbalanced contribution of a single photoreceptor

type will distort a worker's perceptual colour space and may eliminate colour discrimination ability (Rushton 1972, Rodieck 1973). Also contributing to this potential problem is the greater threshold of sensitivity of UV receptors to photon flux over that of blue or green receptors (von Helversen 1972, Backhaus 1991). Although the energy spectra produced by my lamps contained a greater proportion of UV energy than the natural environment, the selection of additional non UV-producing fluorescent tubes is likely to have resulted in a more balanced energy spectrum than that of other flight room designs (van Praagh 1972, Engels 1977, Ruttner 1977, Vesely 1977, Doualt 1978, Pflumm et al. 1978, Czoppelt et al. 1980, Rothe and Nachtigall 1980, Engels and Engels 1987) based on their descriptions of tube specifications and the proportions and arrangement of each type used. Further studies of artificial lighting conditions for honey bees are required to identify the threshold of UV energy necessary to elicit key behaviours, and to determine an appropriate balance of long wavelength energy to better imitate daylight energy spectra.

Illuminance in the flight room at 1.5 m above the floor was 557.3 lx, considerably less than earlier flight room designs where UV energy was not provided: 700 lx (Renner 1955, 1957), 2368 lx (Nye 1962), 2850 lx (Jay 1964b), 891-4520 lx (Nelson and Jay 1967b), but similar to later designs where the choice of fluorescent tubes favoured UV production over that of visible light: 20 lx (Van Praagh and Velthuis 1971), 500 (Doualt 1978), 220 (Pflumm et al. 1978). After conversion to a common unit of comparison (light Watts) which accounted for the relative sensitivity of the receptors in honey bee ommatidia or cones of the human retina (see Table 19), it is observed that 2.2× more energy is perceived by honey bees than humans in the flight room. Thus, although the

room may not appear bright to a human observer, it is considerably “brighter” for bees.

The normal flight behaviour of bees in the flight and rearing room further indicates that two other characteristics of the light energy produced, its angular radiance distribution and frequency of light ripple, satisfied their requirements. Because bees did not exhibit trapping behaviours, I conclude that the indirect positioning of light fixtures and the reflectance of energy from foil-covered surfaces provided a visual environment approximating a natural angular radiance distribution (Verheijen 1958, Velthuis and Verheijen 1963). This conclusion is also supported by the similarity in irradiance values measured within height levels above the floor. The frequency of light ripple was controlled by the electronic ballasts of the fluorescent light fixtures. The use of electronic ballasts confers many advantages previously unattainable with magnetic lamp ballasts. Electronic ballasts have an output frequency of 20 - 40 kHz, far above the flicker fusion frequency of honey bee compound eyes at 265 - 300 Hz (Autrum and Stöcker 1950, Ruck 1958). Previously, the only means of achieving a high frequency light ripple was to wire each of three banks of lights to a separate electrical phase. With 60 Hz power, this approach would marginally raise the light ripple beyond the honey bee’s ability to detect it, and if 50 Hz power was used, the ripple would be at the threshold of detection. In addition, three-phase power is not available in some buildings, and the wiring necessary for its use increases the cost of constructing a flight room. Electronic ballasts also consume less power, are extremely quiet (class A sound rating, Canadian Standards Association), and produce little heat allowing them to be mounted inside lighting fixtures without compromising the cooling capabilities of the room. Electronic ballasts were also used in another recent flight room design, but were found to cause significant reductions

in the spectral power output from lamps (Poppy and Williams 1999). This problem, however, was attributed to improper choice of ballast type for the power requirements of lamps used. The use of higher frequency output of electronic ballasts would also provide a means of producing a suitable visual environment for other fast-flying insects with high flicker fusion frequencies, such as species of Hymenoptera, Diptera and Odonata (Autrum and Stöcker 1950, Autrum 1958, Ruck 1958, Laughlin 1981).

Airflow and Quality

The handling and circulation of air, its rate of exchange and overall quality proved to be excellent for performing experiments in the flight room and represents a substantial improvement over earlier flight room design. An overriding concern in the design of the airflow system was the possibility of sensitizing researchers to airborne allergens. This concern was based on years of previous experience during which individuals had developed serious allergic reactions to bees or their byproducts, after chronic exposure in a flight room (S. C. Jay, personal communication). This situation may have been aggravated by the lack of sufficient fresh air exchange, and by features which diffused air directly over the floor, moving hairs, scales and volatile components of bee faeces into the air. To minimize these problems, my design ensured that the total volume of air in the room was recirculated every 1.28 min, during which time 25.8 % was exhausted and replenished with clean air. In the past, movement of such large volumes of air have created serious problems with drafts that hamper insect flight. Although large volumes of air were circulated at moderate velocity, no draftiness or directional airflow was noticeable in the room. This was achieved by using perforated spiral ducting to diffuse

air from the top of the chamber along its length, and by the use of a large capacity return air duct. Also, air was passed through an electrostatic air cleaner, to remove any particulate matter or airborne contaminants. The use of continuous electrostatic air cleaning, large volumes of fresh air and diffusion of supply air from the top of the room served as a strategy to minimize the concentration of particulates that might function as airborne allergens. Under these conditions, there has been no recurrence of asthmatic symptoms in researchers to bee-produced aeroallergens.

The features of the airflow system also made the flight room an ideal venue for subsequent experimental work. In my studies, I developed bioassays for flying honey bee foragers in which bees chose between dishes containing discretely manipulated pollen-based cues (Pernal 2000a). One cue that was extensively evaluated was pollen odour. The high rate of air exchange in the flight room prevented pollen odours from carrying over between trials, and the non-directional air flow precluded the formation of downwind odour plumes or the forcible intermixing of adjacent odour fields (using point sources of smoke to visualize the effects of air circulation). These features assisted us in evaluating odour-based cues in a constant and repeatable environment, free of confounding influences that would be found outdoors. Such features would also be highly valuable as an arena for examining insect responses to volatile semiochemicals.

Temperature and Humidity Control

Under normal operating conditions, temperature in the bee flight and rearing room could be continuously maintained at any level between 14.0 - 28.0 °C. More extreme temperatures (9.0 - 30.0 °C) could be maintained by reducing the rate of fresh air intake.

By severely limiting air exchange and manually disconnecting the auxiliary electric heater, temperatures as low 5.0 °C were maintained, thereby demonstrating the utility of the room for overwintering studies in which high rates of fresh air intake may not be critical. The maintenance of constant temperatures in a room with such high air exchange continually subjected the heating/cooling compressor to high loads, resulting in periodic breakdowns and eventual replacement. Similarly designed insect rearing facilities should utilize more powerful compressors than ours (3.5 kW) and locate them in closer proximity to airflow systems, so that thermal losses are minimized.

For bees reared in captivity, the maintenance of sufficient relative humidity is essential to prevent reductions in brood area (van Praagh 1975, van Praagh 1982). Although humidity in the flight and rearing room was kept between 45 - 55%, its maintenance, at times, proved too difficult for the automated systems. The two factors that made humidity control difficult were large differences in the humidity of incoming fresh air, and the high rate of fresh air exchange. During winter months, the cold outdoor air in Manitoba air holds little water vapour, and after entering the building and becoming warmed, its relative humidity is very low. Because of this, a large water wheel humidifier with electric heating element was continuously operated to supplement the wall-mounted humidifiers. During spring, summer and fall months humidity control was accomplished without supplementary equipment; only during short intervals of high outdoor humidity in the summer was a portable dehumidifier necessary.

Colony Dynamics and Foraging Behaviour

Unlike other flight room studies that have documented the growth of individual

colonies over several months (e.g. van Praagh 1972, Kefuss 1978, Czoppelt et al. 1980), my observations are based on several different colonies held for shorter intervals of time. Additional data were derived from colonies used in my flight room foraging experiments (Pernal 2000a), when several days or weeks elapsed before the commencement of trials. Growth and food consumption recorded during the conduct of these trials are not described because of the limited access of bees to protein and syrup, thereby confounding observations.

In general, colonies survived well in captivity. Bees were easy to handle without the use of smoke, did not exhibit symptoms of nosema, and had low levels of chalk brood disease. Colonies consumed an average of 23 g of pollen substitute per day and 142 mL of sugar syrup. This rate of pollen collection, corrected for the number of frames of brood in my colonies, is similar to other studies (van Praagh 1972, Czoppelt 1977, Czoppelt et al. 1980), but the consumption of syrup is greater. High rates of syrup collection also necessitated the replacement or supplementation of comb used for syrup storage, to prevent any restriction in the space available for egg-laying by the queen. Increased syrup consumption may reflect the fact that my colonies were transferred into the flight room with few reserves, which would have provided a significant stimulus for foraging. Pollen substitute was fed as a protein source in the flight room so as not to precondition foragers to the odour of certain pollen types during experimental trials (Pernal 2000a). The decreased attractiveness of pollen substitute to foraging bees compared with pollen (Pernal 2000a) may have also influenced the amount collected. Also consistent with previous observations, the collection of food was greater during summer months than in winter (27 vs 18 g per day) (van Praagh 1972; Czoppelt et. al.

1980).

Brood production in the flight room remained relatively constant, with colonies producing an average of 962 cm² of total brood area. In a few colonies, however, brood production remained stable for several weeks, then gradually declined before colonies were removed. The average ratio of eggs + unsealed brood : sealed brood ranged from 0.53 - 0.67, and tended to increase with the length of time a colony was situated in the flight room. The proportion of eggs and unsealed brood to sealed brood in my colonies is larger than would be expected from a stable population with no egg or larval mortality (Gerig 1975). The major mortality factor affecting young brood in other flight room studies (Czoppelt et al. 1980) is thought to be caused by worker cannibalism of eggs and young larvae (van Praagh and Brinkschmidt 1987). Colonies probably regulate their proportion of sealed to unsealed brood to reduce their strength to the minimum size necessary to survive within a restricted flight space (Czoppelt 1987), or experience high larval mortality because of problems associated with the collection of pollen (Velthuis 1977, van Praagh and Brinkschmidt 1987).

Bees foraging in the flight room actively collected protein and syrup from the feeding station and exhibited behaviours similar to those found in nature. Workers collected the finely powdered pollen substitute by hovering directly above the dish and displacing the particles onto their bodies which were then groomed off in flight, or by scrabbling through the substrate (Pernal 2000a). Foragers were capable of locating the feeding station without the addition of artificial scent, save for a small amount of honey placed in the entrance to the boardman feeder when a colony was first introduced into the flight room. Without moving the feeding station closer to the colony, it often took two or

three days before a colony would discover it and start foraging in earnest. Once food was discovered, recruitment dances were performed inside the colony and subsequent foraging flights were characterized by straight line trajectories between the colony and the feeders. Periodicity in foraging was observed with a peak in food collection activity occurring shortly after the time food was presented in the morning, and increasing again in early-mid afternoon. Foraging activity declined toward late afternoon, with any bees remaining outside the colony returning as lights were gradually turned off by the timers. None remained outside the colony by the time all lights were extinguished.

In my experience, colonies remained relatively stable in size and no queens or drones were produced. Queen rearing has been shown to occur in flight rooms in response to queen loss (Czoppelt et al. 1980), or from queen grafts placed in colonies (Jay 1964b). The production of drones by captive colonies has also been documented (Jay 1964b), however this was accomplished by providing colonies that were recently moved into the flight room with drone comb (S. C. Jay, personal communication). Therefore, the production of queens or drones in preparation for swarming has never been achieved indoors.

The inability of colonies to reach population levels suitable for swarming has puzzled many researchers keeping bees in captivity. Several factors, including the quantity and quality of food, photoperiod, and seasonal changes in daylength have been examined for their limiting effects, but none appear to be implicated (Velthuis 1977, Czoppelt 1987). The restriction of brood production has also been addressed by studies that have evaluated the processes of pollen collection in flight rooms. In the first, Velthuis (1977) studied the recruitment dances and density of foragers at feeding stations

and suggested that crowding at a feeding site limited the number of foragers actually engaged in pollen collection. Even though successful foragers were capable of meeting the protein requirements of the colony and storing considerable quantities of pollen, he concluded that the ratio of successful to unsuccessful foragers somehow hampered brood rearing. Unfortunately, a mechanism explaining how these individual forager effects might be translated into colony-level processes was not suggested.

An alternative hypothesis was proposed by van Praagh (1982) and van Praagh and Brinkschmidt (1987) which suggests that colonies are incapable of assessing the protein available for brood rearing because of discontinuities in its pattern of collection. These researchers implied that high ratios of eggs and unsealed larvae to sealed brood (≥ 0.6) could be reduced (< 0.5) by increasing relative humidity in flight rooms from 65 % to 85 %. They showed that this change increased the amount of pollen collected by foragers over time, but these results appear to be specific to coarsely ground pollen (having particles as large as 500 μm), as the collection of finely ground pollen (50 μm) is prevented because of its stickiness when humidity is increased from 45 % to 50 % or more (Czoppelt 1987). It is, however, important to consider that the physical process of pollen collection is not only influenced by humidity, but is inextricably linked to other physical factors such as particle size, shape, stickiness and electrostatic charge (Pernal 2000a). Therefore, generalizations about the way that humidity affects pollen collection cannot necessarily be made without consideration of other interacting factors.

Future Directions

My experiences and those of others have demonstrated the importance of proper

lighting, airflow and humidity for keeping bees in captivity. Although considerable improvement has been achieved since bees were first maintained indoors, several issues require careful investigation if colony growth and behaviour are to parallel the natural environment. Current lighting strategies recognize the honey bee's visual spectrum, and its high requirement for ultraviolet energy. Efforts now must focus on assessing the colour rendering abilities of bees held under artificial conditions to provide a tool for further evaluating the sensory environment of the insect. Quantification of spectral energy within rearing facilities would best be served by complete measurement of energy across the entire visual spectrum of the honey bee, or other insect of study, using a spectroradiometer. These data would be most useful for matching artificial lighting conditions with daylight spectral power distributions.

A second area of investigation should continue to focus on why honey bee colonies cannot increase to a size necessary for the production of queens, drones and eventual swarming. To address this problem, several related factors must be carefully examined. These include measuring colony growth parameters in relation to: the total flight area of the facility; the number, spacing and crowding at feeding stations; the interaction of physical factors affecting the temporal collection of pollen; the actual or perceived need for protein the colony; and the quality of forage available.

The design of a successful controlled environment for honey bees and other insects depends on a thorough understanding of their sensory physiology and behaviour, as well as the capability of technology to satisfy their needs. Based on these concepts, I have developed improvements in air handling, lighting and control systems for honey bees kept in captivity, and suggest that these innovations be incorporated into future flight

room designs. The advantages realized by researchers using controlled environments for the study of insects are numerous, and I hope will merit further development in this field.

Acknowledgements

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Table 19. Irradiance, illuminance and total light energy perceived in the bee flight and rearing room

Height	Irradiance			Total	Illuminance (lx) ^a	Total energy perceived (IW · cm ⁻²) ^b	
	360 nm (UV)	λ_{max} of detection (450 nm (Blue) 550 nm (Green))	450 nm (Blue)			Honey bee ^c	Human ^d
above							
floor (m)							
0.5	62.9	6.4	5.7	75.0	476.2	65.5	32.5
1.5	77.9	10.4	9.1	97.4	557.3	82.1	38.1
1.9	131.9	13.2	11.7	156.8	838.7	137.2	57.3
Overall	10.3	1.1	1				
ratio:							
IW, light watts							

^aAverage of 12 measurements taken along East-West centre line of room and transects 1 m North and South of it.

^bValues represent the area beneath the spectral light power density curve for each species, or total light watts emitted by the source (Murdoch 1985).

^cEstimated by calculating sum of irradiance at each λ_{max} , normalized to the range sensitivities of visual receptors ($R_{\text{uv}} : R_{\text{blue}} : R_{\text{green}} = 6.4 : 1.7 : 1$) (Backhaus 1991, Backhaus and Menzel 1987). Range sensitivity is equal to the reciprocal value of the photon flux needed for a graded potential of half the maximum response in photoreceptors (Laughlin 1981). These coefficients are assumed to maintain the same ratio for dark or daylight-adapted bees (Backhaus 1993).

“Converted from total lux using the constant of $683 \text{ lm} \cdot \text{IW}^{-1}$, from the 1979 General Conference on Weights and Measures definition of the candela (cited in Murdoch 1985).

Fig. 46. Gross features of flight and rearing room, West-facing view (drawn as if East wall was transparent). A self-contained heating and cooling system is situated above the ceiling of the room, and is normally accessed through a maintenance hatch located outside the room. This, and all succeeding figures, are drawn to scale; dimensions in centimetres.

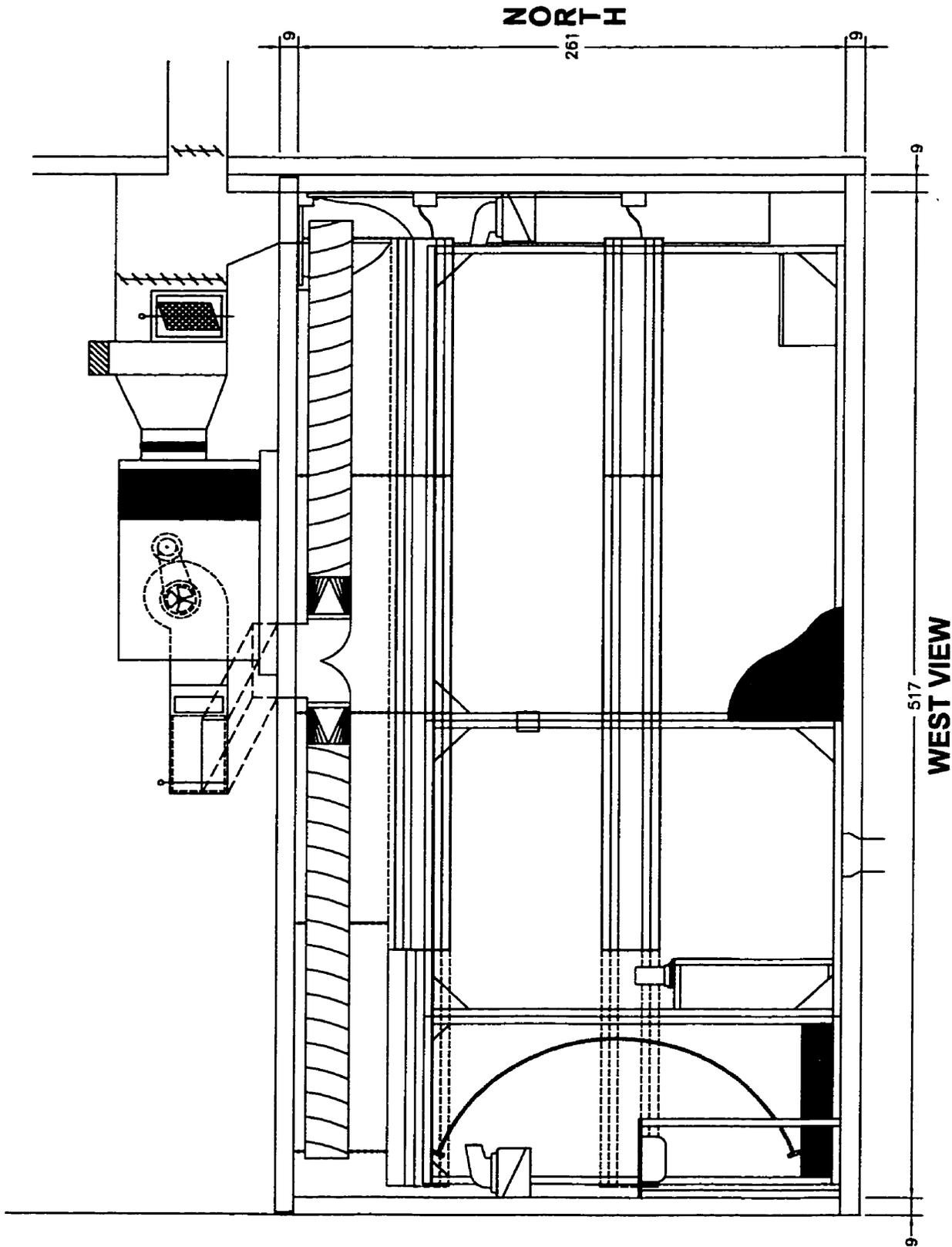


Fig. 47. Gross features of flight and rearing room, top view (drawn without heating and cooling system).

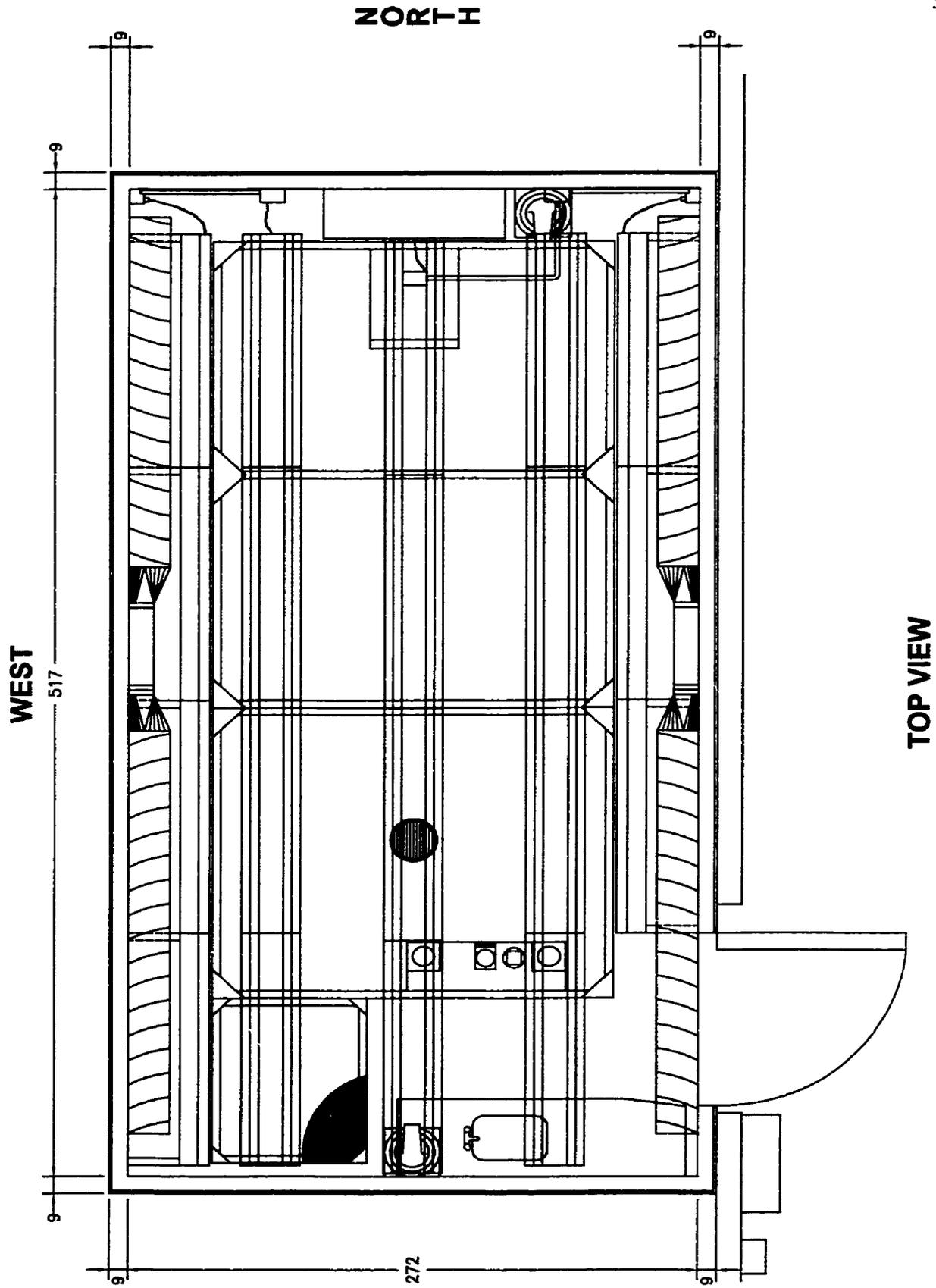


Fig. 48. Position of lighting in flight and rearing room, West-facing view. All fixtures face walls or ceiling (fluorescent tubes not illustrated). A, fluorescent fixtures, West wall; B, fluorescent fixtures, East wall; C, fluorescent fixtures hung from ceiling; D, light weight chain; E, flexible power cord; F, duplex receptacle (15 A); G, PVC electrical conduit. Numbers in bold denote banks of lights controlled by separate electromechanical timers.

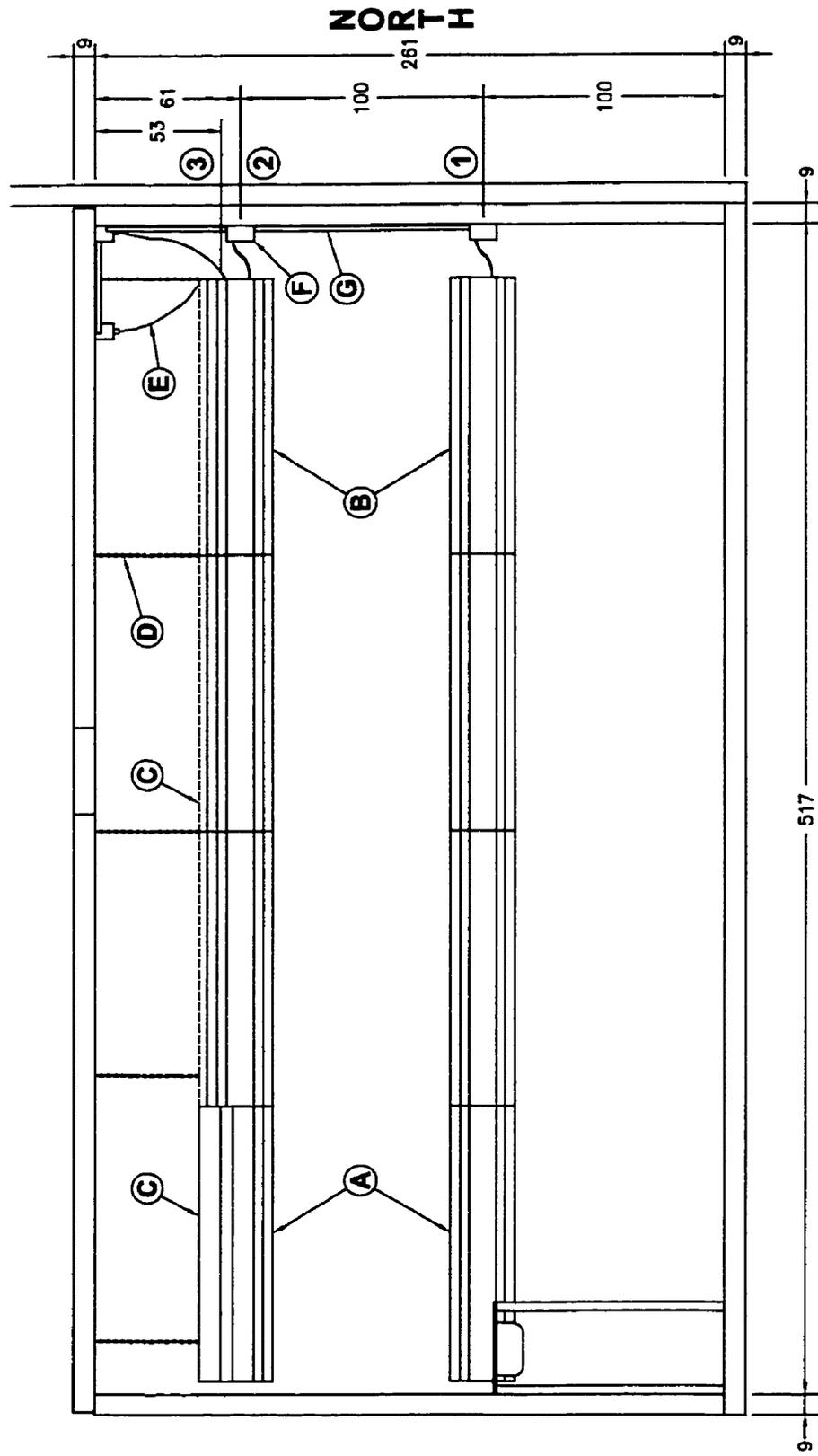
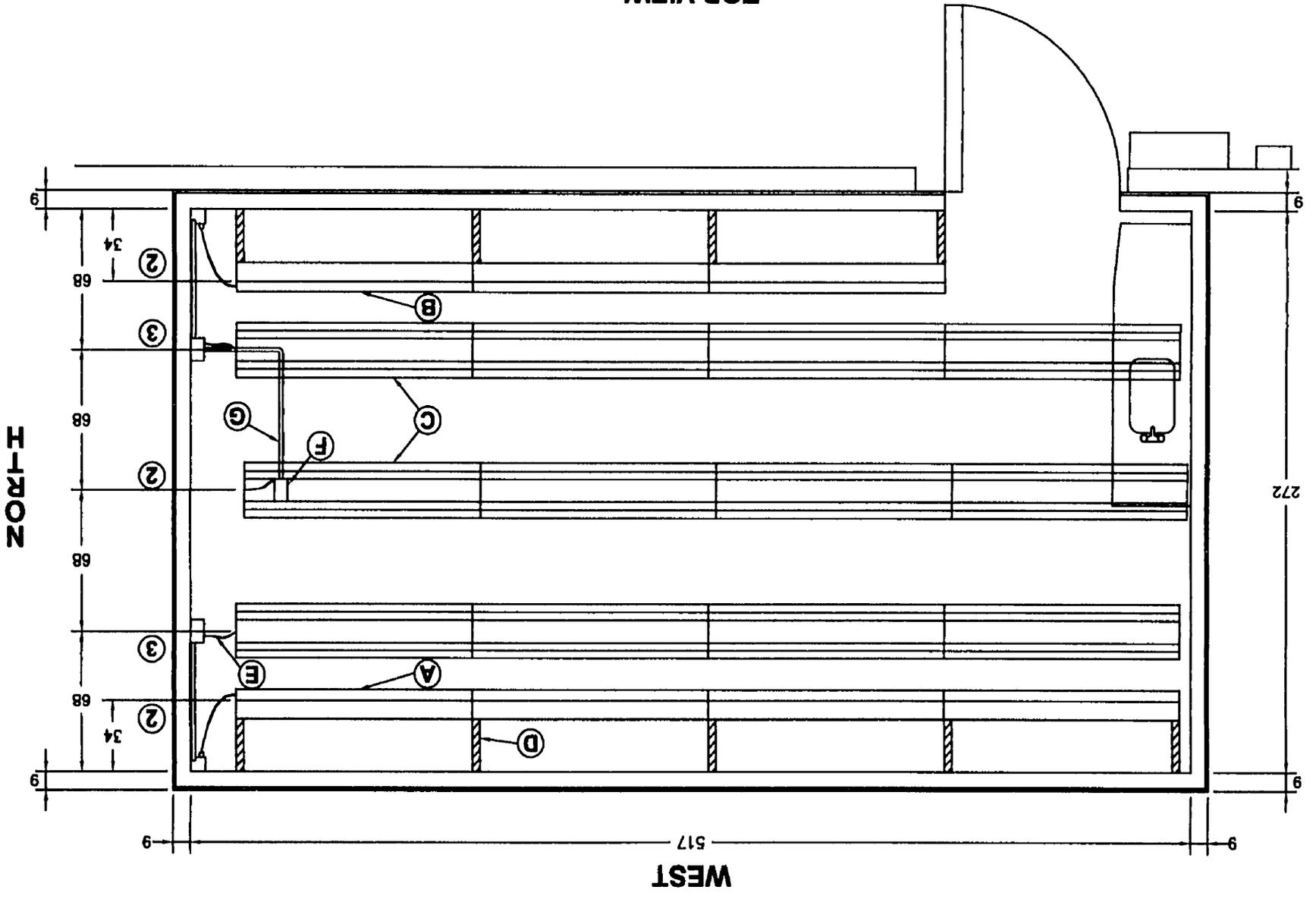


Fig. 49. Position of lighting in flight and rearing room, top view. Legend as in Figure 48, except D, steel wall bracket.

TOP VIEW



E-PLAN

WEST

517

272

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34

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Fig. 50. Heating, cooling, airflow and humidification systems in flight and rearing room, West-facing view. A, humidifier water reservoir; B, humidifier; C, counter with sink; D, reverse osmosis water supply line; E, humidifier water supply lines; F, floor drain; G, perforated spiral duct; H, air-splitting damper; I, ceiling-traversing duct; J, electrical connections for heating element; K, blower; L, electric motor; M, heating/cooling coil; N, flexible ducting connector; O, electrostatic air cleaner; P, fresh air intake damper; Q, recirculating air damper; R, return air duct; S, exhaust air damper; T; thermocouples/humidity sensor (on West wall). Arrows without letters denote direction of airflow.

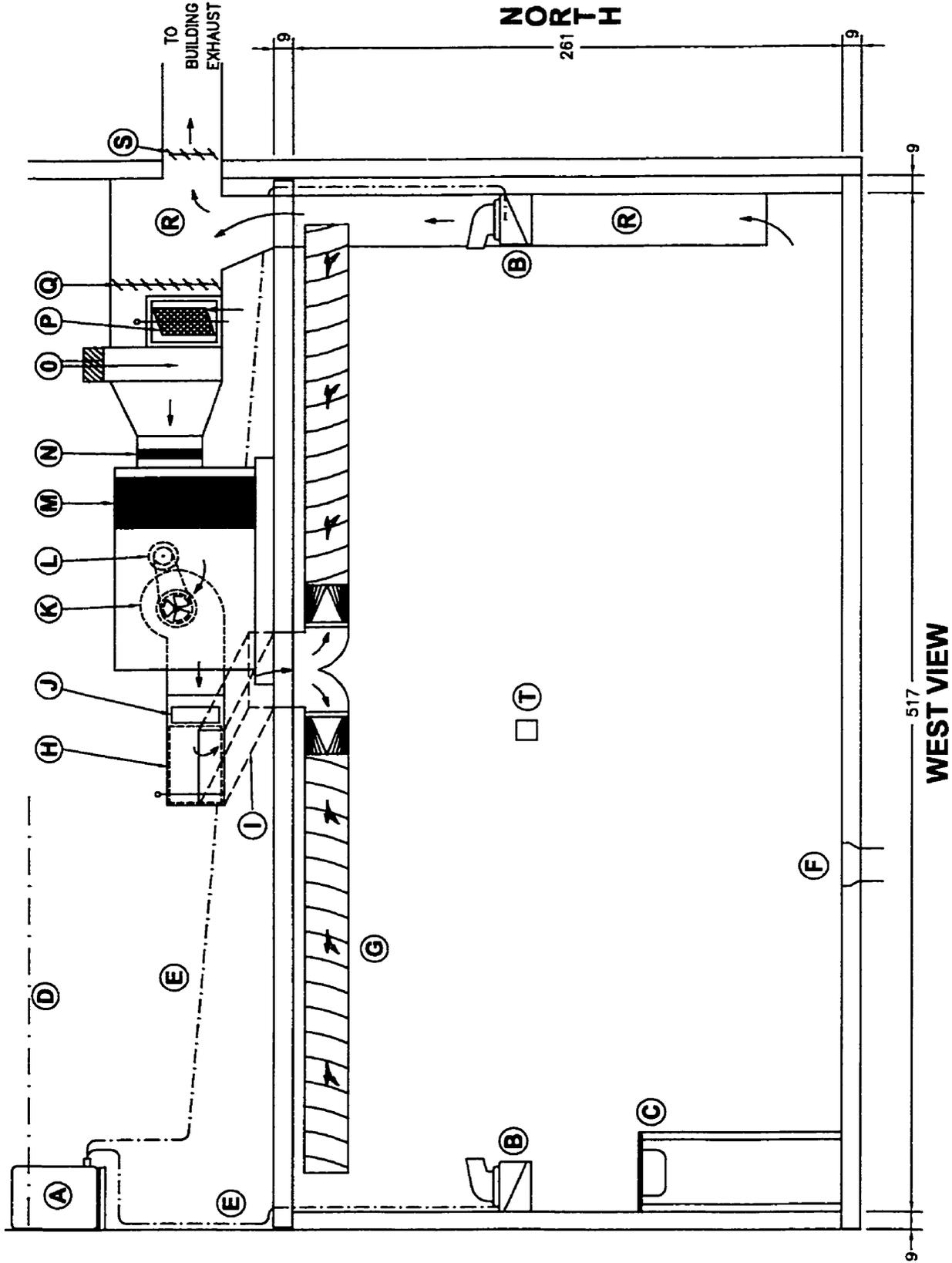
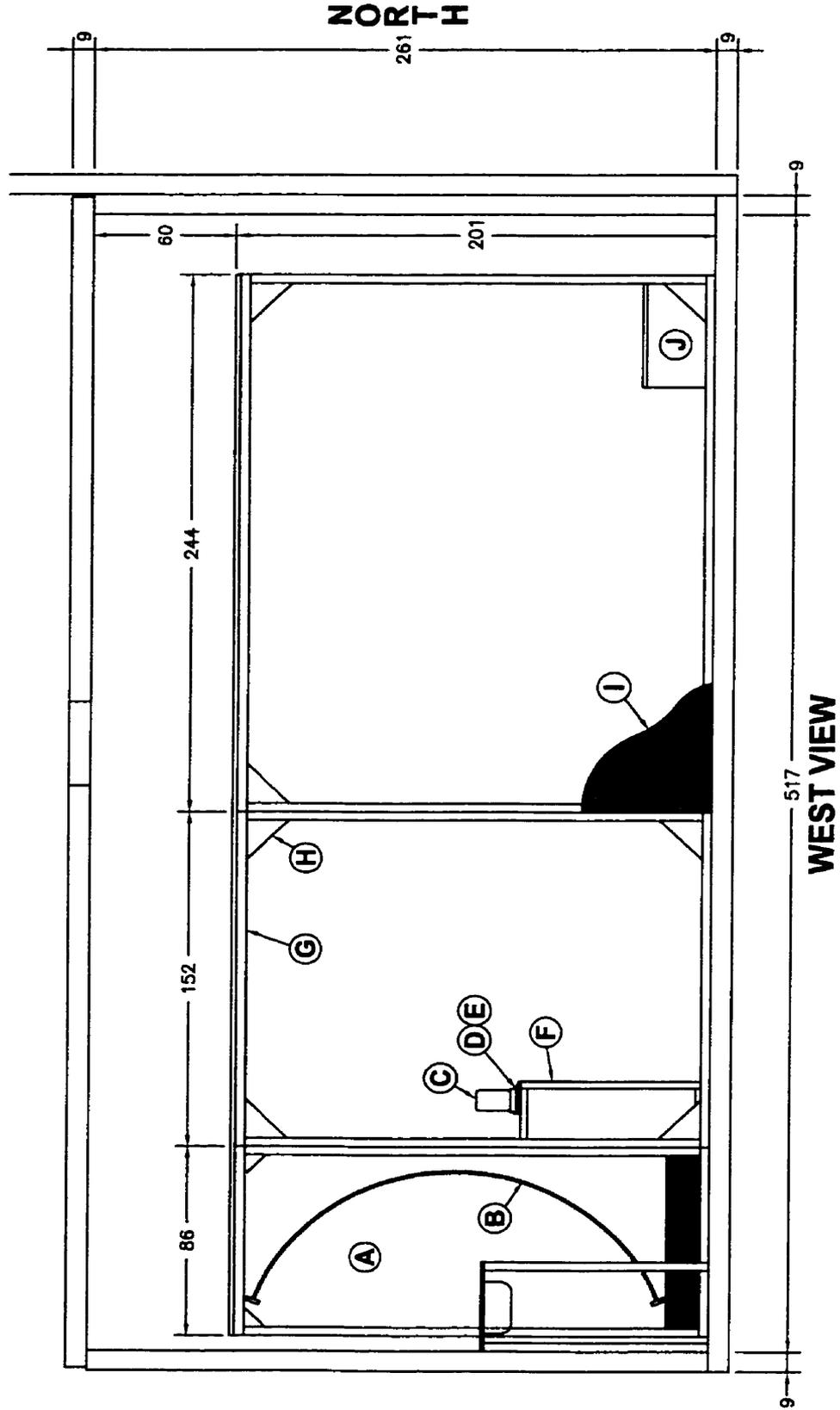


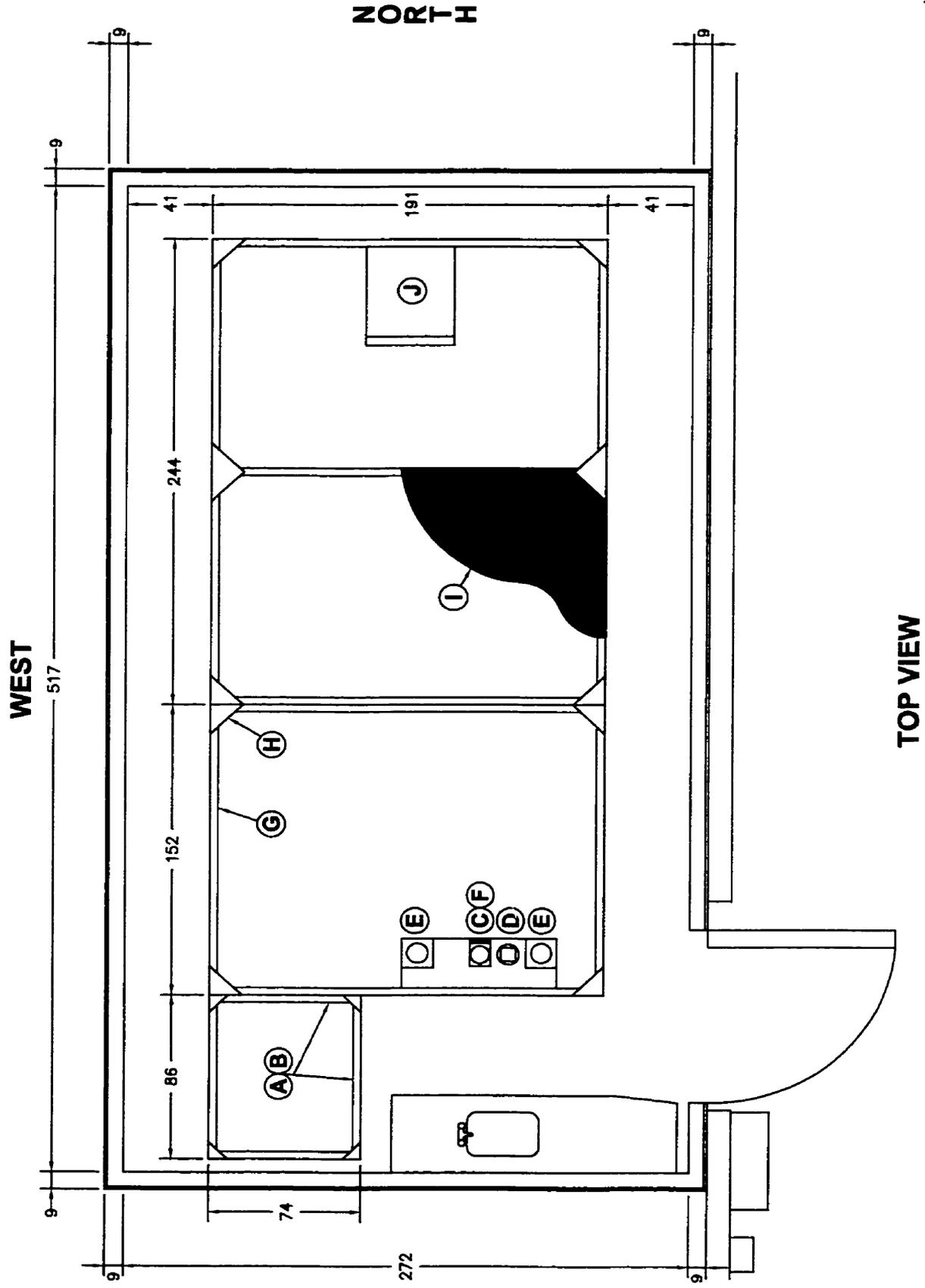
Fig. 51. Heating, cooling, airflow and humidification systems in flight and rearing room, top view. Legend as in Figure 50, with the addition of U, control panel; and V, thermohygrograph.

Fig. 52. Flight cage and feeding station in flight and rearing room, West-facing view.
A, vestibule door; B, zipper; C, syrup feeder; D, water dish; E, protein feeder; F,
orientation cue; G, wooden cage framing member; H, gusset; I, fibreglass screening; J,
bee colony.



WEST VIEW

Fig. 53. Flight cage and feeding station in flight and rearing room, top view. Legend as in Figure 52.



CHAPTER VII

GENERAL DISCUSSION

In this thesis, I have examined how pollen quality affects its utilization and collection by honey bees. These investigations have provided new insights into how pollen quality is evaluated by individual foragers and their colonies, and how colonies match their demand for dietary protein to variability in its supply in the environment.

One of the problems associated with the study of pollen foraging in honey bees is to determine how colonies or individual foragers respond to variation in the nutritional quality of pollen among plant species. The nutritional content of pollen directly influences such fitness-related parameters as the growth and development of hypopharyngeal glands and ovaries in young adult workers. My investigations have shown that gland and ovary development are not always promoted equally by different species of pollen, but that changes in these tissues are directly related to the amount of crude protein ingested by bees. Because the nutritional suitability of pollen for honey bee workers is strongly correlated with its crude protein content, it is a useful parameter for evaluating pollen quality and an important currency for examining colony-level processes. This relationship is supported by the fact that other nutrients supplied by pollen are not growth-limiting factors. The majority of bee-collected pollens contain suitable profiles of the essential amino acids for honey bee development (Gilliam et al. 1980; McCaughey et al. 1980; Solberg and Remedios 1980; Rayner and Langridge 1985; Wille et al. 1985; Day et al. 1990), and those species that are low in protein content are

often deficient in essential nutrients (de Groot 1953, Herbert et al. 1970, Loper and Berdel 1980b, Loper and Cohen 1987). Based on the array of species tested, my studies have also revealed that the amount of pollen consumed by young adult workers does not appear to be related to its protein content. Workers appear to be insensitive to the nutritional value of pollen, as demonstrated by their failure to compensate for diets having low protein content by increasing consumption. Such behaviour also suggests that consumption could be greatly influenced by components of pollen that are independent of nutritional quality, such as lipid-based attractants, phagostimulants and repellants found in the pollenkitt (Schmidt 1985). Based on the importance of pollen protein for honey bee nutrition, the variability of pollen protein in the environment, and the apparent insensitivity of nurse-aged workers to dietary protein content, I chose to manipulate the level of stored protein within colonies both quantitatively and qualitatively allow comparisons between their effects on individual and colony-level foraging.

My manipulations of colony pollen reserves revealed important information about the ability of honey bees to detect and respond to changes in pollen stores. Colonies exhibit similar responses to variation in either the quantity or quality of their pollen stores, thereby demonstrating their sensitivity to a proteinaceous currency. Colonies respond by adjusting their foraging strategy to restore levels of stored protein to meet the requirements of brood production. Where colonies are deprived of pollen or provided low quality pollen, they respond by increasing the proportion of pollen-collecting specialists in their work force, rather than increasing the overall rate of foraging. Individual foragers neither change the amount of pollen they collect nor vary the number of species sampled per foraging trip. Therefore, deficits in protein within the colony are

met by increasing the proportion of pollen-collecting specialists without changes in individual forager effort.

My research also demonstrates the importance of forager experience to the pollen collection activities of the colony. Irrespective of the state of the colony, I discovered that foragers with less foraging experience collect heavier pollen loads, tend to sample more species per foraging trip, and select pollen with slightly higher protein content than experienced foragers. Such differences in the activities of these foraging cohorts are likely related to the difference in age between the younger, inexperienced foragers and older, experienced foragers. Because of the limited lifetime energy budget of honey bees (Neukirch 1982), older foragers are energy-constrained and are likely to employ different foraging strategies than younger foragers. Experienced foragers may collect smaller loads, visit different floral patches and be more “choosy” than inexperienced foragers, effectively optimizing their energetic efficiency and responding to physical processes such as wing wear and degeneration of the flight mechanism (Baker 1976, Collatz and Wilps 1986, Kern 1986, Cartar 1992). In contrast, the less constrained energy budget of novice foragers would better accommodate the greater time spent learning by younger foragers (Dukas and Visscher 1994) and permit them to sample their floral environment more broadly.

I have shown that a colony may effect changes in the size or quality of its pollen reserve by shifting the ratio of inexperienced to experienced foragers in its work force. If the magnitude of this shift is great enough, a change in average weight or protein content over all pollen loads returned to the colony may occur, as shown in other studies (Fewell and Winston 1992, Higo et al. 1992, Eckert et al. 1994). Previously, this was thought to

occur through increased effort on the part of all foragers in response to colony-level stimuli. However I have shown that the effort of foragers does not change in response to such stimuli, and that the allocation of foragers with different levels of experience is the most direct way to adjust the amount or quality of pollen that is returned to the colony. This mechanism has not previously been identified, and fundamentally changes our understanding about how colonies respond to variations in their protein needs. Future studies should carefully evaluate the composition of the foraging force before and after manipulations of colony state, and document the relative contributions of experienced and inexperienced foragers to pollen collection.

Although it is clear that pollen foragers respond to a colony's need for protein, and that foragers of different age cohorts differ in their selection of plants and pollen collecting strategies, very little is known about the way in which foragers use discrete cues to select pollen. In order to evaluate the choices made by foragers in response to specific cues, I devised bioassays that decoupled interactions among floral traits and allowed them to be experimentally manipulated. Confounding effects normally found in natural settings were eliminated by performing bioassays in a honey bee flight and rearing room that provided controlled conditions and high rates of clean air exchange.

During flight room bioassays, individual foragers exhibited considerable fidelity to pollen odours produced by pollenkitt lipids, but showed no species-specific preferences. Pollen-produced odours improve the reproductive success of plants and also increase the efficiency of honey bee foraging by enhancing orientation and constancy to a floral source. Foragers incur fewer costs locating and working flowers with strong pollen odours and perceive the availability of the resource before alighting. Foragers also use

pollen odour to communicate the location of food to nestmates and may recruit nestmates using the odour of pollen alone. I have shown that the odour of pollen is a primary cue evaluated by foragers, and in bioassays in which pollen odour was presented alone, can release pollen collection behaviours in workers. My studies also demonstrate that foraging at a weakly scented food source can be greatly increased or diminished by the presence of a competing food source containing pollen lipid odour, irrespective of other cues present. Foragers were also found to be sensitive to the size of particles collected; those less than 45 μm caused bees to exhibit the greatest range of pollen-collecting behaviours, while those less than 75 μm were physically packed and removed most often. These particle ranges correspond well with the range of particle sizes naturally collected by bees, and probably reflect the optimal size range based on their efficiency of collection. Foragers were also more highly attracted to simulated flowers with lower handling time.

One of the most enlightening findings of these bioassay experiments was the discovery of a set of cues that was hierarchically evaluated by workers. When presented together, the presence of pollen lipid odour predominated over handling time, which in turn was evaluated over particle size. However, the presence of pollen lipid odour was superior to the remaining cues; bees exhibited pollen-collecting behaviours to dishes having pollen lipid odour even if handling time was high and particle size was very large. This reinforces the view that pollen lipid odour is the primary cue evaluated by foragers at flowers.

Another important conclusion derived from flight room bioassays was that individual foragers do not discriminate on the basis of the protein content of a food

source. This supports my field research that showed pollen foragers did not collect pollen with greater or lesser nutritional value in response to colony-level manipulations of the size or quality of the pollen reserves in colonies. These facts strongly support the idea that individual foragers have no way of discriminating the quality of pollen that they collect by means of their individual sensory physiology or by direct feedback from nestmates. This is also congruent with widely reported observations of honey bees collecting substances containing very little or no nutritive value (Shaw 1990). Although I have shown that honey bee foragers are insensitive to the nutritional quality of pollen, their collection preferences are influenced by the colour, size, and the costs associated with handling pollen grains. My results also contest the purported ability of pollen foragers to code for the quality of pollen that they collect in dance information (Waddington et al. 1998). Important to understanding the results of the latter study is the fact that potential differences in recruitment shown between treatments were confounded by the lack of standardization for pollen odour and by other factors that affect the efficiency of pollen collection.

Honey bees are adaptively tuned to levels of stored protein and, possibly, the flow of protein within the colony. Matching overall pollen collection with the nutritional requirements of the colony requires the integration of individual foraging behaviour with a signal indicating the need for protein by the colony. Decreases in the quantity or quality of pollen within the colony serve to stimulate foragers to collect pollen, but do not alter their effort or the foraging choices they make. Other studies have suggested that workers may be stimulated into foraging through direct physical contact with unsealed brood and pollen stores, or perception of the odour from them (Free 1967, Pankiw et al.

1998b, Dreller et al. 1999). Alternatively, pollen collection may be regulated by indirect feedback through an inhibitory signal via the proteinaceous food produced by nurse bees (Camazine 1993, Camazine et al. 1998). My experiments show that pollen foraging is not stimulated by the presence of large areas of empty comb for pollen storage when high quality pollen is present in areas of the colony inaccessible to foragers. This suggests that physical assessment of pollen stores or brood is not the only mechanism by which foragers regulate pollen collection. My findings emphasize that pollen foraging is regulated around a proteinaceous currency and are consistent with the use of volatile stimulatory cues from pollen and brood in association with protein inhibition from nurse bees.

Perhaps the greatest difficulty in understanding the pollen foraging decisions made by honey bees has to do with the integration of individual foraging behaviour with colony state processes. In my bioassay experiments and in other similar studies (Taber 1963, Hopkins et al. 1969, Hohmann 1970, Starrat and Boch 1971), foragers could be induced to collect cellulose powder or substances of no nutritional value by the addition of pollen lipids. Hence, the benefit of collecting large amounts of pollen quickly, must outweigh the potential of foragers becoming constant to a nutritionally deficient food source. Although honey bees can collect substantial quantities of nutritionally inferior species such as kiwifruit (*Actinidia deliciosa* A. Chev.) (Jay and Jay 1984) and members of the Compositae (Loper and Cohen 1987, Schmidt et al. 1987, Taber 1996), there are several ways in which colonies ameliorate the risk of collecting large quantities of poor quality pollen. The information center strategy of foraging (Visscher and Seeley 1982; Seeley 1985a, b; Seeley et al. 1991) illustrates that pollen collectors forage over a wide area

around the hive, and change their patches of floral preference on a daily basis. This general polylectic foraging nature of honey bees may be an adaptation to avoid a scenario in which too many workers become constant to a nutritionally deficient pollen source. The collection of many different pollens also allows a greater mixing of pollen comprising the diet of the brood which would help to fulfil their nutritional requirements, as deficient pollen types would be augmented with superior ones. Furthermore, my results have shown that honey bees can also respond to such risk by increasing the rate of pollen foraging and/or shifting the ratio of experienced to inexperienced foragers.

The research in this thesis has provided new insight into the importance of pollen protein in the nutrition of worker honey bees, and the discrimination of pollen protein by individual foragers and the colony as a whole. It has also shown the importance of pollen protein as a currency by which the foraging of the colony is regulated, by a pathway that is independent of the ability of individual foragers to directly assess the nutritive value of the resource they are collecting. Instead, individual foragers appear to optimize their pollen-collection strategies based on their level of foraging experience and with respect to a number of discrete floral cues, particularly pollen odour. Based on these findings, it is my hope that future investigations will reveal additional details, hitherto unknown, about the intricate world of *Apis mellifera*.

Conclusions

1. The development of hypopharyngeal glands and ovaries in caged honey bee workers is dependent on the quantity of protein consumed from pollen diets. Crude dietary protein can be used as a guideline for evaluating pollen quality. Among the floral species examined in these studies, pollen protein varies greatly. Poor quality pollen is characterized by having low crude protein content ($\leq 15\%$).

2. Pollen protein is not always allocated equally between developing hypopharyngeal glands and ovaries in young adult workers.
3. The consumption of pollen is not directly related to its protein content.
4. The nutritional components of pollen remain unaffected by storage for 1 year at -30°C , under an oxygen-reduced atmosphere.
5. Honey bee colonies respond to reductions in the quality of their stored pollen reserve, and do so in a manner analogous to a reduction in pollen quantity. For decreases in quality, colonies increase the proportion of pollen foragers in the work force, but maintain the same total foraging rate. Individual foragers do not change their behaviour to specialize in collecting higher or lower quality pollen, nor collect from greater or fewer numbers of floral species, in response to such changes. In addition, individual foragers do not exhibit any change in their foraging effort in response to manipulations of the quality or quantity of pollen stores.
6. Colonies respond to reductions in the quality of their stored pollen reserve more slowly than reductions in its quantity. Reductions in the size of the pollen reserve result in increased rates of pollen intake within 12 h, however reductions in the quality of the pollen reserve result in increased rates of pollen intake after only 2 to 3 days.
7. Different cohorts of foragers within the colony differ with respect to pollen foraging traits. Under high pollen demand created through reductions in the quantity or quality of stored pollen, inexperienced foragers collect heavier pollen loads, sample more species per foraging trip and specialize on slightly more nutritious pollen than experienced foragers.
8. Changes in colony-level foraging parameters in response to reductions in pollen quality or quantity are likely to occur through a shift in the ratio of experienced to inexperienced foragers in the foraging population.
9. External pollen lipids stimulate the expression of pollen-collecting behaviours by honey bees and provide important odour cues for orientation to a food source. At the dose used in my experiments, honey bees do not exhibit species-specific preferences for pollen odours. Among adjacent food sources, strong pollen lipid odours may draw foragers away from a weakly scented competitor or, by enhancing the attractiveness of the entire floral arena, increase the rate of visitation to a competitor over what it would receive alone.
10. Honey bees prefer to pack particles $< 75\ \mu\text{m}$ in diameter into their corbiculae, and exhibit the greatest frequency of pollen-collecting behaviours for particles $< 45\ \mu\text{m}$.
11. Honey bee foragers do not discriminate between foods of different protein content. This suggests that individual foragers are incapable of assessing pollen quality, and that

feedback for quality must occur at the level of the colony.

12. Reductions in handling time increase the frequency of behaviours associated with pollen collection by honey bees.

13. When pollen foraging cues are presented simultaneously, pollen lipid odour is the predominant cue used in making pollen foraging decisions. The presence of pollen lipid odour can increase the attractiveness of artificial food sources with no nutritional value, even when handling time is high or particle size is unattractive. Foragers appear to evaluate pollen-based cues that affect its searching and collection efficiency.

15. Honey bee flight and rearing rooms can be improved by the use of electronic lamp ballasts, spectral matching of light sources, high volumes of air exchange, electrostatic air cleaning and computer-controlled operating systems.

Future Research

Continued study in these areas of research should consider the following recommendations:

1. That the interrelationships between pollen protein levels and other pollen-based cues (particularly pollen lipid components) on the collection of pollen by foragers and its consumption by nurse-age bees are elucidated.
2. That the recruitment and behaviour of inexperienced and experienced cohorts of foragers are documented before and after manipulations of colony state variables.
3. That the discrete information coded by the intensity and duration of recruitment dances by pollen foragers is determined, as it applies to the foraging strategy of the colony and the allocation of workers among forage sites.
4. That a currency that is sensitive to the protein status of the colony is incorporated into models describing the regulation of pollen collection.
5. That colony-produced cues used to stimulate or inhibit the collection of pollen by foragers are determined.
6. That the interrelationships between humidity, particle size and surface ornamentation on the amount and efficiency of pollen collected by foragers are determined.
7. That spectroradiometry and colour rendering experiments in indoor flight and rearing rooms are performed to determine the quality of light produced for honey bee colour vision.

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