

DOMESTICATION AND CASTE DIFFERENTIATION
IN BUMBLEBEES

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ABSTRACT

by

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A. Attempts to induce colony founding by B. terricola Kirby, B. nevadensis Cresson, B. fervidus (F.), B. borealis Kirby, B. rufocinctus Cresson, B. ternarius Say, B. perplexus Cresson and B. vagans F. Smith in laboratory nest boxes were made using naturally overwintered, artificially overwintered and non-overwintered queens. Very successful results were obtained with naturally overwintered B. terricola and B. perplexus queens in nest boxes furnished with upholsterer's cotton at room temperature and with naturally overwintered B. ternarius queens in boxes without nest material at 29-30 C. Though only small numbers of naturally overwintered queens of the other five species could be tested, moderately successful results were had with all but B. fervidus.

Colony founding experiments with artificially overwintered queens were most successful with B. terricola, though premature changeover to queen production occurred in many colonies. Non-overwintered queens of five species laid eggs and a continuous laboratory culture of B. rufocinctus

was maintained over four generations.

B. Caste status of imaginal females was investigated in B. terricola, B. perplexus and B. ternarius by holding freshly emerged imagines at 32 C with abundant honey and pollen. Ovary development in the latter two species was negatively correlated with weight gain, and was significantly greater when pairs of B. terricola were confined together than when queens were confined singly. The relevance of these results to imaginal caste determination and to the physiological distinction which has been supposed to exist between castes is discussed.

The distribution and temporal variation of size among the females produced in captive colonies was investigated. As in wild colonies, a more pronounced size dimorphism between castes was found in "pollen-storing" than in "pocket-making" species, even though colonies of pocket-making species were unable to feed larvae in their normal characteristic manner under the conditions imposed by this study. A progressive increase in the size of females reared during colony development was generally found in B. perplexus but not in B. terricola or B. ternarius.

The rôle of changes in the worker/larva ratio in causing changeover from worker to queen production was studied in B. terricola, B. perplexus and B. ternarius. An association between this ratio and female size was found in

all three species and an attempt was made to explain the change of the ratio in terms of the oviposition rate in B. ternarius.

The changeover from worker to queen production was investigated in detail in captive colonies of B. terricola and B. ternarius. The first queens usually emerged from brood batches which also yielded workers. Presumptive queen larvae ceased feeding later than presumptive worker larvae in both species, and had larger fourth-instar head capsules in B. terricola, though not always in B. ternarius. It was concluded that queen determination probably takes place ontogenetically later in B. ternarius than in B. terricola and is perhaps effected in the former species by the exposure of undetermined fourth-instar larvae to a sudden increase in food supply brought about by cessation of feeding of other (worker) larvae in the same brood clump.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEWS

1.1 General

This thesis comprises a re-appraisal of the phenomenon of caste in bumblebees. New data are presented and use is made of analyses of previous work. An attempt has been made to set up an unequivocal formulation of the problems inherent in any study of the caste phenomenon; in particular, attention has been given to the epistemological aspects of these problems, in the hope that the adequacy of the evidential criteria by which the castes are recognised may be appropriately assessed.

Much of the experimental work involved in this study required the initiation and maintenance of bumblebee colonies in the laboratory throughout all months of the year. Accordingly, a great deal of time was devoted to the development of efficient methods for the laboratory rearing of colonies of the bumblebee species which occur commonly in southern Manitoba. The first part of this thesis describes these domestication methods, and the first section of the literature review that follows is devoted to the subject of bumblebee domestication.

1.2 Domestication of bumblebees

The value of bumblebees as pollinators of forage legumes has for a long time stimulated interest in the conservation and augmentation of wild bumblebee populations, while their efficacy in pollinating horticultural plants has resulted recently in a renewed concern that their numbers not be allowed to diminish (VEERMAN & VAN ZON 1965).

STEPHEN (1955) drew attention to the attenuation of population densities of bumblebees by agricultural practices in Manitoba. Even in areas where environmental change has been less drastic than in the settlement of virgin land, yearly variations in bumblebee populations have been reported by BIRD(1944), PEDERSEN & STAPEL (1942 - 1945), VALLE (1948), ÅKERBERG & LESIŃS (1949), JAMIESON (1950), SCHWAN (1953), BINGEFORS & ESKILSSON (1961), HANNINEN (1962), VALLE et al. (1964), while fluctuations in bumblebee numbers from place to place have been observed by many authors (PEDERSEN 1945, SCHWAN 1953, ÅKERBERG & HAHLIN 1953, VALLE et al. 1960, VALLE 1961, UMAERUS & GRAZI 1961, 1962, HANNINEN 1962, VESTAD 1962). The factors associated with the population densities of bumblebees have been much discussed, most recently by HOLM (1966a), who pointed out that at least two of them--availability of nest sites and forage--are susceptible to human control, as has been remarked by STAPEL (1933), SKOVGAARD (1936), STEPHEN (1955), FREE &

BUTLER (1959) and HAWKINS (1962a, b), who have advanced practical suggestions as to how such control might be undertaken. One must support Holm's statement that "it is therefore somewhat surprising that, with the exception of a few cases, these suggestions have passed unheeded."

I concur with the view that if wild bumblebee populations are to be used for the pollination of field crops, habitat improvement projects should play the major part in increasing population densities. In such projects there may be a future for the use of the domicile techniques developed by SLADEN (1912), FRISON (1926), FYE & MEDLER (1954), WILCKE (1953) and HOBBS et al. (1960, 1962), but, though rearing and overwintering of queens in controlled conditions in captivity may serve some purpose in providing a nucleus of bees which may then be encouraged to build up population strength in the wild, schemes involving reliance upon domestication methods as a major source of colonies for pollination "seem to be divorced from reality" (FREE & BUTLER 1959). Even if--as seems likely--current laboratory studies yield domestication methods of an adequate level of efficiency in terms of the proportion of queens which successfully start colonies, the amount of labour required for such methods will probably always be economically prohibitive, except for use in greenhouses and similar restricted areas, such as experimental plots more or less isolated from

alternative sources of bumblebee forage.

The need for laboratory-reared bumblebee colonies for biological studies has been noted by HORBER (1961). Measures taken to increase natural bumblebee populations require adequate knowledge of relevant aspects of the biology of bumblebees, especially of their ecology. In default of such knowledge, it is inevitable that ill-advised and untested notions should appear from time to time, some of which might have serious economic repercussions. I believe one such idea to be CUMBER's (1953) suggestion that abundant food supply in the early stages of colony development may cause premature changeover to the rearing of sexual forms. There is little evidence to support this idea (which probably springs from a misconception regarding the relationship between larval food supply and colony food intake)--indeed, it will be argued in this thesis that the time of changeover to queen rearing is largely independent of colony food intake in some bumblebee species. Nevertheless, Cumber's suggestion has frequently been quoted without dissent (e.g. FREE & BUTLER 1959, HOLM 1966a) in order to support the practical implication that, in managing bumblebee colonies for pollination, the forage available to them prior to the blooming of the seed crop should be kept to a minimum. Such a policy has a degree of credibility similar to the suggestion that honeybee colonies should have their food

intake restricted prior to the main honey flow so that swarming may be prevented.

Controversial issues such as this often suggest controlled laboratory experiments with captive colonies, especially where problems of colony development (which is exceedingly difficult to study in the field) are involved. Much can be done by attracting wild queens to artificial domiciles (see papers on ecology of Bombus subspecies by HOBBS 1964b, 1965a, b, 1966a, b) but for maximum efficiency in the use of time and labour, and for obtaining the greatest possible uniformity in experimental material, colonies initiated and reared in the laboratory--preferably as part of a continuous culture--are required.

The earliest record of a queen bumblebee laying her first eggs in captivity appears to be that of HOFFER (1883). Later authors (LINDHARD 1912, SLADEN 1912, PLATH 1923, 1934, FRISON 1927a, JORDAN 1936, MONTGOMERY 1952, HASSELROT 1952, 1960, WILCKE 1953, VALLE 1955, STEIN 1956, LEHMENSICK 1956, ZAPLETAL 1961, HOLM 1960, 1966b, HOLM & HAAS 1961, HORBER 1961, FRIDEN 1965, POUVREAU 1965b) have developed a number of techniques for starting colonies in the laboratory; their work has been reviewed by HASSELROT (1960) and by HOLM (1966a) who notes that the success achieved by these investigators has been very variable. Holm also states that the difficulty of initiating colonies in the laboratory is the

chief obstacle to making domestication of bumblebees of value to the seed grower, but he does not consider that improvements in technique are likely to bring about an increase in the percentage of queens starting nest-building in laboratory trials; he suggests that "the most important object of future investigations should be to gain a better insight into the factors controlling nest-initiation and the effect of pests and diseases upon the domestication process."

Unfortunately, most of the existing accounts of laboratory domestication experiments have yielded little information on the basic requirements of the queen for colony initiation. MEDLER (1958) stresses the inadequacy of our knowledge regarding the exact nature of the stimuli which induce egg laying and brood rearing. The chief reason for this seems to be that experiments have frequently had to be carried out in an extemporary manner and have often been designed more to simulate natural conditions than to elucidate which features of the natural situation may be dispensed with under laboratory conditions. Also, the majority of laboratory studies on colony founding have been required to supply viable colonies for pollination work, hence more value has been placed on getting as high a percentage of queens started as possible (an objective that is often best achieved by resorting to trial and error methods) than on conducting experiments designed for statistical analysis.

Then again, such a diversity of bumblebee species has been used for domestication trials that it is very difficult to exclude the possibility of interspecific differences when comparing the results of various workers, almost all of whom have noticed that some species seem more ready than others to start colonies in captivity.

Thus, the literature on bumblebee domestication does not contain much information as to why queens failed to oviposit in laboratory experiments, other than records of the occurrence of parasites and disease (e.g. HASSELROT 1960). A major difficulty in determining the causes of oviposition failure is that unless the queens are dissected, it is not possible to assess their ovary development. MEDLER (1962) examined the ovary development of queens in laboratory nest boxes after various durations of confinement; though he found undeveloped ovaries in some queens even after seventy days of confinement, he was not able to attribute all cases of egg-laying failure to insufficient ovary development since the ovaries of many queens contained large eggs (3.0 - 3.5 mm in length). Because many queens (numbers not given) showed signs of egg absorption, Medler concluded that in these insects mature eggs had developed, but were absorbed during the period when not laid. Unfortunately, he did not give the number of queens which did lay eggs.

FREE & BUTLER (1959) tentatively suggested that

HASSELROT's (1952) technique for starting bumblebee colonies in captivity might owe its superiority over that of FRISON (1927a) to the greater degree of insulation which Hasselrot provided. They supported this suggestion by reference to data on the ovary development of workers at different temperatures. However, leaving aside the fact that Frison and Hasselrot worked with different bumblebee species, and that ovary development in workers may not be comparable to that in overwintered queens, the recognition that while oviposition materially implies mature ovaries, the converse is not necessarily true, has rendered of doubtful value any explanation of variations in oviposition success (in different domestication methods) which rests upon the assumption that the various methods differed in their efficacy to stimulate ovary development.

As well as confining queens individually, several authors used pairs of queens, or a queen and one or more workers (not always of the same species) in attempts to get bumblebee colonies started in the laboratory (SLADEN 1912, FRISON 1927a, PLATH 1923, 1934, FREE & BUTLER 1959). It does not seem to be disputed that the presence of workers greatly increases the likelihood of oviposition (though, as Free & Butler point out, the eggs are not always laid by the queen), but somewhat contradictory conclusions have been reached on the desirability of confining queens in pairs.

Sladen found that one queen always killed the other about the time that the first eggs were laid and that the survivor invariably deserted her brood, but Frison and Plath, working with North American species, successfully started colonies in nest boxes containing pairs of queens. However, Frison found that eggs were laid in a smaller percentage (41%) of the boxes containing pairs of queens than in those which contained single queens (59%), though the difference is not statistically significant. Free, on the other hand, obtained greater success (39%) when queens were confined in pairs than when they were confined individually (32%), but once again, the difference is non-significant.

Much ingenuity has been expended upon attempts to provide captive bumblebee queens with suitable nest material. SLADEN (1912) used balls of soft dried grass for his domiciles, and LINDHARD (1912) also furnished the nest chambers of his boxes with dried grass or similar material contained in a hollow cut out of a piece of turf. FRISON (1927a) tried various materials in his nest boxes, finally adopting muslin coated with wax. PLATH (1923, 1934) provided his queens with an inverted tin can under which the pollen lump, located upon a sheet of beeswax, was surrounded by a small quantity of cotton; more cotton was added later as required. HASSELROT (1952) used a ball of cellulose wadding half embedded in moss litter; his design of nest box

has been extensively used by other Scandinavian workers (e.g. VALLE 1955, HOLM 1960, HOLM & HAAS 1961, FRIDEN 1965). HORBER (1961) employed Hasselrot's nest boxes for his experimentally overwintered queens of B. hypnorum, but later abandoned the use of nest material for colony founding experiments conducted in incubators at temperatures up to 35°C. HOLM (1966a) considers that Horber's results support the suggestion that the provision of nest material may play a relatively unimportant part in encouraging queens to start nests in captivity; HASSELROT's (1960) report that some queens built nests in the feeding chambers of his boxes in the absence of nest material gives additional support to Holm's suggestion. The function of nest material as a guide by which the queen's brooding activity may be confined to her eggs and larvae is discussed below.

Not all queens which oviposit in laboratory nest boxes succeed in rearing their brood. In some cases the eggs or larvae are simply neglected, but in others the queen actively destroys the egg cells which she has constructed (VALLE 1955, HASSELROT 1960). Hasselrot considered disturbance to be the major factor associated with the failure of queens to rear their first broods; in a personal communication he wrote that when inspecting the progress of the brood or adding fresh pollen, care must be taken to choose a time when the queen is away from her nest. He recorded (HASSELROT

1960) that in those years (1953 and 1954) when he "allowed a certain coarsening and routine" to enter his method, a greater proportion of queens deserted their brood than in the two years (1952 and 1958) when he reduced disturbance to a minimum.

FRIDEN (1965) obviated the need for inspection of bumblebee nests in their early stages by showing that the rate at which a queen takes sugar syrup from the feeder increases as soon as she has begun to found a colony. But the chief obstacle to eliminating all disturbance is the need to supply fresh pollen to queens which are feeding larvae. Although T. V. COLE (personal communication) reports that he has succeeded in getting colonies started by queens which collected pollen presented in trays in the feeding compartments of their nest boxes, queen bumblebees do not seem to be easily persuaded to collect pollen in this way; so the alternatives are reduced to either placing fresh pollen in the nest cavity itself (in which case, as noted previously, there is a danger that the queen may desert her brood) or to devising a technique by which the queen may be induced to do her own foraging for pollen outside the nest chamber. LINDHARD (1912) allowed his queens to forage from branches of flowers and VALLE (1955), who achieved great success in getting the queens which had oviposited in his nest boxes in 1954 to rear their brood (41 out of 43),

placed fresh flowers in the glass-covered feeding compartments; while PERCIVAL & MORGAN (personal communication) have successfully used artificial flowers as a pollen source for nesting queens. HASSELROT (1960) placed his nest boxes in the field after the queens had commenced nest building and suffered a loss of about half the queens. He attributed this loss partly to the relatively rough treatment to which the nests were exposed during their transport from the laboratory to the field, but since HOLM (1966a) experienced a loss of the same order of magnitude when queens were allowed to fly from colonies which had not been moved at all, it seems more likely that other factors are involved. In any case, it is clear that allowing queens to forage in the field is a most unreliable method of solving the problem of how best to ensure that developing larvae receive adequate supplies of pollen.

Though he did not comment upon it in the text, examination of the tables in HASSELROT's (1960) paper reveals that one colony each of B. jonellus Kirby, B. pratorum L., B. kirbyellus Curt., and B. lapponicus F. produced queens in their first broods. Such colonies would not be expected to reach any great strength, and so the question is raised as to whether the ultimate development of bumblebee colonies started in captivity can be correlated with the behaviour of the queen during the colony-founding period. SLADEN (1912)

found that the colonies which he started in captivity did not become strong; he attributed this to the debilitating effect of confining queens in nest boxes for long periods. HOLM & HAAS (1961) demonstrated a significant negative correlation ($p < 0.001$) between the date on which the queen started nesting and the size reached by the colony (as measured by cocoon counts at the end of the season) in twenty-one colonies of B. terrestris L. which they set out in the field after initiation in captivity, but it is not clear from their account whether or not the length of time spent in nest boxes by the queens before they laid eggs is a factor in this association apart from its effect upon the date of oviposition. In other words, was the colony size associated with the vigour of the queen or with the length of time available for its development once the first eggs had been laid--or with both? Whatever the answer to this question, it is clearly important to be able to control the date at which nests are started, not only in order that colonies of maximum size may be obtained, but also, where the bees are to be used for pollination, that the peak number of workers may be available during the flowering of the crop; hence the advantage of overwintering queens in captivity so that they may be resuscitated from hibernation at the appropriate time.

Attempts to mate and overwinter queen bumblebees in

captivity were made by LINDHARD (1912), SLADEN (1912), FRISON (1927b), PLATH (1927), CUMBER (1953), MEDLER (1958), and HASSELROT (1960), but except for Lindhard none of these workers succeeded in developing efficient techniques for artificial hibernation. The recent highly successful methods by which queens may be overwintered fall into two groups: HOLM (1960) and HOLM & HAAS (1961) used the same method as Lindhard--they allowed mated queens to choose their own hibernation sites in piles of moss or in boxes containing turf or earth and moss which were located in a greenhouse--while ZAPLETAL (1961) and HORBER (1961) stored queens individually in containers, with provision for adequate humidity, at low temperatures. POUVREAU (1965a) used both methods on queens of several European species; he allowed some queens to choose their own overwintering sites in a greenhouse in which were placed wooden boxes containing various materials such as soil and moss, while others were held at +7°C in glass beakers partly embedded in vermiculite. As part of a study of the ecology of the subgenus Bombias Robertson, HOBBS (1965a) combined features from each method by allowing queens of B. nevadensis Cresson to dig their own hibernacula in the ground under a wire screen cage; the bees were later unearthed and placed in containers with damp vermiculite according to Horber's technique. HOLM (1966a) notes that the percentages of queens surviving artificial hibernation

in these investigations are sufficiently great to bring overwintering in captivity within the bounds of practical possibility.

For the attainment of maximal rate of increase and to provide a convenient source of experimental material, one of the ultimate objectives of bumblebee domestication should be to achieve perennial indoor cultures in which mated queens are induced to start colonies without having undergone hibernation. That ovary development may take place in queens which have not undergone low temperature dormancy has been established for B. affinis Cresson by PLATH (1934), for B. terrestris L. by CUMBER (1963), and for B. hypnorum L. by HORBER (1961), who maintained a continuous culture of this species over five generations; but no systematic study has yet been made of the difficulties inherent in obtaining vigorous colonies from such "non-diapause" queens.

During 1964-1966 I attempted the domestication of the following eight North American bumblebee species¹:

Genus BOMBUS Latreille

B. terricola Kirby

¹ The classification adopted here is that proposed by MILLIRON (1961).

Genus MEGABOMBUS Dalla Torre

Subgenus BOMBIAS Robertson

B. nevadensis Cresson

Subgenus MEGABOMBUS Dalla Torre

B. fervidus (Fabricius)

B. borealis Kirby

Genus PYROBOMBUS Dalla Torre

Subgenus CULLUMANOBOMBUS Vogt

B. rufocinctus Cresson

Subgenus PYROBOMBUS Dalla Torre

B. perplexus Cresson

B. ternarius Say

B. vagans F. Smith

Since this work was undertaken to obtain material for observation and experiment, most attention was paid to rearing and maintaining colonies in the laboratory; though queens were mated and overwintered in captivity, no experiments were attempted in order to arrive at optimal techniques for these purposes. Moreover, most of the early experiments on colony founding were undertaken in a trial-and-error fashion; insofar as they yielded useful biological information, these experiments are described below, but some of the attempts made at the outset of the domestication program have been omitted since they were largely unproductive.

1.3 Caste differentiation in bumblebees

The family-society or colony, characteristic of all species of non-parasitic bumblebees, constitutes a highly integrated unit, whose function potentialities are well adapted to the achievement of a reproductive rate sufficient to ensure survival in an environment where seasonal changes preclude a continuous generation cycle. Thus bumblebees are typically insects of the temperate and arctic zones; and even among the comparatively few tropical species, some, at least, preserve an annual cycle similar to that of their non-tropical relatives, in that colony founding by young mated queens does not take place until the end of the dry season (DIAS 1958).

As in other integrated insect societies, functional efficiency in a bumblebee colony is obtained by division of labour among its members. A primary subdivision of female bees into reproductive and non-reproductive subsets occurs in all normal colonies; by analogy with other social insects, these subsets are known as castes (queen- and worker-respectively). Further subdivision of the worker caste has been recognised by several authors, notably COVILLE (1890), RICHARDS (1946), CUMBER (1949) and BRIAN (1952).

Both the phylogeny and ontogeny of insect societies has excited much interest and speculation for a long time (e.g. DARWIN 1859, WHEELER 1928, RICHARDS 1953 et al.) One

of the most fascinating aspects of the development of colonies of most social insects, including those of bumblebees, is that two or more castes may arise from genetically identical eggs (though in Melipona caste is probably determined genetically, according to KERR 1950), a fact which has stimulated much research into the mechanisms which underlie the determination of caste during development (see reviews by BRIAN 1957 and WEAVER 1966).

The term "caste differentiation" possesses a process-product ambiguity. It may be used to mean the set of events by which one caste becomes differentiated from another during ontogeny; alternatively, the term may refer to the state of a population of adult social insects in which caste polymorphism occurs so that some individuals are morphologically or behaviourally differentiated from others. Under one interpretation of caste this ambiguity, which has probably never caused much confusion, disappears; however, the double connotation of caste differentiation should be emphasised because it lies at the root of the problems encountered in studies of the developmental origin of castes, for it is necessary to be able to arrive at a working definition, whereby individuals of different castes may be recognised, if one is to elucidate their mode of origin. In many social insects (ants, honeybees, etc.) there is sufficient discontinuity in morphology between castes to allow their easy

recognition; intermediate forms are rare. But in bumblebees, as in social wasps, the difference between the worker- and the queen-caste is largely a matter of size and behaviour; female bumblebees of intermediate status occur, and it is not always easy to assign them to one caste or the other. It is necessary to consider, therefore, whether the two bumblebee castes should be regarded as regions on a continuum, in which case bees of intermediate size pose no special problem, or alternatively, whether a fundamental discontinuity (e.g. one established at an early stage in development) separates workers from queens, so that female bees of intermediate size represent a mixture of two polymorphic phases.

The ontological problem thus presented must be set in an appropriate epistemological framework. How can we formulate definitions for the two female bumblebee castes, and what assumptions about the relationships between the castes are made when such definitions are used in the course of a study of caste determination? These questions call for immediate attention because of the danger of letting ontological preconceptions influence examination of the caste phenomenon. To assume, for example, that the caste differentiation (product-usage) found among adult bumblebees must arise from a qualitative separation of developmental pathways in the larvae can lead to a question-begging circularity derived from an unnoticed shift in defining character-

istics; we proceed as if we had already adopted definitions for the castes and so arrive at a supposedly synthetic statement describing the association between caste and larval development which actually turns out to be reducible to a series of tautologies. Reduced to absurdity, the argument might run as follows:

"There are two castes in bumblebees, a queen-caste which mates, survives the winter and founds a new colony, and a worker-caste which does none of these things but, instead, performs foraging and brood rearing duties. These two castes must result from different patterns of larval development. In many species of Bombus, two patterns of larval development are apparent, one leading to small pupae and the other leading to large pupae; therefore, the difference between the two castes must depend upon larval morphogenesis--queens result from larvae achieving large size and workers arise from those that don't."

The weakness of this argument is that it starts off using a functional definition of caste but later substitutes a definition based upon size.

FRISON (1927b) and WHEELER (1928) regarded the distinction between castes in bumblebees as an entirely quantitative phenomenon resulting from differences in the amount of food fed to the larvae. However, CUMBER (1949), on the basis of an experimental investigation of weight changes in

females of B. agrorum F., claimed to have demonstrated that queens and workers were "two very different physiological types". Cumber's results will be discussed in detail in a later section of this thesis, but it should be noted here that he did not explicitly state that the difference between his two physiological types was a qualitative one, though this seems to be implied by his remarks.

Even apart from Cumber's experiment, several features of bumblebee biology are not immediately reconcilable with the idea of an underlying continuum as a basis for caste expression. In most areas where bumblebees abound, a classificatory separation (apparently on a size basis) may be observed between those bees which assist in foraging and brood rearing, and those which, after mating, survive the winter to found new colonies the following year. Moreover, though many bumblebee species exhibit a more or less continuous size distribution within the female sex, in others the female bees are clearly separable into two size groups with (normally) no intermediates, as has been shown by numerous authors (e.g. RICHARDS 1946, 1953, CUMBER 1949, FREE 1955a and LØKEN 1961). For the European species, these two species groups correspond very well with SLADEN's (1912) subdivision of Bombus species into "pollen storing" and "pocket making".

Thus we have evidence of two discontinuities,

suggesting either no continuum, or a continuum in which the appearance of bees in the central region is suppressed, and in which some or all bees are subject to one or other of two fates during their imaginal life. In either case problems concerning development are raised, but for the moment it is worth considering whether either of the two discontinuities could serve as a basis for a working definition of caste.

Clearly, if we adopt the criterion of whether or not fertilised female bees survive the winter to start new colonies in the spring, we are committed to regarding caste-status as a process rather than as a set of predicates attributable to a bee at any particular time other than when the process has been concluded. It may not be necessary, however, to entertain such a Whiteheadian concept of caste except as a final arbiter by which a derived definition, based upon empirically discovered predicate-process correspondences, may be validated; it is well known, for instance, that the possession of food reserve in the form of abdominal fat body is associated with the ability of bumblebees to survive the winter (see CUMBER 1949 and FREE & BUTLER 1959) and if it could be established that the presence of such fat body precluded a bee from engaging in brood rearing or foraging, and that the presence or absence of fat body was an "all-or-none" phenomenon, a definition in terms of this characteristic might be admissible.

Of course, some properties which might be used as defining characteristics (such as having been fertilised, having survived the winter and having founded a colony in the spring) are implied by the process definition. Nevertheless, the association between these properties--and hence the usefulness of the process definition--is an empirical matter and must be tested experimentally. For example, because of the toll taken by parasites, predators and bad weather, many bees which hibernate successfully fail to found colonies in the spring. Also, HOBBS (1965b) has observed an overwintered queen of B. rufocinctus Cresson which produced only males in her first brood, suggesting that she had probably failed to mate before she began hibernation.

It seems, then, that some provisional assumptions may have to be made in the selection of a defining characteristic from those implied by our process definition, but that the arbitrariness thus introduced can be justified as a temporary measure pending further research. I suggest that a fairly safe assumption to make is that any female bee which enters into hibernation by constructing a hibernaculum should be provisionally considered as a potential source of a new colony in the following year and hence eligible for

denotation by the term "gyne" (defined by BRIAN 1957 as "a sexual female that is not socially a functional reproductive"); further research would then be necessary to discover the validity of this assumption.

The great advantage gained by adopting such a criterion for use as a primary defining characteristic is that so far as is known at present, hibernaculum construction either occurs or it does not; no cases have been recorded of bumblebees abandoning half-constructed hibernacula in order to return to their maternal colonies to take up foraging or brood-rearing duties. Thus we are provided with a separation of female bees into two classes, which can then be examined by objective statistical tests to discover the strength of their association with other characteristics, such as size, ovary development and food reserve status. However, hibernaculum construction is not a very easy criterion to test experimentally, and it would perhaps be preferable in any case to avoid a behavioural criterion in favour of a physiological one if possible. In view of Cumber's experiments with B. agrorum, deposition of fat-body suggests itself as a possible physiological criterion; experimental work on fat-body deposition is described below.

It might be thought that in those bumblebee species where two distinct size groups of female bees are found, size dimorphism should constitute a convenient starting

point for defining the two castes, especially since such a definition would agree with that generally accepted at the present time. For WHEELER (1928) writes that "we can hardly speak of polymorphism in the social Aculeates till a worker caste makes its appearance as a distinct morphological expression of the behaviouristic and physiological division of labour among the . . . female members of the colony"; in bumblebees, as noted by BRIAN (1957) this distinct morphological expression is mainly a matter of size. However, apart from the fact that a definition based upon size alone cannot yield a clear-cut separation between castes in all bumblebee species, there is the inherent danger that by adopting a purely morphological classification we may cease to regard division of labour as the most fundamental manifestation of caste. From a selectionist viewpoint, it would be "putting the cart before the horse" to suppose that the morphological attributes of castes in social insects should be treated as more fundamental than the functional properties which they express. In any case, Wheeler's dictum is too restrictive, for in some species of Halictus a clear functional separation between worker and queen castes occurs, though unaccompanied by morphological distinction; in fact PLATEAUX-QUENU (1960) has demonstrated that caste-status in H. marginatus is established by imaginal differentiation.

It is important, therefore, to find out how well the functional discontinuity between bees which hibernate and those which forage and/or tend brood is correlated with individual size. PLATH (1934) reported the occurrence of small individuals of B. vagans in the early spring of several years and was able to show in 1924 that one of these abnormally small females had founded a colony and laid fertilised eggs. In April of 1964 I found "worker-sized" individuals of both B. hortorum L. and B. agrorum F. (PLOWRIGHT 1966) which must have recently emerged from hibernation, so though neither of these two species show a very pronounced size dimorphism, it is clear that at least some bees which would be defined as workers by their size must be recognised as queens by their ability to overwinter. Conversely, though CUMBER (1949) was of the opinion that a period of diapause coinciding in part with hibernation was necessary before the queen bumblebee might develop ovaries (it is to be presumed that Cumber intended a definition of "queen" based on size) and though FRISON (1928) was unsuccessful when he tried to induce young queens to lay eggs and start a colony the same season in which they were reared, nevertheless oviposition was recorded by PLATH (1934) by young queens of B. affinis Cresson. Furthermore, HORBER (1961) showed that B. hypnorum L. queens could start colonies in captivity, and if, as YARROW (personal

communication) maintains, it is true that B. pratorum L. and B. jonellus Kirby are double-brooded in some cases in England, the second series of colonies must be started by bumblebees which have not had to withstand hibernation; however, some delay may be necessary between emergence and oviposition, as seems to be the case in B. incarum which occurs in the South American tropics (DIAS 1958). CUMBER (1963) recorded that many bees of large size in a colony of B. terrestris L. which he observed in New Zealand failed to mate, and instead took up worker duties such as foraging, caring for groups of eggs and larvae and laying unfertilised eggs. Cumber attributed the abnormal behaviour of these bees, which he called "gypsy-queens", to their failure to mate on account of the absence of males at the time of year they were reared. FREE & BUTLER (1959) also considered mating to be a factor influencing the ultimate fate of bumblebees, for they state that mating probably causes physiological changes to occur which enable queens to survive the winter, but the evidence for this--that all queens dissected in the spring so far have been found to be fertilised--is weak, and has recently been put in doubt by Hobbs' observation on a B. rufocinctus queen (quoted above).

CUMBER (1949) suggested that all large-size female bees which had accumulated fat body in early imaginal life were potentially capable of mating but that the reverse was

not necessarily true, as was borne out by his discovery of a fertilised "worker" (231 mgm) in a nest of B. hortorum.

To summarise: though large-size in female bumblebees is generally correlated with the undertaking of queen rôle (fat-body deposition, mating, hibernation, etc.) and small size with the assumption of worker functions (foraging and brood-rearing without prior hibernation, etc.), sufficient exceptions have been found to show that caste is only partly determined by size. So for "pollen-storing" bumblebees, in which a sharp size dimorphism occurs, we are able to distinguish caste in two ways--by size or by function; for the sake of clarity in this thesis, I shall use the terms "f-queen" and "f-worker" for castes defined according to function, and "s-queen" and "s-worker" for castes defined by size. Thus an individual may be s-queen-f-queen, s-worker-f-worker, s-queen-f-worker, or s-worker-f-queen. In those species where no sharp size dimorphism occurs, size must, of course, be treated as a continuous variable; here, a dichotomous separation between castes, if such can be found, must be based upon function.

The problems tackled in this thesis fall, therefore, into two groups:

- 1) How is the size of female bumblebees determined--does the morphology of small bees differ from that of large ones other than in size; i.e. is the

discontinuity apparent in "pollen-storing" species due to an underlying difference in the morphogenetic pathways leading to s-queens and s-workers, or does the discontinuity result from suppression of the intermediate range in a size continuum?

2) How and when is the functional fate of a female bumblebee established? In particular, are the fates of s-queens-f-workers and s-workers-f-queens in "pollen-storers" determined during imaginal life or earlier?

Most of the experiments and observations described here were done upon three "pollen-storing" species, B. terricola Kirby, B. perplexus Cresson and B. ternarius Say, representing two subgenera (Bombus Latreille and Pyrobombus D.T. respectively). The three species, which strongly resemble their European analogues, B. terrestris L., B. pratorum L. and B. lapidarius L. in methods of brood-rearing and choice of nesting sites, differ from each other in several important respects--notably in the development of their larvae and the manner of egg-cell construction by the queens. A description of their specific peculiarities is given below (Section 3.2).

CHAPTER II

EXPERIMENTS ON BUMBLEBEE DOMESTICATION

2.1 Techniques used for mating bumblebees in captivity

Methods for mating each of the seven species studied were devised by trial-and-error; queens and males were introduced into one of the several sizes of mating cage which were tested, and the incidence of copulation during the following hours was observed. For the more "difficult" species (see below), the cage design was considered satisfactory if, after a few days, over 50% of the queens were fertilised.

Though no experiments were carried out specifically to compare the readiness of each species to mate in captivity, differences in mating susceptibility (which were confirmed by the small number of fertilisation checks which were undertaken) were so striking that they are described here.

In B. borealis, B. fervidus and B. rufocinctus, attempts at copulation (sometimes successful) frequently took place in the nest boxes, but, apparently, only when these were illuminated by daylight or fluorescent light. The first and last of these three species (B. fervidus was not tested) could be mated successfully in small (1.2 m x 0.6 m x 0.6 m) plastic cages in which honey and pollen were supplied. The mating activity shown by males of B. borealis

in such cages was so great that up to 8 were sometimes seen attempting to copulate simultaneously with a single queen; very often the participants arranged themselves in a pile. These multiple attempts at copulation did not seem to vitiate the successful insemination of the queen by the male which was actually in coitu with her, although the wear and tear caused by such events usually removed most of the pile from her abdomen. All ten B. borealis queens and all but one out of 18 B. rufocinctus queens which were dissected after a few days in plastic mating cages were found (on dissection) to have sperm in their spermathecae.

In B. perplexus, attempted copulation in nest boxes was only rarely seen--but mating in 1.2 m x 0.6 m x 0.6 m plastic cages seemed to be fairly successful, though 3 out of six queens which subsequently laid eggs in laboratory nest boxes (see Section 2.312) were unfertilised.

B. terricola, B. nevadensis, B. ternarius and B. vagans were never observed to mate in nest boxes, and copulation rarely occurred in 1.2 m x 0.6 m x 0.6 m plastic cages. In August-September 1964, moderately successful results were obtained by releasing large numbers of B. terricola and B. ternarius queens and males in an outdoor greenhouse, but in 1965 an indoor 3.6 m x 1.8 m x 1.8 m wire screen cage, illuminated by external banks of fluorescent lights, was used. Strips of fabric were hung from the roof

of the cage, to provide suitable resting places, and several dishes of honey and lumps of pollen dough were placed at various locations on the roof, walls and floor.

Out of about 150 B. terricola queens released into the wire screen cage with an equivalent number of males, a sample of 24 were removed for dissection after five days; 16 of these were fertilised (67%).

On August 25, 1965, about 75 B. ternarius queens were released in the cage which already held approximately 200 males of that species. Within an hour, 24 pairs were found in coitu, many of them on the floor. Jars were gently placed over the copulating bees (pairs on the roof and walls were brought down onto the floor) and the queens were later used for colony founding experiments (see Sections 2.311 and 2.312). In spite of the fact that all 24 queens were known to have copulated, five were found to be unfertilised when dissected some months later, possibly because the confinement of the copulating couples under jars had inhibited the passage of sperm.

Efficient methods for mating bumblebees in captivity have already been devised, notably by HOLM (1960), who observed that multiple mating is common in B. lapidarius and B. terrestris. I do not consider that the rather crude methods described above make any useful practical contribution to the subject of captive bumblebee mating, but the

interspecific variability in mating susceptibility which is revealed by these methods is interesting in view of the wide range of species-characteristic patterns of mating behaviour known to occur in the wild (see review in FREE & BUTLER 1959).

2.2 Method used for overwintering queens in captivity

The method used was an adaptation of that described by HORBER (1961) and later employed by HOBBS (1965a).

Queen bumblebees were removed from the cages in which they had been mated and placed singly in 120 ml glass bottles with plastic screw-tops, two-thirds filled with moist vermiculite (approx. 10 g--moistened with 10 ml distilled water--per bottle).

Mortality of hibernating bees was generally not recorded, but on November 25, 1965, when removing queens to be used for colony-founding experiments, data (see Table I) were obtained on the survival of queens which had been put in hibernation during the last week in August 1965. The mortality in B. terricola, B. ternarius and B. perplexus is significantly greater than in B. nevadensis and B. borealis. It is interesting to note that B. nevadensis and B. borealis queens seemed very reluctant to start colonies without prior hibernation (Section 2.311). Very likely the greater hibernation survival in these species is associated with their having the well-developed fat body characteristic of queens

TABLE I

MORTALITY IN BUMBLEBEE QUEENS
STORED FOR THREE MONTHS AT 1° C

Species	Living	Dead	Total	Values of chi-square (corrected for continuity)			
<u>B. nevadensis</u>	12	0	12	9.7**	5.6*	17.9***	20.0***
<u>B. borealis</u>	21	3	24				
<u>B. terricola</u>	22	27	49				
<u>B. ternarius</u>	17	13	30				
<u>B. perplexus</u>	6	22	28				

* = $P < 0.05$ ** = $P < 0.01$ *** = $P < 0.001$

with undeveloped ovaries.

Better hibernation survival with the last three species (as with B. rufocinctus and B. vagans, which were also poor hibernators) might have been obtained if a) the bottles and vermiculite had been sterilised, and b) care had been taken to select only those queens which were relatively heavy for their size (see HORBER 1961). As it was, an adequate supply of stored queens was available on most occasions for use in colony-founding experiments.

2.3 Experiments on colony founding by queens in captivity

2.31 Experiments using nest boxes

2.311 Colony founding by non-overwintered queens

Several series of experiments were carried out in an attempt to induce colony initiation by bumblebee queens which had not hibernated. Some of these experiments were so unsuccessful that they will not be described here; the rest, which yielded slightly better results, are briefly described for the sake of the biological conclusions which may be drawn from them.

Series I

A. Queens and males of B. terricola, B. fervidus, B. perplexus and B. ternarius were reared from wild colonies brought into the laboratory during August 1964, and were allowed to mate in a greenhouse, where a number of the queens (of B. terricola, B. perplexus and B. ternarius)

became broody and constructed wax honey pots near the pollen lumps which had been placed in the angles between the roof and walls of the greenhouse. Eggs were laid by queens of B. terricola and B. perplexus but were not successfully reared (cf. Section 2.32). Most of the B. fervidus queens (none of which became broody) were found torpid in piles of moss upon the floor, as also were many queens of the other three species which had not become broody. Unfortunately, when the bees were removed from the greenhouse to start colony founding experiments in mid-September, no attempt was made to distinguish between torpid and broody queens.

The queens were installed singly, or in pairs, in a container consisting of two half-pint waxed paper cartons connected by a short tube and kept at room temperature (ca. 21° C). A lump (about 0.75 g) of pollen dough (pollen moistened with honey solution) was put in one carton which was covered to exclude light. The other carton contained a plastic vial-cap filled with honey solution which was renewed daily.

A summary of the results, which are presented in detail in the Appendix (Table XXV) is given in Table II. An overall incidence of egg laying of 32% was obtained. Unfortunately, the need for periodic renewal of pollen was not appreciated, nor the desirability of a high environmental temperature for brood-rearing; consequently none of the

TABLE II

INCIDENCE OF EGG-LAYING IN NON-OVERWINTERED
QUEENS OF FOUR SPECIES OF Bombus

Species	No. of queens per box	Total no. of boxes	No. of boxes in which eggs were laid
<u>B. terricola</u>	1	29	8
	2	14	8
<u>B. fervidus</u>	1	9	0
	2	2	0
<u>B. perplexus</u>	1	3	2
	2	2	0
<u>B. ternarius</u>	1	6	1
	2	3	3

queens succeeded in rearing adults in this experiment, though one of B. ternarius and two of B. terricola did eventually produce adults when moved to nest boxes containing cellulose wadding. Two of these queens reared only males, suggesting that they had not mated. One of the two B. perplexus queens which laid eggs had reared half-grown larvae by the time she died.

B. Three B. rufocinctus queens were removed from a captive colony which had been reared December 1964 and were mated in a plastic cage on the laboratory bench. Each queen was confined in a nest box of Series II type (see Section 2.313 and Fig. 1) with a piece of comb and two workers from the maternal colony. Two of these queens began to lay within a few days but in the third box the only eggs laid were those of workers.

One of the two queens which oviposited died while her first-brood workers were still pupae, but the other successfully mothered a large colony.

C. Twenty B. terricola, ten B. nevadensis, ten B. borealis and ten B. ternarius queens which had been reared from captive colonies in August 1965 and allowed to mate in 1.2 m x 0.6 m x 0.6 m plastic cages in the laboratory, were installed singly in Series II boxes at 30° C.

Five of the B. ternarius queens and one B. nevadensis eventually laid eggs, though not until at least 30 days after

the experiment was begun, but no queens of B. terricola or B. borealis showed any colony founding activity.

Three out of the five ovipositing B. ternarius queens reared only males and were found on dissection to be unfertilised; one died before her larvae had pupated, and the other died after rearing one queen and one worker.

Discussion

The laying of eggs by non-overwintered bumblebee queens may be associated with caste differentiation (see Section 3.3). Except that they may have been mated, oviposition by queens in the season during which they have been reared, at least under experimental conditions such as were used here, seems to be analogous with oviposition by laying workers. The same is true of the two B. affinis queens which PLATH (1934) reported to have taken over the egg-laying duties of the colonies in which they reared, following the death of their mothers.

Yet, in its entirety, colony founding under natural conditions involves much more than oviposition. If, as is likely (see PLOWRIGHT 1966), some species in Europe (e.g. B. pratorum L. and B. jonellus Kirby) show double-broodedness, then current season queens must presumably carry out the entire sequence of colony-founding behaviour normally undertaken by overwintered queens; by doing so, they become distinguishable from laying workers.

The matter is further complicated by the question of diapause (defined here as that period during the imaginal life of a female bumblebee during which ovary development is inhibited by internal physiological factors which may or may not be environmentally induced). In Section 3.3 it is suggested that the presence of fat body may be antagonistic to ovary development and that a queen which has accumulated much fat body must suffer its partial breakdown before she can oviposit. We would therefore expect diapause to be broken by hibernation, as is the case, but it is important to note (as pointed out by FREE & BUTLER 1959) that in early nesting European species such as B. pratorum, the first part of "hibernation" spans the hottest months of the year. Furthermore, the incidence of egg laying in the unmated queens described in Section 3.3 indicates that oviposition is stimulated by the presence of a second queen.

Presumably, the conditions in the greenhouse used for mating in August 1964 were greatly conducive to precocious ovary development, perhaps because of the presence of so many bees (≥ 100) together. But it is not possible to say whether what occurred within the greenhouse should be regarded as (a) imaginal differentiation of "queens" into "laying workers", (b) the truncation of a diapause state, or (c) both.

From a practical standpoint, the failure of the bees

which oviposited after being taken from the greenhouse to rear their brood is adequately explained by the unsuitability of the method used to induce them to found colonies. With suitable refinement, there seems no reason why the experiments described here should not be used as a basis for a workable method for perpetuating a succession of bumblebee colonies and so avoiding the hiatus caused by queens overwintering for several months.

2.312 Colony founding by queens overwintered in captivity

I Twenty B. terricola queens, reared from captive colonies in July 1965, were mated in laboratory cages (see Section 2.1) and stored over damp vermiculite (see Section 2.2) at 1°C for 8 weeks. They were placed singly in Series II boxes (see Section 2.313) at 30°C; fresh pollen was supplied every third day until the sixtieth day, on which the survivors were dissected. Only one of these bees laid eggs, but she failed to rear the larvae resulting from them. Of the eight bees surviving on the sixtieth day, four had well developed ovaries which appeared to contain resorbed eggs; the remainder had slightly developed ovaries and small amounts of more or less yellowish fat body.

II Fifty five B. terricola queens were mated and stored over damp vermiculite at 1°C (see Section 2.2) for thirty weeks. After weighing, 45 of the queens were placed in Series II boxes at 30 C for sixty days. The date of ovipos-

oviposition and composition of the first brood was recorded for each bee that laid eggs. On the sixty first day the radial cell lengths of each queen were measured, and all queens which had not oviposited were dissected, their ovaries weighed using RICHARDS' (1946) method, and the length of the longest egg measured under a binocular microscope.

The data are given in Table III. Correlation coefficients and partial correlation coefficients relating the number of days between installation and oviposition (T), the radial cell lengths (R), and the logarithms of the initial weights (W) of the 24 queens which oviposited were calculated:

$$r_{WT} = 0.1541 \text{ N.S.}$$

$$r_{WR} = 0.6379 **$$

$$r_{RT} = 0.1607 \text{ N.S.}$$

$$r_{WT.R} = 0.0678 \text{ N.S.}$$

Though there was considerable variation in the ratio of body weight to wing dimension among these 24 bees, there is no evidence that queens which are heavy for their size take a longer or shorter time to oviposit than those which are underweight. However, the experiment supplies no information on the ovary development of these bees; since the ovaries of the 8 queens which had not oviposited by the sixtieth day were well developed and four (Table IV) showed signs of egg resorption, it is possible that the relationship

TABLE III

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DATA ON B. terricola QUEENS PLACED IN SERIES II BOXES
AFTER OVERWINTERING IN CAPTIVITY

Box no.	No. of days between re- suscitation and egg- laying	Weight on resusci- tation mgm	Length of radial cell 1 unit = 0.05 mm	No. of days between re- suscitation and death	Survived until 60th day with- out laying eggs
1		434	71		+
2	10	495	74		
3		497	73	3	
4	14	538	76		
5	11	404	72		
6	13	478	74		
7		573	75	1	
8		517	75	3	
9	36	711	79		
10		487	79	3	
11	19	455	72		
12	8	750	78		
13		580	78	1	
14		572	76		+
15	9	615	79		
16		582	78	9	
17		527	77	3	
18		624	79		+
19		646	78		+
20	10	458	76		
21		451	76	1	
22		428	74		+
23		610	80		+
24	12	452	74		
25	15	553	78		
26	11	424	73		
27	7	569	75		
28	28	540	78		
29		562	77	12	
30	9	418	71		
31	10	615	79		
32		559	74		+
33	5	660	75		
34		407	77		+
35	10	550	80		
36		539	77	3	
37	11	437	75		
38		504	76	12	
39	10	507	81		
40		609	73	10	
41		498	77	10	
42	11	466	78		
43	12	434	74		
44	14	497	73		
45	9	609	77		

TABLE IV

OVARY DEVELOPMENT IN EIGHT B. terricola QUEENS
WHICH FAILED TO OVIPOSIT BY THE 60th DAY

Body weight		Weight of ovaries	Length of longest egg	Resorption of eggs
Day 1	Day 60			
mgm	mgm	mgm	mm	
437	534	27	2.5	+
572	662	26	1.9	-
624	625	26	3.1	+
646	604	40	3.2	-
428	555	32	3.4	-
610	746	12	2.4	+
559	554	25	2.9	+
407	587	18	2.6	-

between maturation of ovaries and time of oviposition is not very precise (see MEDLER 1962a).

Table V gives the number, sex and caste of the first broods reared by 20 of the 24 queens which laid eggs during the experiment. Four of these queens produced only males and were found on dissection to be unfertilised, and four queens failed to rear the eggs which they had laid. The mean number of first-brood eggs in the six boxes in which these were counted was 6.8 per queen. Six of the 32 first-brood egg cells which were opened for egg counting contained more than one egg (3, 2, 2, 3, 2, 2); the remainder each contained single eggs. The mean number of larvae hatched per queen for the 16 queens which reared their eggs was 4.6 (SE = 0.52). Only five queens reared workers, while three queens produced males as well as females in their first broods.

The remaining ten queens were installed at the same time in Series III boxes with cellulose wadding nest material. Two bees died and one had not laid eggs by the twentieth day, when it was dissected and found to have well developed ovaries containing the remnants of absorbed eggs. Data for the seven queens which started colonies are given in Table VI. The mean number of first-brood eggs laid per queen was 7.0, which does not differ significantly from the corresponding mean (6.8) for the 6 Series II queens whose

COMPOSITION OF FIRST BROODS REARED BY 20 ARTIFICIALLY OVERWINTERED

B. terricola QUEENS IN SERIES II BOXES AT 30° C

Box no.	No. of eggs laid	No. of larvae hatched	No. of larvae ejected	No. of adults emerged			
				queens	workers	males	total
25	NR	0					0
28	NR	0					0
43	NR	0					0
45	NR	0					0
6	NR	5				5	5
11	NR	4				4	4
30	NR	7	4			3	3
33	8	5				5	5
4	NR	3		2	1		3
5	NR	3	3				0
12	9	7	2	5			5
15	4	1		1			1
20	5	4			1	3	4
26	NR	4	1	3			3
27	8	4	1	3			3
31	NR	5		4	1		5
36	NR	8	3		2	3	5
40	NR	2		1	1		2
44	NR	8	4	4			4
46	6	3		2		1	3

NR = not recorded

TABLE VI

COLONY FOUNDING DATA ON EIGHT ARTIFICIALLY
OVERWINTERED B. terricola QUEENS IN SERIES III BOXES

Box no.	No. of days between re- suscitation and ovipos- ition	Composition of first brood				
		No. of eggs laid	No. of larvae hatched	No. of adults emerged		
				queens	workers	males total
1	7	6	6		6	6
2	8	9	9		5	5
3	9	6	6	1	5	6
4	10	8	8		8	8
5	10	8	7	1	6	7
7	9	9	9		9	9
8	9	6	4		4	4

first-brood eggs were counted. However, the mean number of first-brood larvae (7.0) hatched by the 7 queens in Series III boxes significantly exceeds ($p < 0.02$) the mean number (4.56) reared by the artificially overwintered queens which succeeded in hatching larvae in the Series II boxes, but is not significantly different from the mean number (7.1) hatched by 9 naturally overwintered queens in Series III boxes in June 1965, nor from the mean number (6.0) hatched by 10 naturally overwintered queens in Series III boxes in May-June 1966. Moreover, the mean time elapsing between installation and oviposition (8.9 days) in the artificially overwintered queens in Series III boxes was not significantly different from the mean times taken by naturally overwintered queens in Series III boxes (6.2 days in 1965 and 7.0 days in 1966); nor was the length (2.70 mm) of the radial cells of the first-brood workers of the artificially overwintered queens (see Table VII) significantly different from that of the workers of either of the two groups of naturally overwintered queens in Series III boxes (2.73 mm in both 1965 and 1966).

Only one queen reared males in her first brood. As soon as these had all emerged, she was dissected; her spermatheca contained no sperm.

The composition of the first broods of the remaining six queens was more typical (an average of 6.3 workers was

TABLE VII

LENGTHS OF RADIAL CELLS OF FORE-WINGS OF FIRST-BROOD FEMALES REARED
BY ARTIFICIALLY OVERWINTERED B. terricola QUEENS IN SERIES III BOXES

No. of colony	Radial cell lengths (1 unit = 0.051 mm)		
	Maternal queen	First-brood workers	First-brood queens
1	79	50 53 56 52 56 45	
3	77	53 54 51 59 54	69
4	76	53 44 52 51 57 54 52 52	
5	77	51 56 55 52 54 53	69
7	79	55 45 50 55 53 49 52 49 57	
8	77	53 56 55 54	

reared per colony) than those of the preceding series of B. terricola queens in Series II boxes. Nevertheless, 5 out of the 6 Series III colonies changed over to rearing queens and males in their second broods (see Table VIII), and the sixth changed over in the third brood, so no vigorous colonies were obtained.

III On several occasions, attempts were made to get colonies started by using overwintered queens of other species.

B. rufocinctus queens and males were reared from 4 colonies which had been brought in from the field during August 1965. Because most of the males were killed before they could be removed from their nest boxes (see Section 3.2), a further supply of males was collected in the neighbourhood of Winnipeg in early September. The bees were allowed to mate in a 2.4 m x 1.2 m x 1.2 m plastic cage illuminated by fluorescent lights, in which piles of earth and Sphagnum moss were placed (see HOLM 1960). Most of the queens were found torpid in the moss in late September; they were stored over damp vermiculite at 1°C (see Section 2.2) for four weeks, after which 28 of the survivors were placed in pairs in nest boxes made from paper cartons (Section 2.311) at room temperature (ca. 21°C). Since few of these bees showed any inclination to start colonies, the temperature was gradually raised to 29°C and the relative humidity maintained at about 50%; also, pollen was renewed more

TABLE VIII

COMPOSITION OF SECOND BROODS OF SIX ARTIFICIALLY
OVERWINTERED B. terricola QUEENS IN SERIES III BOXES

No. of colony	Total number of second- brood-larvae hatched	No. of larvae ejected	No. of adults reared			
			queens	workers	males	total
1	26	7	5	6	8	19
3	29	17	1	9	2	12
4	24			24		24
5	29			12	17	29
7	31	15	3	7	6	16
8	38	17	5	11	5	21

frequently (every third day) in the later stages than at the start of the experiment.

Consequently, the results shown in Table IX cannot be taken to indicate that a long period of time is necessary for B. rufocinctus queens to become physiologically ready to oviposit following insufficient duration of low-temperature treatment; for the considerable delays which elapsed between installation and oviposition in some of the queens may have been associated with unsuitable conditions for egg laying.

Though adults were reared in six boxes, only one medium sized colony was obtained (No. 4, which eventually produced 29 workers, 3 queens and 54 males; most of the males resulted from the eggs of laying workers after the death of the queen). Of the other five colonies, two yielded queens and males in the second brood, and in two the queens died soon after the first workers had emerged.

Two out of three B. rufocinctus queens, revived from hibernation in March 1965, laid eggs in Series II boxes at 29°C, but only one reared adults. A large colony was eventually obtained from this queen.

Two out of twelve B. nevadensis queens laid eggs in Series II boxes at 30°C in February 1966, but one was unfertilised. The other produced a small colony (5 workers, 4 queens and 3 males).

Two medium sized colonies of B. ternarius were

COLONY FOUNDING BY PAIRS OF B. rufocinctus QUEENS

AFTER FOUR WEEKS IN ARTIFICIAL HIBERNATION

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	10	+	non-brooding queen removed on day 34
2	11	+	non-brooding queen removed on day 43
3	46	-	first egg cells on floor - not reared; later brood deserted by queen
4	38	+	removed non-brooding queen after pupation of brood
5	71	-	queen died on day 81
6	22	-	repeated egg cells but none successfully reared
7	60	-	worker added on day 55
8	37	+	brood mostly neglected
9	18	+	first egg cells unsucces- ful
10	no eggs laid	-	one queen died on day 28; discontinued on day 55
11	" "	-	one queen died on day 39; discontinued on day 55
12	" "	-	one queen died on day 40; discontinued on day 55
13	6	-	brood neglected and larvae thrown out
14	5	+	
Boxes in which eggs were laid : number = 11			
% of total = 79			

obtained from a total of 15 artificially overwintered queens in Series II boxes at 30°C in February 1966. Eggs were laid by other queens of this species in the same trial but either they were not successfully reared or only males were produced.

Five groups of B. perplexus queens (consisting of 6, 5, 5, 4 and 4 bees respectively) were put in containers formed from two glass-topped wooden boxes (each box--int. dimensions 20 cm x 12.5 cm x 10 cm) with honey and pollen at 30 C in February 1966. Much fighting occurred in all groups and not more than 3 queens in any one group survived more than a week. Eggs were laid in each group, but in two groups only males were produced and the ovipositing queens were found to be unfertilised. In the other three groups, adult females were reared, but in only one were workers (3) produced, and even in this box the second brood was entirely male (possibly because the original laying queen was superseded).

Two B. borealis queens were put in Series III boxes during February 1966, after artificial hibernation. One queen laid eggs (which she failed to rear) after three weeks; the other showed no nest founding behaviour up to the fifth week, when the experiment was discontinued.

Discussion

HOLM (1960), HORBER (1961) and ZAPLETAL (1961) have

succeeded in rearing colonies from queens overwintered in captivity. Many of the colonies of B. terrestris and B. lapidarius started from such queens by HOLM & HAAS (1961) reached a large size. But disappointing results were obtained in the present series of experiments; except in the final trial with B. terricola queens in Series III boxes, the incidence of colony initiation was low and few of the colonies which were started reached any size. In most cases larval mortality was high; few, if any, workers were reared, and the changeover to production of sexual forms occurred abnormally early.

However, for the maintenance of a continuous laboratory culture of bumblebees, large colonies are not essential. Nests containing few workers (as in the B. terricola trials in Series III boxes) are easy to handle and will usually yield satisfactory numbers of males and queens if kept in complete captivity and well fed. Moreover, time is saved by premature changeover to queen production.

But for pollination, colonies should be as strong as possible. Few of the colonies which I reared from artificially overwintered queens could have contributed more than a very meagre foraging population. Too little information is available for many conclusions to be drawn as to why so few strong colonies were obtained, but the striking difference between first broods of B. terricola colonies in

Series II boxes and those in Series III boxes indicates that the treatment used to induce colony founding was of great importance. For naturally overwintered queens of B. terricola the Series III method seems much more effective than any of the others tried (see Section 2.313); quite possibly this difference in effectiveness is even more pronounced for queens which are physiologically less prepared to start colonies than those which have emerged from natural hibernation, but since the tests with artificially overwintered queens were not run at the same time as those with naturally overwintered queens, differences in handling technique may also have played some part.

Nevertheless, the six mated artificially overwintered B. terricola queens which started colonies in Series III boxes produced first broods comparable to those reared by naturally overwintered queens. Why, then, did these colonies change over to rearing queens and males so early? No full answer can be given at present, but ejection of larvae and low oviposition rate were probably partly responsible. Also, the incidence of premature changeover seemed to be greater in 1966 even among colonies started by naturally overwintered queens in Series III boxes than it was in 1965 (see Section 2.313), indicating that the handling technique may not have been uniform. Finally, the conditions for mating B. terricola in the laboratory may not have been

ideal (see Section 2.1); exhaustion of sperm could have resulted in an unusually early production of males.

The greater incidence of oviposition in the B. terricola queens in Series II boxes started in February 1966 than in those started in October 1965 suggests that the duration of low temperature storage may be correlated with the ability of queens to start colonies; alternatively, the improved results in February 1966 could be associated with the selection effect brought about by the death of more of the less vigorous bees by the later date than had occurred by the time the bees for the earlier trial were removed from hibernation.

To summarise: a number of factors may have resulted in the generally unsuccessful attempts to obtain vigorous colonies from queens overwintered in captivity. Further experiments, controlled wherever possible by concurrent trials on naturally overwintered queens, are needed to disentangle the rather complex problems in this aspect of bumblebee domestication. Nevertheless, the results from a very small number of B. terricola queens tested in February 1966 gives hope that for this species at least, successful colony founding by laboratory overwintered bees may soon become a feasible method for rearing vigorous out-of-season colonies.

2.313 Colony founding by naturally overwintered queens

Queens of B. terricola, B. nevadensis, B. fervidus, B. borealis, B. vagans, B. rufocinctus, B. perplexus, and B. ternarius were caught in the field within a few weeks of spring emergence in May and June of 1965 and 1966, and were transported in gauze-covered jars or in polythene tubes kept on ice in a styrofoam picnic cooler. On arrival in the laboratory the queens were either (a) placed immediately in nest boxes, or (b) stored over damp vermiculite at 1°C until needed, or (c) confined at room temperature with access to food in a 1.2 m x 0.6 m x 0.6 m plastic cage for not more than 24 hours before installation in nest boxes.

Two designs of nest box were used, one without nest material (Series II) and the other with nest material (Series III).

Series II

Queens were installed singly or in pairs in a wooden container consisting of two 7.5 cm x 7.5 cm x 5 cm boxes (internal dimensions) with glass roofs and corrugated paper floors (Fig. 1) at 29°C and 50% R.H. All boxes were kept in total darkness with minimal disturbance.

Honey solution (equal volumes of honey and water) was provided in glass gravity-feeding tubes inserted through the wall of the outer box. By sterilising the filled tubes in a pressure cooker it was found unnecessary to replace them more

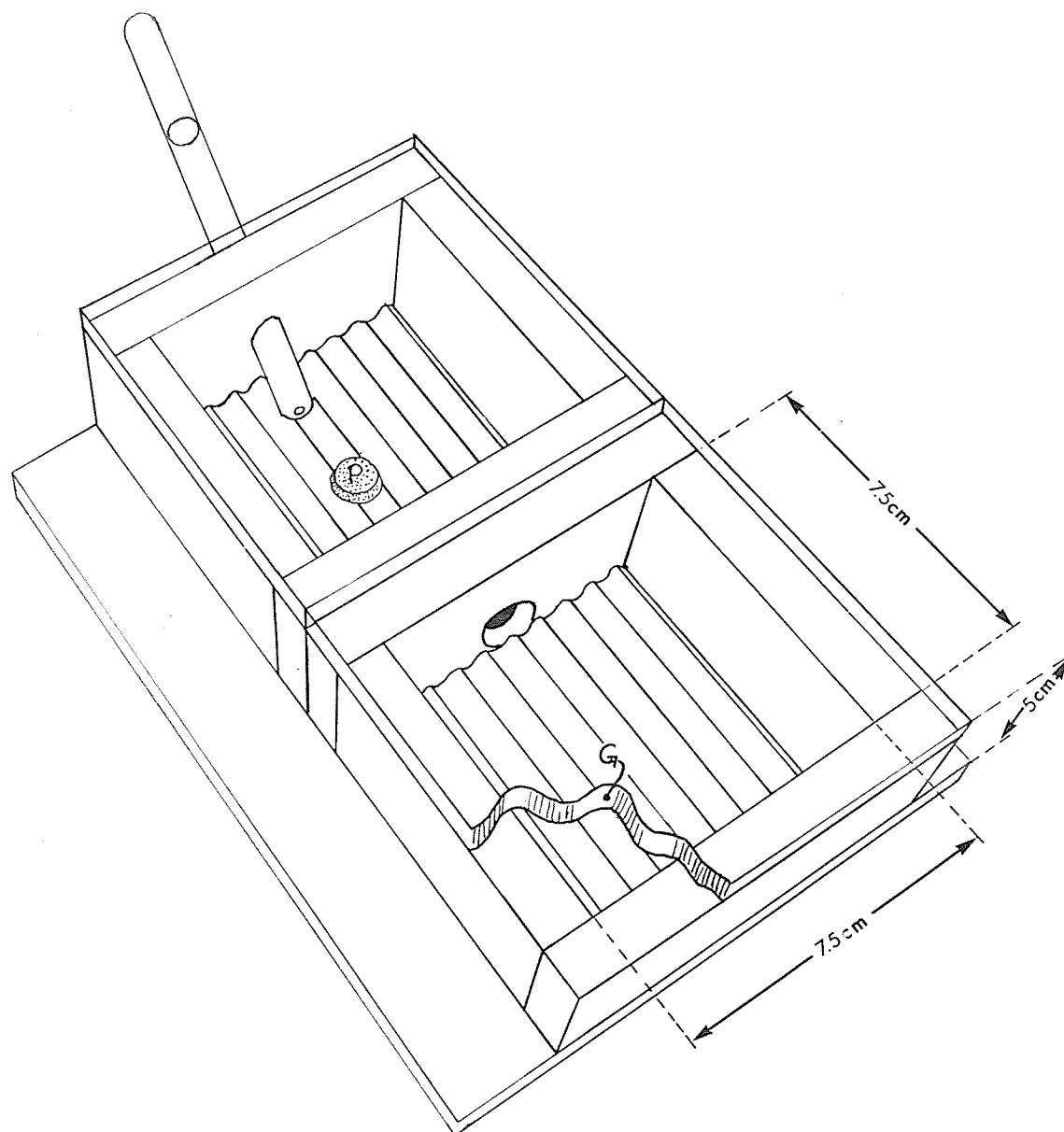


Fig. 1.- Nest box used to induce colony founding in Series II queens.

G = glass

P = pollen lump

often than once every third day.

In early trials, the pollen lump (consisting of pollen moistened with honey solution) was placed in the inner box, but several queens built their eggs cells directly on the corrugated cardboard floor of the outer box. In later experiments the pollen was located in the outer box, adjacent to the feeding tube, so that the inner chamber was used only for defaecation.

The pollen lumps were renewed every three days, or more often where queens persistently fragmented their lumps.

In 8 trials with single or paired queens of B. terricola and 11 trials with B. ternarius, the inner box contained cellulose wadding in which a central cavity contained the pollen lump.

From about three days after a queen had laid eggs, small pieces of pollen dough were introduced into the nest box on alternate days; these were put as far away from the brood as possible to avoid egg cells being built on them.

During the development of the first brood, some queens neglected larvae and/or tore down the wax envelope surrounding them. Although many of these rejected larvae were underweight for their instar, they appeared healthy; on seven occasions I either replaced such larvae in the original envelope or constructed a new one made of beeswax and pollen. They were always accepted by their mother, but were often neglected again later. I also transferred egg cells

to pollen lumps in instances where they had been constructed elsewhere, but they were always destroyed or ignored after introduction.

Following FRISON (1927a), an attempt was made to induce broodiness in ten queens of B. ternarius by confining them together in a cage with plenty of pollen and honey. After one week the eight survivors were installed singly in nest boxes at the same time as a control series of eight queens brought in from the field immediately after capture.

SERIES III

Two wooden boxes (at ca. 21° C) were used for each bee, the larger (17.5 cm x 12.5 cm x 10 cm internal dimensions) holding the feeding tubes and lined with corrugated cardboard and a glass roof. The smaller chamber (7.5 cm x 7.5 cm x 5 cm internal dimensions) was lined with upholsterer's cotton (Fig. 2). The sides and roof of the cavity thus formed were smeared with honey solution in order to simulate the natural situation in which the nest material appears to act as a temporary food store. A pollen lump (about 0.75 g) was placed in the centre of the cavity. Each queen was left undisturbed for the first three days after installation, and the progress of her nesting activity was assessed by the appearance of the faeces which she deposited in the antechamber, oviposition being always preceded by the production of large amounts of pollen-containing faeces. If

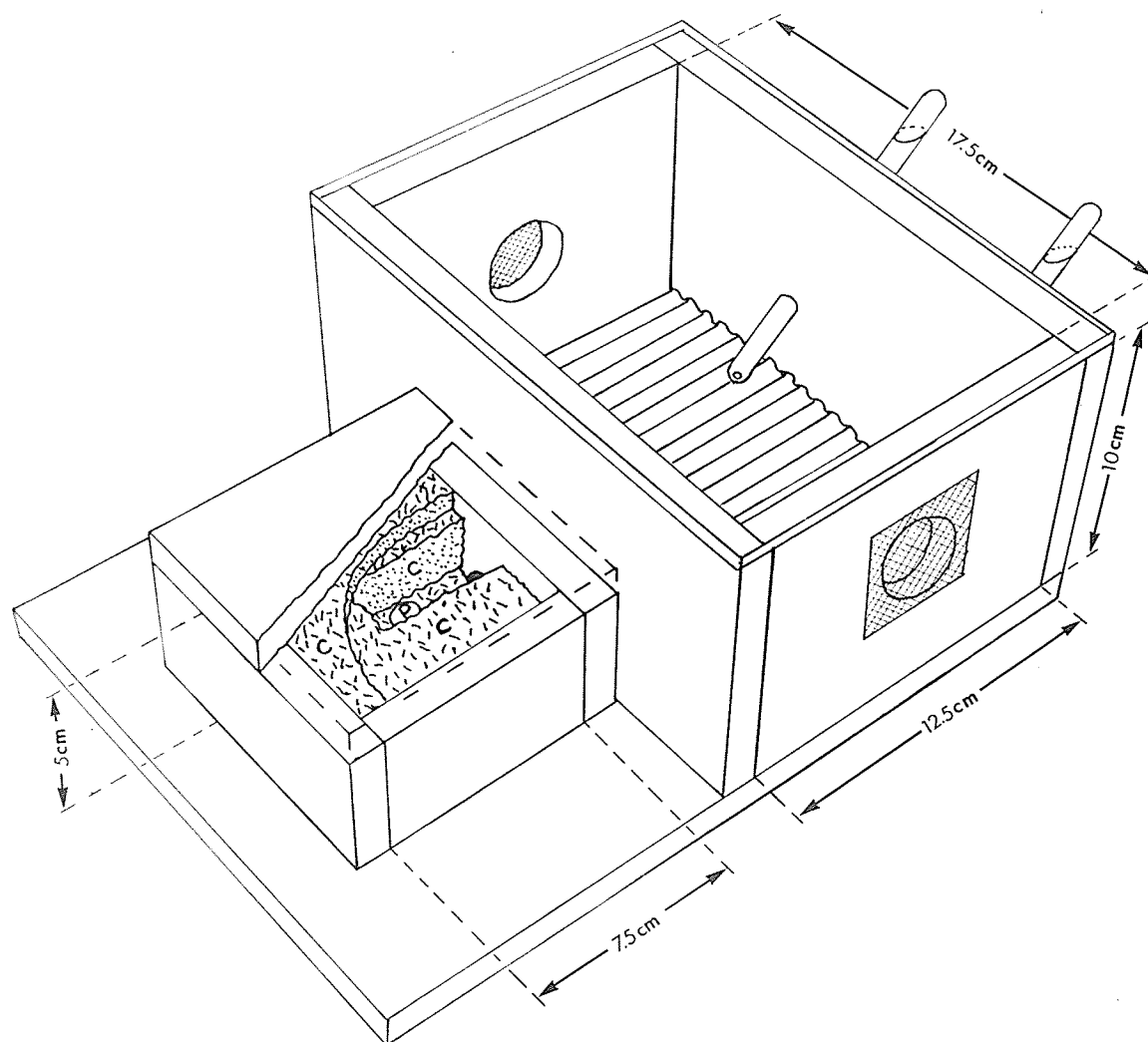


Fig. 2.- Nest box used to induce colony founding in Series III queens.

C = upholsterer's cotton

P = pollen lump

no eggs had been laid by the third day, a new pollen lump was supplied, and the nest material was changed. This process was repeated every third day until the queen had laid her first eggs. After this, pollen was supplied on alternate days in increasing quantities until the first workers emerged, when it was necessary to supply pollen every day. When giving pollen to brooding queens, care was taken not to obstruct their "brooding grooves". In the earlier trials of this series, pollen replenishment was undertaken only when the queen had left her nest to feed or defaecate, but it was found that this precaution was unnecessary. None of the 67 queens which laid eggs deserted her brood, although 6 died before the emergence of their first pupae; in three cases these were reared by a foster-queen.

In 1965, after the first or second group of workers had emerged, some boxes containing B. terricola and B. perplexus colonies were put out-of-doors with the nest box placed inside the antechamber and the excess space filled with nest material. A wooden lid covered with polythene sheet was stapled on to provide waterproofing. A count was made of the number of cocoons produced by these colonies (Table X).

The remaining colonies were kept within the laboratory to provide material for experiment. As these colonies became larger, the nest material was removed and the

TABLE X

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COCOONS PRODUCED BY COLONIES STARTED BY NATURALLY OVERWINTERED
QUEENS IN Series III BOXES AND SET OUT IN THE FIELD IN JUNE 1965

1. B. terricola

Box no.	No. of cocoons					
	empty		unemerged			total
	queens	worker/males	queens	workers	males	
1	16	118	2	2	1	139
3	9	54			2	65
4	2	412				414
5	155	205	2			362
6	14	519			64	599
7	11	71				82
8		210				210
10		7				7 ¹
12	14	167				181
13	13	314				327
14	74	199	24		9	306
19	29	113	7 ²		3 ²	152
20	50	135	7		11	203
21	2	110			8	120

¹ Colony died soon after being set out

² Killed by Brachycoma

2. B. perplexus

Box no.	No. of cocoons					total
	empty		unemerged			
	queens	worker/males	queens	workers	males	
1	3	37				40
5	comb destroyed by mice during winter storage					
6	3	273				276
7	comb destroyed by wax moth					
10	comb destroyed by mice during winter storage					

colonies were kept in darkness at 30°C, thus facilitating inspection and the addition of pollen. Where necessary, the Series III colonies were given larger nest boxes (17.5 cm x 12.5 cm x 10 cm) so that the comb could expand evenly.

One colony of B. perplexus and of B. terricola were connected to separate 1.2 m x 0.6 m x 0.6 m plastic cages in which petri dishes containing honey solution and wood-wool were placed. Apart from the slight saving in time gained when feeding honey solution, no advantage could be found in this method of housing the colonies, since by keeping them in darkness much smaller containers can be used.

Results and discussion

The results are summarised in Table XI, which does not include the two B. terricola queens which were parasitised by nematodes. Tables XXVI and XXVII in the Appendix give more detailed information for each nest box. Table XII gives the number of first-brood workers and their radial cell measurements for 11 Series III colonies of B. terricola and 11 of B. perplexus.

The data for B. ternarius in Series IIA₁, B, C, D, were examined by analysis of variance, each queen (or pair of queens) being scored 1 or 0 according to whether or not eggs were laid within 21 days after installation.

SUMMARY OF RESULTS OBTAINED IN COLONY-FOUNDING TRIALS

WITH NATURALLY OVERWINTERED QUEENS (Series II & III) ¹

Species	Series	Year	Group	No. of queens per box	Total no. of boxes	No. of boxes in which eggs were laid	No. of boxes in which adults emerged
<u>B. terricola</u>	II	1965	A	1	9	7	7
			B	1	4	1	1
			C	2	6	5	3
			D	2	4	2	2
	III	1965		1	30	28	28
	III	1966		1	11	11	10
<u>nevadensis</u>	II	1965		1	1	1	1
<u>borealis</u>	III	1965		1	2	2	2
	III	1966		1	3	2	1
<u>rufocinctus</u>	III	1965		1	9	5	4
<u>perplexus</u>	II	1965		1	1	1	1
	III	1965		1	10	10	10
	III	1966		1	7	7	7
<u>ternarius</u>	II	1965	A ₁	1	8	5	5
			A ₂	1	8	6	5
			B	1	5	1	1
			C	2	8	7	5
			D	2	6	5	4
	III	1965		1	3	0	0
	III	1966		1	3	3	2
<u>vagens</u>	II	1965		1	3	3	2
	III	1965		1	2	0	0

¹ For a detailed presentation of the results summarised in this table, see Appendix Tables XXVI and XXVII.

LENGTHS OF RADIAL CELLS OF FOREWINGS OF FIRST-BROOD BEES
 REARED BY NATURALLY OVERWINTERED QUEENS IN SERIES III BOXES

1. B. terricola 1965

Colony no.	No. of bees in first brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
5	6	50 51 51 54 56 53	52.5
6	9	55 57 48 56 55 53 57 53 47	53.4
10	7	53 47 49 43 56 54 54	50.9

1966

2	5	56 50 53 49 56	52.8
3	5	54 50 55 47 54	52.0
5	8	51 55 59 49 54 50 57 49	51.3
6	9	42 57 57 48 49 52 55 55 54	51.2
7	4	59 56 61 56	58.0
8	10	49 47 50 47 49 53 52 45 46 45	48.3
9	2	61 66	63.5
10	7	57 56 53 56 56 56 56	55.7

TABLE XII contd.

2. B. perplexus 1965

Colony no.	No. of bees in first brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
5	9	56 55 51 52 54 48 47 50 49	51.1
6	4	59 63 61 61	61.0
7	8	55 49 52 52 48 52 48 49	51.1
10	7	47 52 48 48 46 46 47	47.8

1966

1	8	50 49 53 52 48 50 49 46	49.6
2	6	54 53 52 50 52 52	52.2
3	7	53 44 44 42 47 50 60	49.6
4	7	45 51 52 49 55 47 51	50.0
5	8	50 55 48 50 49 50 51 54	50.9
6	6	53 51 52 54 54 48	52.0
7	5	50 49 52 52 50	50.6

<u>Source of variance</u>	<u>D.F.</u>	<u>Estimated Variance</u>
No. of queens per box	1	1.0549 *
Presence or absence of nest material	1	0.2727
Residual	24	0.1947

* = $p < 0.05$

Thus, paired queens gave significantly better results than solitary queens. Nest material had no significant effect; since four of the five pairs which oviposited in boxes containing nest material (Series IID) laid their eggs on the floor of the outer compartment rather than within the nest material, the provision of nest material at 29°C seems to have served no useful purpose.

The greater success obtained by confining B. ternarius queens in pairs does not necessarily imply that oviposition was stimulated by social interaction; the improved results may have arisen from the fact that in boxes containing pairs of queens, only the bees which oviposited first contributed data for statistical analysis. Thus when the 13 single bees in Series IIA₁, B, are arranged in sixty sets of pairs obtained by drawing random combinations of the numbers 1-13, and the mean incidence of success for each set of six pairs calculated by scoring 1 for any pair including a queen which laid eggs during the 21 day period, and 0 for any pair in which neither queen oviposited, the mean of all 60 sets

(0.759) is not significantly different from the mean (0.857) of the 14 pairs in Series IIC, D, but significantly exceeds ($p < 0.05$) the mean (0.462) for the 13 single bees (Series IIA, B) from which the 60 pair combinations were taken.

The mean times which elapsed between installation and oviposition of the B. ternarius queens that laid eggs in Series IIA₁ and IIA₂ were 7.0 and 32.5 days respectively ($p < 0.01$), indicating that confining queens together in a cage before installation in nest boxes inhibited colony founding.

Of the queens in Series II which did not lay eggs within 21 days, 3 B. ternarius and 6 B. terricola were dissected; all except for 2 B. terricola parasitised by nematodes had well developed ovaries and in some it appeared that egg resorption had occurred (see MEDLER 1962a). Four randomly selected unsuccessful B. terricola queens from the same series were transferred after 21 days to Series III boxes, where they laid eggs within 5 days; whereas four controls which were left in their Series II boxes failed to start colonies during the 5 day period and eventually died without ovipositing.

In each of 6 pairs of B. terricola and 12 pairs of B. ternarius which oviposited in Series IIC, D, one queen died within the 21 day test period. The mean time of death was 0.64 days after the first eggs were laid (S.E. = 0.88

days with 17 d.f.).

Several queens of B. terricola in both series reared queens in their first brood in 1965; but in every such box, at least two workers were produced as well, and, in contrast to the trials with artificially overwintered queens (see Section 2.312), in all but two colonies the second brood consisted entirely of workers. In 1966, a record was kept of the composition of first and second broods in B. terricola and B. perplexus colonies started in Series III boxes (Table XIX). For some unexplained reason, the incidence of queens in the second broods seemed to be much higher in this year than in 1965.

Comments on experimental methods

Though the use of waxed paper cartons at 21°C was unfavourable for brood rearing, many queens of B. terricola, B. ternarius and B. rufocinctus in Series I laid eggs, suggesting that obtaining colonies from non-overwintered queens of these species will be feasible using improved techniques.

The wooden containers used in Series II were convenient for handling and the corrugated paper floor covering absorbed faeces well and was easy to renew. Since most of the queens used only the rear chamber for defaecation, in all but the earliest trials the pollen lump was placed near the feeding tube in the outer chamber.

However, some modification of the Series II design is

required to prevent queens building their first egg cells upon the floor. Those that did so usually built an excessive number of cells so that the larvae hatching from the earliest cells were neglected, or the number of larvae exceeded that which the queen could rear unaided. This led to the ejection of some or all of the larvae unless the excess were removed by hand, or one or two workers added from another colony.

On the other hand, a queen which had constructed her first egg cells upon the pollen lump rarely built more cells upon the floor, though the addition of further pollen often stimulated renewed oviposition unless the new pollen was placed at a distance from the original lump; so it seems that the covering of the pollen lump with egg cells, at least in B. terricola and B. ternarius, inhibits further egg laying. Thus some type of nest material may be necessary, even at high temperature, if only to ensure that queens build their first egg cells upon the pollen lumps rather than on the floors of their boxes; but cellulose wadding seems unsuitable for this purpose.

Attempts to save moribund first broods in Series II by adding workers, repairing torn larval clumps, transferring larvae and adding wax were mostly very successful, but such delicate manipulations would not be possible when dealing with large numbers of colonies since too much time

is required to carry them out successfully.

The design of the Series III boxes is quite similar to that of HASSELROT (1952). However, Hasselrot (personal communication) states that with his method great care is required when adding fresh pollen or inspecting the progress of the brood, otherwise the queen may desert her progeny. Though queens in Series III were frequently inspected while brooding eggs or young larvae, none deserted her brood; this may have been associated with the much smaller size of the Series III nest chambers than those which Hasselrot used, though until more work has been done it will not be possible to rule out interspecific differences. Smearing the nest material with honey appears to have a beneficial effect in inhibiting the tunnelling activities of the queen and in slowing down the drying out of the pollen dough.

Other considerations

Failure of queen bumblebees to start vigorous colonies in captivity may result from:

- a) their failure to oviposit within the duration of the experiment
- b) long delay between confinement and oviposition
- c) failure to rear the first brood
- d) abnormally early production of sexual forms.

Unless queens which have failed to oviposit are dissected, it is not possible to ascertain whether their

failure results from non-development of their ovaries or from lack of suitable stimuli for egg laying. Since dissection of the unparasitised queens which had failed to oviposit in Series II showed that their ovaries were well developed, and since the unsuccessful Series II queens quickly started colonies when transferred to Series III boxes, it seems that unsuitability of nest box design was at fault here. However, many non-overwintered queens of these two species which had failed to oviposit in Series II boxes were found to have undeveloped ovaries (see Section 2.311) even after long periods of confinement, and MEDLER (1962a) recorded that 15 out of 32 queens of various species confined in laboratory boxes for more than 40 days contained only small (0-1.5 mm) eggs, i.e. their ovaries were comparatively undeveloped. It thus appears that inadequate ovary development may be the major obstacle to colony founding in captivity in some cases, though there was no evidence for this in the overwintered queens tested in Series II and III.

SLADEN (1912) and FREE & BUTLER (1959) reported that oviposition occurred more often when several queens or one queen and one (or more) workers were confined together than when queens were in solitary confinement. However, FRISON (1927a) obtained a greater (though non-significant) incidence of colony founding in boxes housing single queens than in boxes containing pairs of queens. My results from

B. ternarius support the observations of Sladen and Free, but there is no evidence that the greater incidence of oviposition in the Series II boxes containing pairs of queens results from anything other than the biasing of data caused by including only the result from the first queen to oviposit within each pair. Further experiments are necessary to determine whether oviposition is stimulated or inhibited when B. ternarius queens are confined in pairs, but the experiment in which 10 B. ternarius queens were confined together in a cage for a week before being placed in nest boxes shows that oviposition was thereby delayed, perhaps because one queen had established "dominance" (see FREE 1955c) over her seven companions, resulting in the inhibition of development of their ovaries.

SLADEN (1912) found that when pairs of queens were confined together, one killed the other about the time that the first eggs were laid. Sladen's results are confirmed for B. terricola and B. ternarius, in which the mean time of death for the queens of the pairs in Series II did not differ significantly from the time at which the first eggs were laid. However, contrary to Sladen's findings, 14 out of the surviving 19 members of these pairs successfully reared adults. Other authors (e.g. FRISON 1927a, PLATH 1923, 1934, FREE & BUTLER 1959) have also found that the death of one queen does not always occur when two are confined together

(some pairs of artificially hibernated B. rufocinctus queens co-existed until after the first workers emerged--see Section 2.311).

FREE & BUTLER (1959) suggested that HASSELROT's (1952) method for getting colonies started in captivity might in part be due to the degree of insulation which was provided, since it had been shown that ovary development in worker bumblebees was accelerated by high temperatures. Although no attempt seems to have been made to compare the incidence of colony founding at high and low temperatures, HORBER (1961) reported that though most of the 24 queens of B. hypnorum, from which he attempted to get colonies, started building nests when they were exposed in an unheated greenhouse, real progress was only observed after the nests had been moved to a rearing cabinet where the temperature could be kept over 25°C and later between 33-35°C. However, Horber's results may not have had anything to do with ovary development.

The laying of eggs by queens in Series II boxes was always preceded by a period of "broodiness" during which they flattened themselves over their pollen lumps or on the floors of their containers while undergoing rhythmic abdominal movements. Similar behaviour was observed in all of the Series III queens which were inspected shortly before oviposition. Presumably, this broodiness was associated

with heat production, though whether it had any connection with ovary development is unknown.

In the colony founding experiments with non-overwintered queens (see Section 2.311), failure of queens to rear their first broods was thought to be partly due to too low an environmental temperature. Though larval ejection took its toll in many nest boxes, survival of first broods in Series II was much better than in experiments conducted at room temperature, so it seems that whether or not it encourages oviposition, a high rearing temperature should be used for starting bumblebee colonies without nest material, because it allows survival of eggs and larvae even when queens fail to incubate them.

SLADEN (1912) noted that the colonies which he reared from queens in captivity did not reach large size; he attributed this to the weakening effect of long confinement on the queens. HOLM & HAAS (1961) showed that among the colonies of B. terrestris L. which they reared in captivity and set out in the field, those which had been started later in the year produced fewer cocoons than those started earlier. One of the reasons for this was probably that the later colonies would have had less of the season remaining in which to complete their development, but possibly declining vigour in the queens could have played a part. In the present series of colony-founding experiments (see also

Section 2.312), premature changeover to rearing sexual forms (and consequently smaller colonies) was a constant problem with some species. The factors associated with premature changeover are discussed below (Section 3.51). So far as first broods are concerned, it seems desirable to get as many adults reared as possible, since the size of first-brood females is negatively correlated with their number (Section 3.511); i.e. queens are less likely to appear in numerically large first broods than in small ones. Though detailed records of the composition of all first broods in Series II and Series III boxes were not kept in 1965, it was noted that in B. terricola, the first broods in Series III boxes were larger and had a lower incidence of queens than those in Series II boxes. Similar results were obtained in trials with artificially overwintered queens (Section 2.312), where it was shown that egg and larval mortality were the chief factors associated with this difference between the two colony founding methods.

Thus, since B. terricola queens seem also more ready to oviposit in Series III boxes than in Series II boxes, the use of the former seems to be preferable until some method for reducing egg and larval mortality at high temperature without nest material can be devised. However, B. ternarius appears to be very reluctant to start colonies in Series III boxes (though fortunately the problem of premature changeover

does not seem to be so acute with this species), so there may be a case for building improvements into the Series II method in order that better first-brood survival can be obtained. Furthermore, more information is needed on the development of natural colonies, for though CUMBER (1953) presented data on the fate of 80 wild colonies of B. agrorum F. in England, very little similar information is available for wild colonies of B. terricola or B. ternarius in Manitoba, against which the developmental histories of colonies started in captivity might be compared (but see Section 3.2). In any case, some degree of variability in the pattern of colony development is to be expected under natural conditions in order to ensure survival of the species despite climatic vagaries. Possibly the number of small colonies obtained in domestication experiments is just an expression of such variability. Also, an unknown, but probably large, proportion of naturally overwintered queens fail to rear colonies in the wild state; presumably many of these bees are lacking in vigour and would not have produced large colonies even if they had been more successful.

In view of the outstanding success in getting colonies of B. terricola and B. perplexus started in Series III boxes, it seems fair to reject HOLM's (1966a) suggestion that improvements in technique are unlikely to bring about much improvement in getting colonies started by bumblebee

queens in captivity. The results in the Series III boxes are very much better than those obtained by any other workers, though it must be noted that possibly B. terricola and B. perplexus are unusually favourable species for colony founding experiments.

2.32 Colony founding by queens in unenclosed situations

In late August, 1964, approximately 100 queens of B. terricola, 50 of B. ternarius, 25 of B. fervidus and 20 of B. perplexus were confined with males in an outdoor greenhouse, with liberal supplies of honey solution and pollen lumps placed in the angles between walls and roof. After a week many queens of B. terricola, B. ternarius and two queens of B. perplexus had become "broody" on or near the pollen lumps. Numerous wax honeypots were constructed a few days later, and three egg cells were built upon three of the pollen lumps by B. terricola queens. Also, a group of queens of all three species had assembled in a fold of the polythene floor covering beneath a wooden board; although there was no pollen lump in this situation, wax honeypots were built and eggs were laid by a B. perplexus queen. However, none of the eggs survived more than a few days; since the cells were torn down, it is assumed that the eggs had been eaten.

A further attempt was made to obtain colonies from bees in mating cages by confining 20 queens and 20 males of

B. rufocinctus in a 2.4 m x 1.2 m x 1.2 m plastic cage in the laboratory. After several weeks some of the queens were broody, wax honeypots were built and an egg cell was constructed on the floor of the cage. However, this cell was destroyed by the bees a few days later.

During late May, 1965, ten naturally overwintered queens were caught in the wild and put in each of two 60 cm x 30 cm x 30 cm plastic cages with honey and pollen. One of these two groups was used for colony founding experiments (see Section 2.313), but the other was left for two weeks, at the end of which time only 2 queens were still living. One of these bees had constructed an egg cell upon the paper-covered floor of the cage. A small lump of pollen was placed adjacent to this egg cell and a hollowed out block of polystyrene foam was set over pollen and eggs. The other surviving queen was removed and disturbance was kept to a minimum.

After the first brood of workers (three bees) had emerged, the brood was covered with upholsterer's cotton, following removal of the foam block. Pollen lumps were put on the cage floor a few inches from the nest and honey was supplied in petri-dishes containing wood-wool. The colony, which was allowed to proceed to completion, eventually produced 37 workers, 10 queens and 78 males. The mother queen died comparatively early in the development of her colony,

so that most of the males resulted from eggs laid by workers.

In one Series III box in 1966 (see Table XXVII), a naturally overwintered B. terricola queen built egg cells on the lower surface of the hole connecting the antechamber with the nest chamber, but did not succeed in rearing her first brood. HASSELROT (1960) also reported that some queens built egg cells in the feeding compartments of his nest boxes in the absence of nest material.

I do not suggest that oviposition by bumblebee queens in open situations could be made the basis for an efficient colony founding technique. Nevertheless, the phenomenon is interesting in that it shows, as noted by HOLM (1966a), that whatever may be the factors operative in eliciting egg laying in captive bumblebee queens, the presence of an enclosed cavity within a mass of nest material is not always essential.

CHAPTER III

STUDIES ON CASTE DIFFERENTIATION IN BUMBLEBEES

3.1 Explanation of terms

A number of terms used frequently in the following sections of this thesis require definition and explanation if ambiguity is to be avoided.

The terms "s-queen", "s-worker", "f-queen" and "f-worker" have already been defined (see Section 1.3). Where the simple terms "queen" and "worker" are used, they are to be taken to denote individuals whose caste status is no longer in doubt - e.g., foundress queens and bees which have assumed foraging or nursing duties.

The term "first brood" or "first brood batch" denotes the insects resulting from the eggs which are laid by a foundress queen as she starts her colony. When she has laid her complement of first brood eggs, a bumblebee queen normally lays no more until the first brood larvae have constructed their cocoons.

The term "second brood" includes all individuals resulting from eggs laid in cells upon first brood cocoons. The second brood eggs usually occupy a number of egg cells, often built in groups ("egg clumps" = "egg batches") at separate locations on the first brood cocoon clump. In this denotation I depart from normal usage in order to obtain objectively a sequential series of stages from the more or less continuous

process of colony development. Thus, the term "third broods" refers to all those bees which result from eggs laid in cells constructed on second brood cocoon clumps; and so on.

Fig. 3, which describes the development of an actual B. ternarius colony (No. 49), will help to make the above explanation clearer. Each egg clump (consisting of several egg cells) is represented by a letter of the alphabet which indicates the temporal relationship of its construction to that of other egg clumps of the same brood. The location of each egg clump is indicated by a line joining it to a clump in the previous brood. Thus the cocoons resulting from the second brood egg clump C had built upon them three third brood egg clumps - M, N and O. The numbered caste symbols following each clump represent the number and caste of the adult bees which emerged from it.

The term "mixed clump" is used to describe brood clumps which yield adults of both queens and workers. The presence of such clumps is characteristic of colonies which are changing over from worker to queen production.

3.2 The development of captive colonies of seven North American bumblebee species

Since the distinction between castes and their mode of origin is somewhat variable among different bumblebee subgenera, and even between species in a single subgenus, it is necessary to give an account of those features of

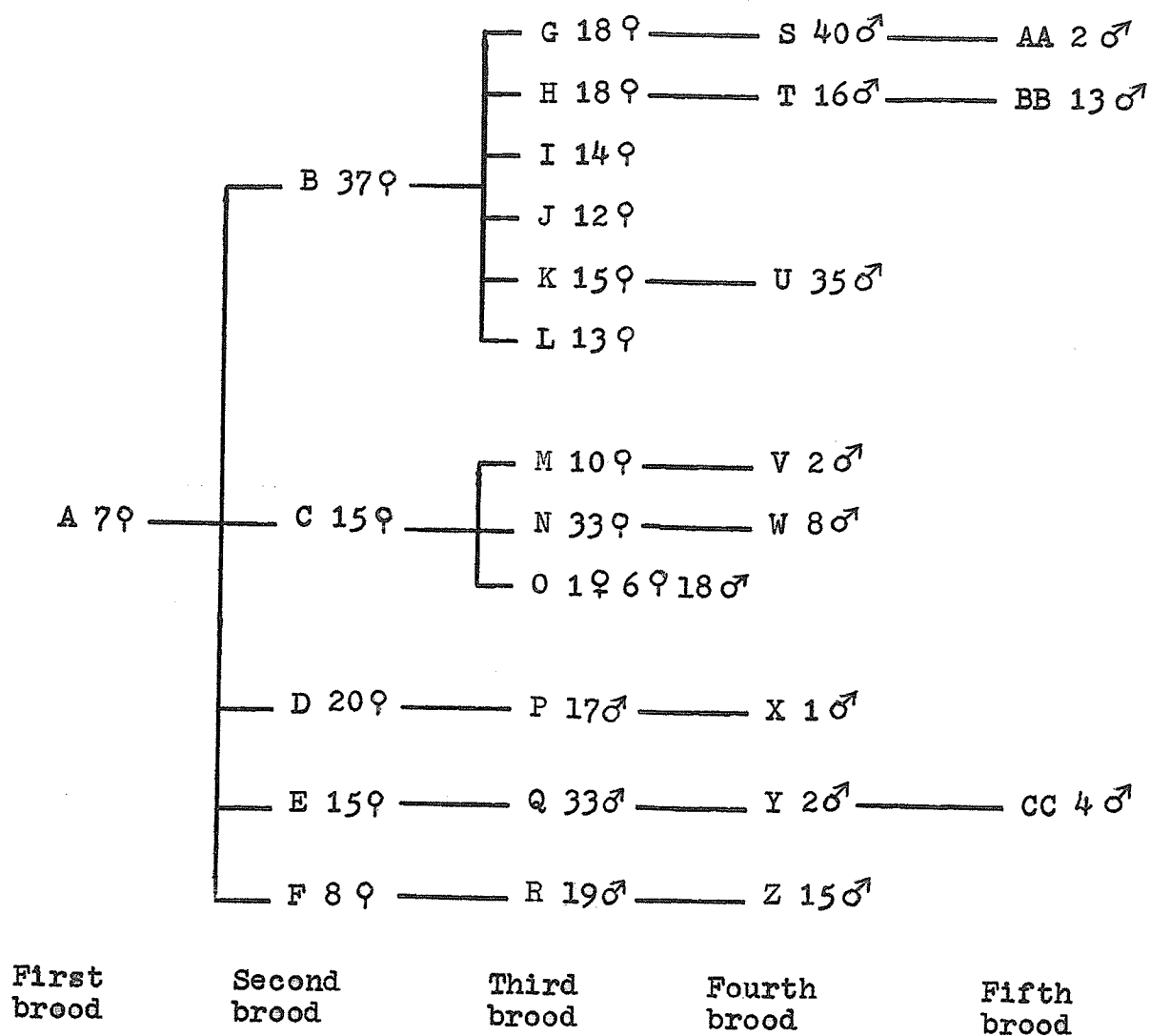


Fig. 3.- Analysis of brood stages in a captive colony of *B. ternarius*.

colony development in each of the species studied which seem to be relevant to the process of caste determination.

In none of the colonies on which these observations were based were the bees allowed to forage normally. Pollen was presented in honey-moistened lumps on or adjacent to the comb, and honey was supplied in gravity feeding tubes or in dishes containing wood-wool. Except in the early stages of their development (see Section 2.313) most of the colonies were housed in pairs of glass-covered wooden boxes (20cm x 12.5cm x 10cm internal dimensions), one of which contained the comb and held the feeding tubes, while the other served as an area for defaecation. Where nest material had been used in colony initiation, it was removed shortly after the first brood of workers had emerged; thereafter, each colony was kept in darkness at an ambient temperature of 29°C (1965) or 30°C (1966).

No doubt the abnormal treatment which the colonies received had some influence upon the course of their development. The "pocket-making" species, which feed their larvae by dropping their corbicular pollen-loads directly into wax pockets constructed on the side of the larval clumps, were unable to do so under the experimental conditions. Furthermore, since the bees were not allowed to fly, the normal division of labour among the workers must have been considerably modified, probably resulting in a higher

proportion of nurse bees than would occur in a wild colony. Lastly, because a perpetual surplus of pollen and honey was available to the captive colonies, the effects of fluctuating colony food-intake on colony development were presumably eliminated, or at least very much reduced.

Nevertheless, except for a few aberrant features which will be described below, the pattern of colony development in most of the colonies appeared to resemble that undergone by bumblebee colonies in the wild state. Almost all the captive colonies produced both queens and males, and though most colonies were killed shortly after the mother queen had died, many reached large size, in spite of the overcrowding which probably truncated the final phase of colony growth.

Fortunately, the colony development of three of the species which I studied (B. nevadensis, B. rufocinctus and B. borealis) has been investigated under almost natural conditions by HOBBS (1965 a & b, 1966 c), so some interesting comparisons may be made.

1) B. terricola Kirby

This species, which is extremely abundant and widely distributed over the province of Manitoba (NEAVE 1933), closely resembles the European B. terrestris L., to which it is closely related. B. terricola is a "pollen storer", i.e., the larvae in all broods are fed by regurgitation,

and pollen is stored in wax cylinders (see Fig. 4).

26 colonies of B. terricola in 1965 and 12 in 1966 were started, using naturally overwintered queens, and allowed to develop in captivity.

HOBBS (1964a) stated that queens of the subgenus Bombus Latr. laid only one egg per cell in their first broods. Most of the first brood egg-cells of B. terricola that were opened contained single eggs, but a few contained more than one egg, both in Series II and Series III boxes. Doubtless the provision of a lump of pollen dough for the use of captive queens alters their normal pattern of first brood egg-cell construction.

A peculiarity in the construction of later egg cells, (especially those built on cocoons of the second and third batches) noted only in this species, was that the queen did not always build her cells directly on cocoons. In many cases one egg cell was located on a cocoon while others were built successively, one on another, using the first cell as a starting point. Thus a chain of six or more egg cells was often produced, spanning from one cocoon clump to another (Fig. 5); in this way, a certain degree of independence between oviposition rate and the availability of cocoons to serve as bases for egg-cell construction may be achieved in B. terricola. In all observed instances, egg cells in which the queen oviposited were built by herself alone.



Fig. 4.- Comb of a naturally started B. terricola colony removed from a wooden bumblebee domicile in Winnipeg, showing centrally placed pollen cylinders.

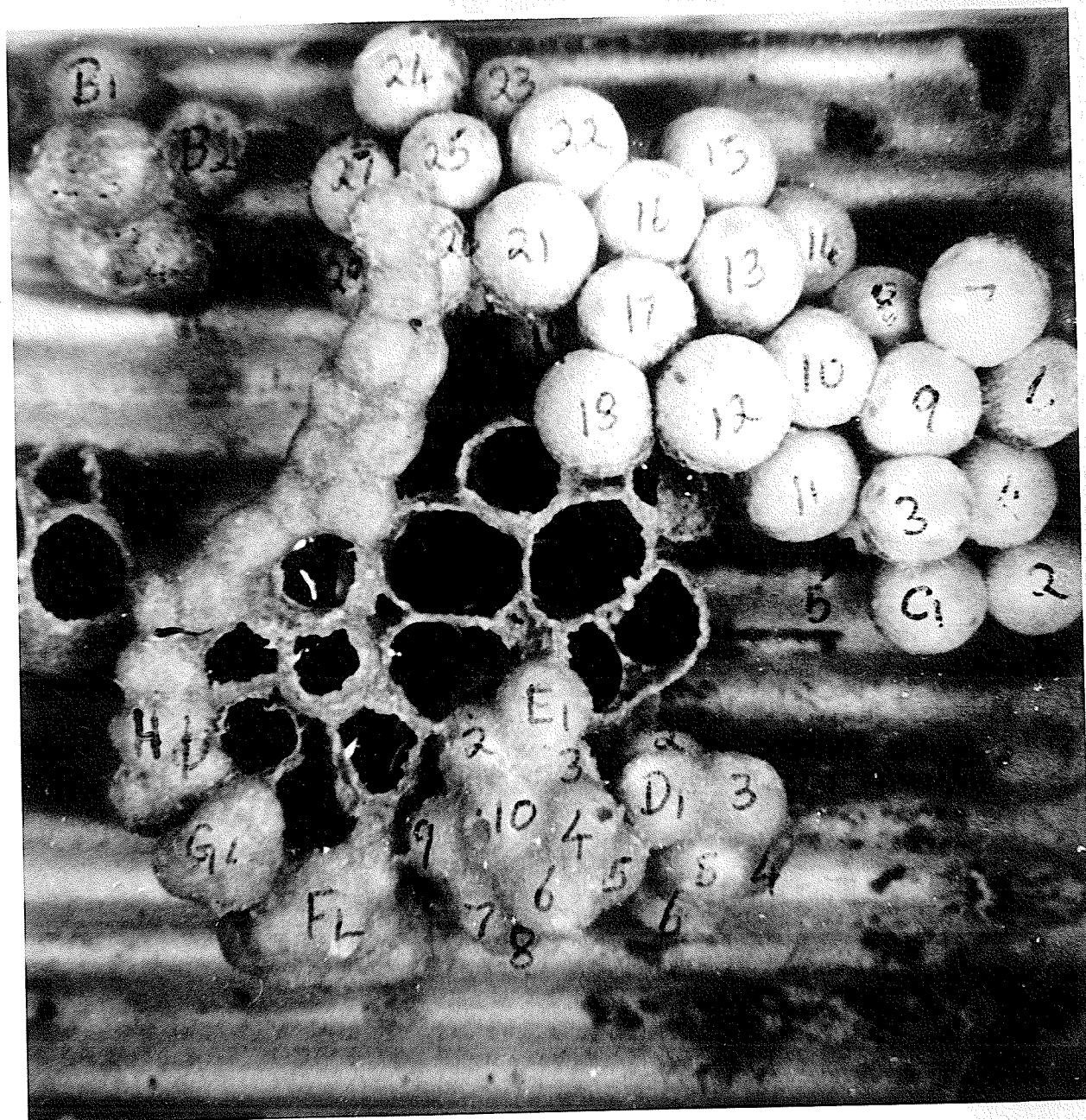


Fig. 5.- Comb of an artificially started colony of B. terrestris in a Series II box, showing egg cells (along edge of comb, left centre) spanning from one cocoon clump to another.

The larvae of this species, initially located in a communal wax envelope (Fig. 6), become separated from each other earlier in the fourth instar than in B. rufocinctus, B. ternarius or B. vagans. At the time of this separation, larvae destined to become s-workers have already begun to spin silk cocoons but most male and presumptive s-queen larvae separate into individual wax cells, each of which generally has an open "window" through which the larva continues to be fed (Fig. 7).

Ejection of larvae was often observed in B. terricola, especially from colonies in first- and second-brood stages. In almost every case, larval ejection was accompanied by premature changeover from worker to queen production, though the converse was not always true. Egg-eating by workers (see FREE & BUTLER 1959) was observed on many occasions, as was the reduction or disappearance of egg-cells built by the queen - presumably also due to worker activity. No laying workers of B. terricola were found, though it is possible that they may have escaped detection; in any case, the mother queens of this species were mostly long-lived and all but a few were still living when the colonies were terminated in August. Animosity between workers or between the mother queen and her workers was never seen in any B. terricola colony.

The changeover from worker to queen production in this



Fig. 6.- Comb of a B. terricola colony in a Series III box, showing empty first-brood cocoons, second-brood worker and queen cocoons, third-brood eggs and young larvae.



Fig. 7.- Comb of a B. terricola colony in a Series II box, showing second-brood worker, male and queen cocoons and third-brood eggs, young and advanced larvae.

species was usually accompanied by the appearance of mixed clumps of queen and worker pupae, but in some colonies the rearing of the first queens coincided with the rearing of the first males. (Fig. 7)

B. terricola is not a very prolific producer of wax - a fact which is possibly associated with the early separation of fourth-instar larvae. In all the captive colonies, the wax was dark brown and rather fragmentary, perhaps partly because of admixture of shredded corrugated cardboard.

The maximum number of workers produced by any captive colony was 94, but two wild colonies removed from domiciles on 1.8.66 contained 104 and 87 workers. Judging from numbers of empty cocoons, both these colonies probably contained less than half the number of workers which had been reared altogether.

2) B. nevadensis

Manitoba is on the edge of the range of this species (NEAVE 1933), but one queen was taken on Caragana near Morden during June 1965. The peculiarities of this species have been described by FRISON (1917 & 1918) (B. auricomus Robt. is now considered to be conspecific with nevadensis) and by HOBBS (1965 a).

The average size of female bees increases as the colonies progress, but no size discontinuity between worker and queens exists in B. nevadensis. W.P. STEPHEN (personal

communication) reports that in some colonies of this species, more than one egg may be laid in each cell, but in the two colonies which I observed, each larva (and presumably, therefore, each egg) occupied its own wax cell, although sometimes two adjacent cells had one wall in common.

In both of the colonies which were reared in captivity, all larvae except those of the first brood (which were able to consume the pollen lump on which the first egg cells were constructed) were fed entirely by regurgitation. Though wax pockets were built on the sides of several larval cells, these were never used, presumably because of the abnormal method of pollen-intake by the colonies.

3) B. borealis

Two colonies of this species were reared from queens taken near Winnipeg in June 1965. The ecology of the species of the subgenus Subterraneo-bombus (to which B. borealis and its close relative B. appositus Cresson belong) which occur in southern Alberta has been described by HOBBS (1966 b). My observations on colony development in B. borealis agree closely with Hobbs' account (based mostly on B. appositus Cresson) though, as in B. nevadensis, the feeding of larvae by pollen placed in wax pockets built onto the side of the larval clumps, characteristic of B. borealis colonies in the wild state, was not performed in my colonies because of the unusual way in which the colonies obtained their pollen.

PLATH (1934), who attempted to classify bumblebee species according to their modes of brood rearing, expressed surprise at SLADEN's (1912) observation that fourth instar larvae of B. latreill^{el}lus Kirby (= B. subterraneus (L)) became separated from each other in incomplete wax envelopes before pupation, in much the same way as do those of B. terrestris L. and B. lucorum L. However, HOBBS (1966 b) showed that larvae of the two North American species of the subgenus Subterraneo-bombus, B. borealis and B. appositus, behaved as Sladen had described for their European relative. Separation of all fourth instar larvae was observed in both my captive colonies of B. borealis.

As in many other "pocket-making" bumblebee species, there is no sharp size dimorphism between s-workers and s-queens in B. borealis (see Section 3.4). Female bees of successive broods showed a gradual increase in mean size, until eventually all the female larvae achieved the maximum size recorded - i.e., that of s-queens (see Section 3.42).

B. borealis does not appear to be a very prolific producer of wax, which may explain the "windows" which are left in the wax envelopes which cover the late fourth instar larvae.

4) B. rufocinctus

This species is extremely common in southern Manitoba, where it exists in a bewildering variety of colour forms

which grade into each other to a large extent. B. rufocinctus emerges from hibernation later than the earliest species (B. terricola, B. ternarius, B. perplexus and B. vagans), at about the same time as B. fervidus and B. borealis. HOBBS (1965 b), who has studied the ecology of B. rufocinctus in southern Alberta, comments upon its adaptability in the choice of nest sites. Around Winnipeg, colonies of the species were found in a number of situations, ranging from subterranean cavities, through abandoned car-seats, to old bird nests in the gable-ends of a garden shed and in a switchbox six feet above ground on an electricity pole.

Four colonies of B. rufocinctus were started by naturally overwintered queens in the laboratory in 1965 and were kept in captivity for observation.

In contrast to the preceding two species, B. rufocinctus is a "pollen storer" - pollen is stored in wax cylinders within the nest, and the larvae of all broods except the first are fed their pollen by regurgitation, i.e., not in wax pockets built onto their clumps.

Except for the earlier changeover to queen production, colony development of this species very much resembles that in B. ternarius and B. vagans (see below). Fourth instar larvae remain enclosed in a communal wax envelope until they pupate, and the onset of queen rearing is marked by mixed clumps of pupae - i.e., both s-workers and s-queens may emerge

from the same pupal group. As in the two analagous species, there is a pronounced size dimorphism within the female bees of B. rufocinctus and intermediates are rare.

As noted by HOBBS (1965 b), this species is a copious producer of wax. In all seven colonies found in the wild state, the wax was dark greenish-black, but in laboratory reared colonies, the wax was generally light brown, though never so pale as in B. ternarius. The colour of the wax probably depends upon the nature of the pollen and other material incorporated into it; an analysis of B. rufocinctus wax revealed that only 30% by weight could be removed by repeated leaching with chloroform; the insoluble material included much fibrous matter (probably scraped from the corrugated flooring) as well as a great deal of pollen.

So far as could be ascertained, the queens constructed their egg-cells unaided in B. rufocinctus. Though egg-eating by workers was never seen, hostility between workers and between workers and queen was very common; moreover, laying workers seemed to appear very early in B. rufocinctus, sometimes even before the mother queen had ceased laying.

As in B. perplexus (see below), males of B. rufocinctus were attacked and killed by the workers (or by young queens) unless removed from the colony within a few days after emergence. In nature, such hostility probably would not develop since the males would leave the nest a few days

after becoming adult; it is hard to see what biological advantage could result from this behaviour, other than that in both B. perplexus and B. rufocinctus, incestual mating within the nest is quite common (though possibly only in the presence of daylight, for while such mating was never observed under red illumination, there was usually much copulatory activity when the colonies were exposed to fluorescent lighting or daylight), so possibly worker hostility may encourage the males to leave the nest and thus find mates from other colonies. However, in neither B. borealis nor B. fervidus colonies (in both of which species incestual mating occurs frequently) were workers ever observed to attack the males.

5) B. perplexus

Alone of the species studied, B. perplexus appeared to be far commoner within the city and suburbs of Winnipeg than in the surrounding countryside, but it was also taken in the Whiteshell provincial park and near Stonewall, Manitoba. As in B. rufocinctus, this species is very flexible in its choice of nesting sites, which in the Winnipeg area included a rolled-up carpet laid across the rafters of a garage and a disused squirrel's nest (twenty feet above ground), as well as more conventional locations. In this way, B. perplexus somewhat resembles its European relative B. pratorum L., as it does also in its pattern of colony development.

In both 1965 and 1966, seven colonies of this species were reared from naturally overwintered queens and maintained in the laboratory for observation.

It does not seem to be known whether B. perplexus is a "pocket-maker" in the wild state. In captive colonies, though larval feeding in the manner used by pocket-making species was impossible, wax pockets (apparently similar to those built by pocket-making bumblebees) were observed on several occasions in B. perplexus colonies, always by the side of first- or second-brood larval groups. However, the conclusion that B. perplexus is a pocket-maker during part of its colony development should not be accepted until colonies in the natural state have been examined. In any case, large wax cells containing pollen are usually present in the later stages of the development of wild colonies. (Fig. 8)

FREE & BUTLER (1959) remarked of B. pratorum that the size distinction between queens and workers was not as sharply defined as had previously been supposed. In my captive colonies of B. perplexus, though mixed clumps of pupae (s-queens and s-workers) were usually produced at the changeover to queen production, the separation into two non-overlapping size groups was not as clear as in B. rufocinctus, B. ternarius, or B. vagans; female bees of intermediate size occurred frequently in colonies at

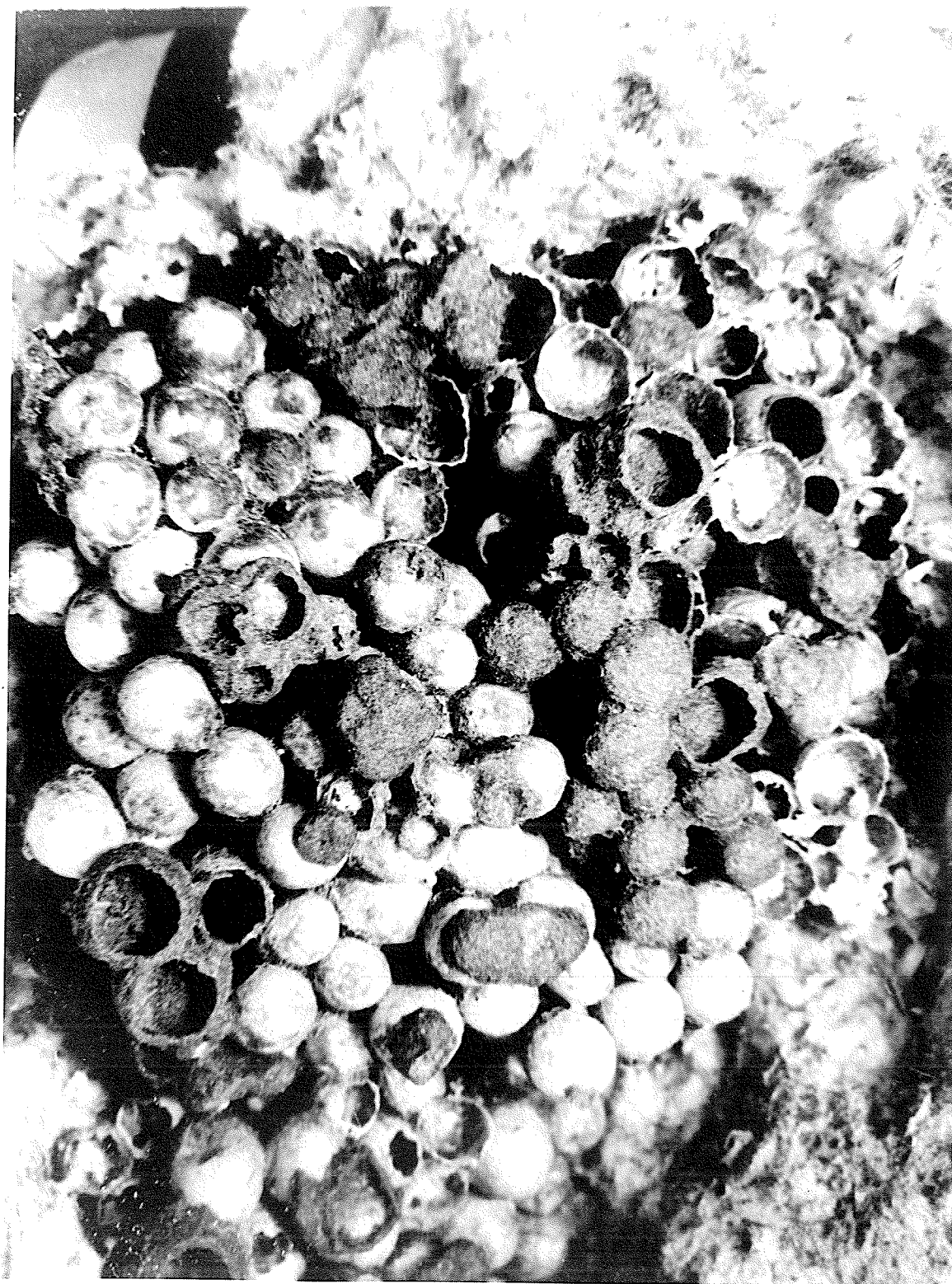


Fig. 8.- Comb of a naturally started B. perplexus colony removed from a wooden bumblebee domicile in Winnipeg, showing round wax cells used for pollen storage (on edge of comb--lower left).

the point of changeover in B. perplexus. As in B. ternarius (and probably in B. rufocinctus and B. vagans also) the larger pupae in mixed clumps resulted from larvae which pupated later than those which gave small pupae. (See Section 3.52)

In contrast to B. ternarius, queens of B. perplexus appear to construct their egg cells unaided by the workers. Eating of eggs (which the queen had laid) by workers was often observed in captive colonies of B. perplexus, even though the colonies were never allowed to run short of pollen. On two occasions, in two different colonies, the queen was seen to attack a worker in the process of egg-eating; in each case, the workers thus attacked were the objects of further hostility by the queen, even when not molesting her eggs, suggesting that their ovaries may have been developed in association with the failing dominance of the queen. (See FREE 1955 c)

Of all the species studies, colonies of B. perplexus appeared to be the most prone to throw out larvae of the second and later broods. In one colony, ejection of larvae coincided with a temporary pollen shortage, but in four others in which larval ejection was recorded, at no time was there any lack of pollen or honey. As in B. terricola (the other species which habitually threw out larvae), this behaviour was often associated with changeover

to queen rearing (see Section 3.513); in fact, no colony of either species which was still producing workers was observed to throw out any larvae other than occasional ones which had apparently died in their cells. However, in some colonies of both species, the changeover from worker to queen rearing was accomplished without ejection of larvae.

Because larvae destined to be thrown out generally had a major part of the wax envelope surrounding them removed during the days which preceded their ejection, it is tempting to suggest that larval ejection is triggered by shortage of wax, especially since neither B. perplexus nor B. terricola are copious wax producers. It is not known for certain whether colonies of B. perplexus or B. terricola ever throw out larvae in the wild state, but several dead larvae were found near the entrance of a B. perplexus colony captured in the field in August 1966.

Perhaps associated with the sparing production of wax by B. perplexus, the larvae separate from each other early in the fourth instar, and the adult bees start to remove wax from them well before they have ceased to feed (see Figs. 9 & 10). Larvae destined to become s-queens usually commenced spinning their cocoons when they were relatively smaller than similar larvae in B. terricola; the partly-spun cocoons of B. perplexus must have been quite expandable to have allowed the considerable size increase

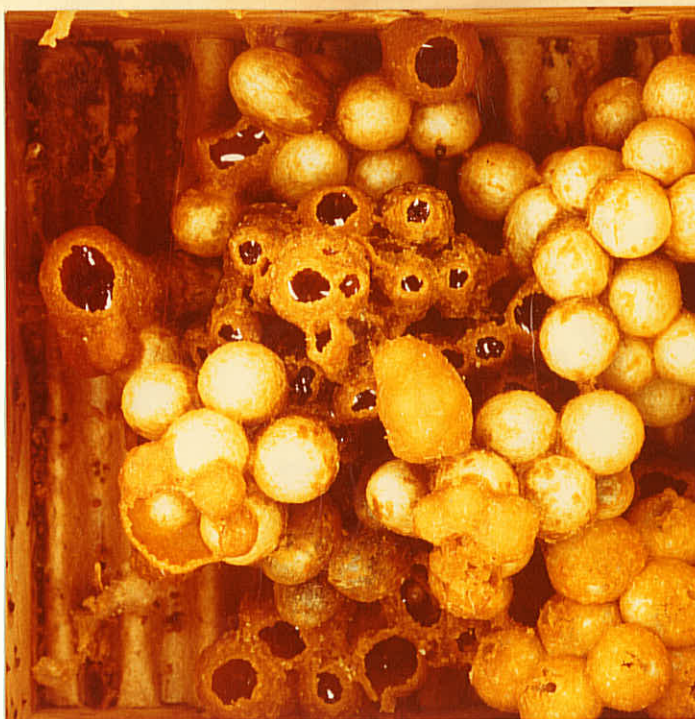


Fig. 9.- Comb of a B. perplexus colony in a Series II box, showing separated fourth-instar larvae (lower right).



Fig. 10.- Comb of a B. perplexus colony, showing fourth-instar larvae at a later stage than those in Fig. 9. (lower right).

that took place in presumptive s-queen larvae after spinning had commenced.

As in B. rufocinctus, B. perplexus males were usually attacked and killed by workers unless removed from their colonies within a few days after emergence. Though less liable to copulate within their colony-boxes than B. rufocinctus, males and young queens of B. perplexus mated readily in 1.2m x 0.6m x 0.6m plastic cages on the laboratory bench.

The maximum number of workers produced in captive colonies of B. perplexus was 30 (2 colonies), but wild colonies certainly achieve larger worker population than this. A colony removed from a domicile on 31.7.66 contained 35 workers, but the number of empty cocoons indicated that a total of about 100 had probably been reared.

6) B. ternarius

B. ternarius appears to be nearly as abundant as B. terricola in south-eastern Manitoba, and like that species, it emerges from hibernation early. As far as I can determine, all B. ternarius nests reported near Winnipeg have been situated below ground, generally connected to the surface by narrow tunnels. Thus the species appears to be a typical subterranean nesting bumblebee, similar to the European B. lapidarius L., which it also resembles in the large size of its colonies and in its mode of colony development.

More colonies of this species (27) were reared than of any other except B. terricola. All colonies of

B. ternarius were kept in the laboratory for observation and a daily photographic record was kept of 6 of them throughout part of their development. Though it was not feasible to observe all colonies with equal thoroughness, interesting findings were checked by examining as many colonies as possible. There was much variation in the number of workers reared per colony; of the colonies whose populations were counted, the largest produced 234 workers, but even this may not have been the most populous of all 27 colonies. Unfortunately no data are available as to the size attained by wild colonies of B. ternarius in Manitoba, so it is impossible to determine whether the captive colonies reached comparable dimensions. MEDLER (1963) quotes "more than 100" as the number of workers reared by a B. ternarius colony observed by PLATH (1934).

Certainly, some queens of this species are capable of a very high oviposition rate. This may be partly a result of the fact that (in captivity at least) when the colonies become populous, the queen no longer has to construct all her egg cells; this function is partially taken over by workers who build characteristic wax rosettes on top of the cocoons. Should the queen fail to lay in these cells, they are often enlarged and used for honey storage.

Captive colonies of B. ternarius were very prone to develop laying workers, and s-workers of this species, when hatched and kept in isolation, often underwent ovary development and laid eggs (see Section 3.31). In at least two larger colonies, it was established that eggs were laid concurrently by the mother-queen and one or more of her filial workers, operating in widely spaced regions of the nest-box. Though egg-eating was never observed (and since no egg clumps were ever seen to be reduced in size, it probably never occurred) hostility between workers or between queen and workers was common. In B. ternarius, such hostility was sometimes fatal to one or both protagonists; in one colony, the maternal queen was attacked and stung to death by one of her worker offspring. Such extreme revolutionary activities were never observed to have been performed by the workers of any of the other six species studied.

Though B. ternarius queens in Series II boxes often ejected some or all of their first-brood larvae, perhaps because they could not secrete sufficient wax to keep pace with the expanding larval envelope, larvae were almost never thrown out after the first workers had emerged. In the one colony where second-brood larvae were ejected, these were either moribund or dead before they were thrown out.

B. ternarius is a "pollen storing" species and colonies generally show a marked size discontinuity between s-workers and s-queens (see Section 3.41). As in B. rufocinctus, the members of each larval group remained covered by a communal wax envelope until their cocoons were fully spun. Sometimes as many as thirty larvae (resulting from several egg cells) shared the same envelope. The changeover from worker to queen rearing was usually achieved by the production of mixed clumps of s-queen and s-worker cocoons, though in a few colonies a period of male production intervened between worker and queen rearing, and in others the first s-queen cocoons appeared in clumps with males only. The manner in which mixed s-worker - s-queen cocoon clumps arose was investigated in detail (see Sections 3.522 and 3.523).

This species seems to be a moderately prolific producer of wax, though not as much so as B. rufocinctus. In all captive colonies of B. ternarius the wax was pale yellow, but in two colonies taken in the wild state, the wax was darker and brown.

7) B. vagans

Only two colonies of this species were reared; they were not observed intensively, because much is already known of the colony development of B. vagans (FRISON 1930), which shows no great differences from that of B. ternarius.

3.3 The establishment of caste-status in imaginal females

In an attempt to duplicate the findings of CUMBER (1949), a series of experiments to study the process of fat-body deposition in newly emerged female bees were carried out.

Cocoons were removed from colonies of B. terricola, B. perplexus and B. ternarius and placed in an incubator at 32°C and 60% R.H. As soon as possible after emergence, the bees were confined individually or in pairs in glass-topped boxes with pollen and honey (which was renewed on alternative days) and held at 32°C and R.H. 60% (approx.). Each bee was weighed daily (B. ternarius) or on alternate days (B. perplexus and B. terricola) until the twentieth day after installation, when it was killed and dissected, the state of its fat body noted, and its ovaries weighed (see RICHARDS 1946).

I. In the first experiment (Groups I_Q and I_W) six "mixed clumps" (i.e., those containing both s-queens and s-workers) of cocoons were taken from four B. ternarius colonies on the point of changeover from worker to queen production. These cocoons yielded ten s-queens and seventeen s-workers, together with many males (which were discarded). After weighing, and before they had access to honey or pollen, all bees were confined individually, with access to honey solution, but in six of the boxes containing s-queens (Subgroup I_{Qa}) and six containing s-workers (Subgroup I_{Wa}) no honey or pollen was supplied for the first two hours;

at the end of this time, the bees were reweighed and honey and pollen were then added. Thus an estimate of the capacity of the honey-stomachs was obtained for these bees. In the remaining boxes (Subgroups I_{Qb} and I_{Wb}) pollen was supplied at the time that the bees were installed.

At a later date, a further sixteen s-queen cocoons (Group II_Q) were removed from another B. ternarius colony; the bees emerging from these were treated in the same way as subgroups I_{Qb} and I_{Wb} .

The results for B. ternarius are given in Table XIII. Eggs were laid by a significantly greater proportion of the s-workers (13 out of 17) than of the s-queens (1 out of 25) ($P < 0.001$). The least weight gain exhibited by any s-queen (143 mgm) is greater than the greatest gain (93 mgm) achieved by any of subgroup I_{Qa} during the first two-hour period, but the mean of the weights gained by the s-workers does not differ significantly from that of the weights gained by the subgroup I_{Wa} during the first two-hour period. There is no evidence, then, that the s-workers underwent any increase in weight during the experiment other than the initial weight gain achieved (presumably) by filling their honey-stomachs. Among the s-queens, however, all individuals showed a greater weight gain than could be accounted for by distension of their honey-stomachs.

RADIAL CELL LENGTHS, INITIAL AND MAXIMUM BODY WEIGHTS AND WEIGHTS
OF OVARIES OF B. ternarius FEMALES HELD AT 32° C FOR TWENTY DAYS

Group no.	Length of radial cell	Initial body weight	Weight gained during first two hours	Maximum body weight	Weight-gain (maximum - initial wt.)	Weight of ovaries
	1 unit = 0.051 mm	mgm	mgm	mgm	mgm	mgm
I _Q	a	67.0	323	70	475 *	152
		68.0	317	75	493	176
		68.0	286	70	593	307
		68.0	341	74	583	242
		67.5	340	93	620	280
		67.5	304	76	630	326
	b	68.0	401		643	242
		67.5	336		625	289
		68.5	349		649	300
		68.5	355		685	330
	II _Q	66.0	380		628	248
		69.0	409		635	226
		72.0	489		664	175
		70.0	445		660	215
		66.0	323		512	189
		70.0	435		647	212
		67.5	403		593	190
		71.0	450		664	214
		71.5	391		700	309
		70.0	353		644	291
		72.5	375		528	143
		70.5	439		632	193
		72.0	433		643	210
		72.0	444		627	183
I _W	a	70.0	346		588	242
		70.0	405		607	202
		49.0	101	46	158 *	57
		54.0	154	49	204 *	50
		51.5	129	49	205 *	76
		51.5	138	69	214 *	76
	b	50.0	119	74	193	74
		51.0	122	57	182	60
		53.0	158		202 *	44
		52.0	148		188	40
		49.5	114		157 *	43
		53.0	147		182 *	35
		51.0	142		194 *	52
		50.0	130		187 *	57
		51.5	142		194 *	60
		51.5	151		210 *	59
		53.5	165		235	70
		49.0	115		169 *	54
		48.0	115		158 *	43

* = eggs laid during the 20 day period

The four s-queens whose ovaries were well developed (22, 16, 16 and 18 mgm) differed from those with undeveloped ovaries in possessing less abundant fat body of a more or less yellow colour, in contrast to the copious clear white fat body of the latter bees. The fat body of the workers (where detectable) resembled that of the s-queens whose ovaries were well developed.

To test the association between ovary-weight and weight-gain in the s-queens, it was necessary to find out whether the two groups (I_Q and II_Q) could be treated together, since the mean radial cell length in Group II_Q significantly exceeded that in Group I_Q ($t = 2.897^{**}$ for 24 d.f.). Correlation coefficients were calculated for initial weight upon radial cell length for each group:

Group	r	d.f.	z
I_Q	0.3119 ^{NS}	8	0.32
II_Q	0.4675 *	14	0.59

The difference between the z-values of the correlation coefficients was non-significant when compared with the standard deviation of differences between two values of z for 7 and 13 degrees of freedom ($t = 0.4675$ for 20 d.f.). Hence it appears that the two groups may legitimately be combined for the estimation of other parameters.

The correlation coefficient for ovary weight on weight-gain was -0.5835 for 24 d.f., indicating that ovary

development is negatively correlated with the differences between initial and maximum weight.

II A second experiment was run with eight s-queens (all taken from one colony) of B. perplexus (see Table XIV). Once again, a significant negative correlation, -0.8565 ($P < 0.01$), was found between the weight gained and the weight of the ovaries on the twentieth day.

III In a final experiment, seventy B. terricola s-queens were obtained from colonies reared in the laboratory from artificially overwintered queens in January - March 1966. Because of the large numbers involved, it was not possible to transfer cocoons to the incubator (as in the other two species); instead, the colonies were inspected daily, and all newly-emerged s-queens were removed. After randomisation, each day's total was distributed among three treatments (A, B, C) in a 2:2:3 ratio, so that treatments A and B each received 2 out of every 7 bees, the remaining 3 going to treatment C thus:

DAY	1	2	3	4
DAILY TOTAL (No. of bees)	4	9	6	2
TREATMENT ALLOCATION	AABB	CCCAABBCC	CAABBC	CC

In this way, bias due to starting replicates with treatments at different times was eliminated as far as possible.

TABLE XIV

RADIAL CELL LENGTHS, INITIAL AND MAXIMUM BODY WEIGHTS AND WEIGHTS
OF OVARIES OF EIGHT B. perplexus S-QUEENS HELD AT 32° C FOR 20 DAYS

Length of radial cell	Initial body weight	Maximum body weight	Weight gain (Maximum - initial wt.)	Weight of ovaries
1 unit = 0.051 mm	mgm	mgm	mgm	mgm
80	503	518	15	24
77	400	573	173	4
79	433	527	94	18
81	483	620	137	3
77	467	704	237	2
79	503	713	210	5
79	398	565	167	3
76	381	579	198	3

All bees were weighed on removal from their colonies and on alternate days thereafter. In treatments A and B, a single s-queen was put in each box, but in treatment C the s-queens were installed in pairs. Bees in treatments B and C were dissected on the twentieth day and those in treatment A on the thirty-fifth day after their installation.

One bee in A and two bees in B died during the experiment, and one bee from B escaped and was lost; thus Table XV gives data for 19 s-queens in A, 17 in B and 30 in C.

An analysis of variance showed no significant difference between means of the lengths of the radial cells nor between the mean maximum weights of the three groups.

A highly significant value of χ^2 was obtained by applying Bartlett's test to the ovary-weight data. Using the procedure suggested by HEALY & TAYLOR (1962), the most appropriate transformation was found to be:

$$z = x - 0.1618$$

Analysis of variance of the transformed values for ovary weights gave:

<u>Source</u>	S.o.S.	d.f.	Est. variance	F
Total	52,959,014	65		
Between treatments	5,707,762	2	2,853,881	3.81*
Residual	47,251,252	63	750,020	

$$\text{LSD}_{5\%} = \begin{cases} 578 & (\text{A, B}) \\ 526 & (\text{B, C}) \end{cases}$$

RADIAL CELL LENGTHS, MAXIMUM BODY WEIGHTS AND WEIGHTS OF OVARIES

OF B. terricola S-QUEENS HELD AT 32° C

Group A : individually confined and dissected after 35 days

Length of radial cell	Maximum body weight	Fat-body status	Weight of ovaries on 35th day	Transformed values of ovary weights
1 unit = 0.051 mm	mgm		mgm	$10^4 \cdot \text{mgm}^{-0.1618}$
77.0	869	MLY	16	6386
75.0	834	CW	3	8371
66.5	567	CW	5	7707
67.0	532	CW	3	8371
76.0	928	MLY	13	6604
67.0	523	CLY	10	6889
65.5	429	MLY	3	8371
76.0	845	CW	7	7300
64.0	425	MLY	12	6689
78.5	766	CW	9	7008
77.0	913	MLY	12	6689
74.0	594	SDY	8	7144
70.0	741	MLY	13	6604
73.0	532	CLY	4	7991
67.5	627	CW	5	7707
79.0	764	CW	5	7707
77.5	853	CW	7	7300
75.5	773	CW	9	7008
64.5	478	CW	5	7707

C = copious M = moderately abundant S = sparse W = white LY = light yellow

DY = dark yellow

Group B : individually confined and dissected after 20 days

Length of radial cell	Maximum body weight	Fat body status	Weight of ovaries on 20th day	Transformed values of ovary weights
1 unit = 0.051 mm	mgm		mgm	$10^4 \cdot \text{mgm}^{-0.1618}$
77.5	603	CW	6	7484
80.0	771	CW	5	7707
73.0	672	CW	3	8371
75.0	780	CW	6	7484
75.5	743	MLY	7	7300
73.0	650	CW	4	7991
77.5	716	CW	3	8371
71.0	599	MLY	6	7484
72.0	750	CW	5	7707
70.0	578	CW	3	8371
73.0	759	CW	2	8939
69.0	437	MLY	3	8371
75.0	681	CW	4	7991
73.0	750	CW	3	8371
68.5	425	MLY	3	8371
74.0	678	CW	2	8939
72.0	574	CW	4	7991

C = copious M = moderately abundant W = white LY = light yellow

Group C : confined in pairs and dissected after 20 days

Length of radial cell	Maximum body weight	Fat-body status	Weight of ovaries on 20th day	Transformed values of ovary weights
1 unit = 0.051 mm	mgm		mgm	$10^4 \cdot \text{mgm}^{-0.1618}$
76.0	753	CLY	8	7144
76.0	767	CW	4	7991
80.0	744	CW	3	8371
75.5	704	CW	4	7991
77.0	883	CW	6	7484
74.0	504	MLY	7	7300
75.0	716	SDY	17 *	6323
76.5	727	CW	3	8371
67.0	569	CW	4	7991
70.0	439	SDY	24 *	5980
75.5	763	CW	1	10000
76.0	566	SDY	13 *	6604
72.5	609	CW	5	7707
67.0	511	MLY	14	6525
76.5	859	CW	5	7707
73.0	570	SDY	22 *	6064
74.5	742	CW	3	8371
76.0	678	CW	5	7707
75.0	686	CW	4	7991
73.0	706	SDY	17 *	6323
80.0	819	CW	3	8371
76.0	754	CW	5	7707
71.5	726	CW	2	8939
77.0	657	CLY	8	7144
69.0	486	SDY	31 *	5737
77.0	612	SDY	58 *	5184
70.0	474	SDY	7	7300
73.0	734	CW	2	8939
71.0	578	CW	3	8371
71.0	488	SDY	29 *	5799

* = bee laid eggs during the 20 day period

C = copious M = moderately abundant S = sparse W = white

LY = light yellow DY = dark yellow

The least significant difference for $P < 0.05$ separates the mean ovary weights of the groups thus:

$$\bar{x}_A > \bar{x}_B$$

$$\bar{x}_C > \bar{x}_B$$

but the mean ovary weight for treatment A does not differ significantly from that for treatment C.

Since the newly emerged bees were not weighed before feeding, it was not considered appropriate to test the correlation between ovary weight and the difference between initial and maximum weights, since much variation had been observed in the weight increase during the first twenty-four hours of imaginal life, presumably partly due to myconium discharge.

To get at the relationship between ovary development and weight gain, partial correlation coefficients were calculated, relating ovary weight (O) to maximum weight (W), keeping radial cell length (R) constant:

<u>A:</u>				d.f.
	r_{RW}	=	0.85761***	17
	r_{WO}	=	0.42860 ^{NS}	17
	r_{RO}	=	0.26480 ^{NS}	17
	$r_{WO.R}$	=	0.4063 ^{NS}	16

<u>B:</u>	$r_{RW} = 0.67208^{***}$	d.f. 15
	$r_{WO} = 0.2288^{NS}$	15
	$r_{RO} = 0.36015^{NS}$	15
	$r_{WO.R} = -0.0192^{NS}$	14
<u>C:</u>	$r_{RW} = 0.71056^{***}$	28
	$r_{WO} = -0.46519^{**}$	28
	$r_{RO} = -0.16835^{NS}$	28
	$r_{WO.R} = -0.4983^{**}$	27

Thus, only among the s-queens confined in pairs (Group C) was there any significant association between weight-gain and ovary development. Eggs were laid by 8 s-queens in this group, but not by any in groups A or B. The fat body was sparse, deep yellow and speckled with brown droplets (c.f. CUMBER 1949) in all the eight B. terricola s-queens which oviposited and in some others in both groups A and B which had well-developed ovaries (see Table XV). But in others, generally with moderately developed ovaries, the fat body was copious or moderately abundant with a light yellow tinge.

Discussion

CUMBER (1949) placed all the adult female bees from two colonies of B. agrorum F. in jars with, in one case, honey, and in the other, honey and pollen. The bees were weighed at the beginning and end of the experiments, which

which lasted five and seven days respectively. Frequency distributions by weight and by weight/wing length ratio showed that the larger individuals had gained weight, whereas the weights of the smaller bees had not altered much. Cumber claimed that his experiments had shown that it was possible to widen the gap between the peaks of the bimodal frequency distribution of weight normally found in "pocket-making" species of bumblebee. He found, however, that even after his feeding treatment, there still remained some individuals of doubtful status, whose weights lay in the trough between the peaks of the bimodal distribution; but when these individuals were dissected it was evident "that we are concerned with two very different physiological types. Those which are workers have the scanty light-yellow fat bodies typical of all workers, the queens have the prolific white fat bodies, and the distinction in all cases could not be more striking. . . . Thus a division on a physiological basis may be made It is felt that this latter division is more satisfactory than one based on size alone. A few intermediate values still persist. Dissection of these, however, showed that the true division between queen and worker (based on the appearance of the fat bodies) occurred at the first zero point on the left of the queen peak".

Clearly, Cumber's results are of great importance, but his interpretation of the situation may be questioned. First, it needs to be emphasised that the individuals exposed to his feeding treatment were not all of the same age; the smaller bees were presumably on the whole older than the large ones. Moreover, since the bees were confined together communally, it is possible that inter-action between individuals could have influenced the results. These objections prevent the drawing of any conclusions as to the ontogeny of caste differences from Cumber's data, but do not necessarily prevent us from accepting Cumber's claim that an effective separation between the physiological states of queens and workers has been demonstrated, provided that we recognise that this separation only applies (pending further experimental data) to the specific conditions imposed by the application of his feeding treatment. We must also note that while an effective dichotomy between bees of two classes seems to be provided by the colour and amount of fat body (though not by weight - see below), no evidence of the isomorphism between this dichotomy and that presumed to exist between queens and workers, other than correspondence with size (a continuous variable), has been provided by Cumber's experiments. I do not doubt that those bees which possessed copious white fat body were potentially functional queens and those with scanty yellow fat body were

functional workers (actual or potential), but the fact remains that unless we decide to take the state of the fat body as a definiens of caste, there is no justification for the claim that bees with scanty yellow fat body were "true workers", etc. Furthermore, it is not impossible that had Cumber's experiments been continued over a longer period, some of the bees with copious white fat body might have graduated to the "scanty light-yellow" condition, or, conversely, that had the smaller bees been given the feeding treatment earlier in their imaginal lives, they might have developed copious white fat bodies. WHEELER (1928) discusses "nutritional castration" (suggested by MARCHAL 1897) in relation to the suppression of reproductive activity in the worker caste, but the concept seems equally attractive when applied to the suppression of fat body development. In Cumber's experiments, where the bees were removed from their brood, the curtailment of nursing activity might be expected to result in fat body accumulation in any bees which were of a suitable age.

For these reasons, it was thought necessary to repeat Cumber's experiment under more precisely controlled conditions. No evidence was found in the pattern of daily weight records to suggest development of fat body in the s-workers of B. ternarius, but in view of the weight fluctuations due (presumably) to changes in gut and honey-stomach contents, it is doubtful whether fat body development would have been

detectable in so small a sample by this method. But none of these bees had copious white fat body on the twentieth day after pupal emergence; moreover, all except two laid eggs during the experiment. Since the laying of unfertilised eggs without diapause is a worker characteristic, the f-worker status of the B. ternarius s-workers is further confirmed, but the outstanding question (which will require further work before an answer is forthcoming) remains: was their f-worker fate irrevocably determined by the time of pupal emergence?

For s-queens of all three species (B. ternarius, B. perplexus and B. terricola) the experiments suggest that caste-differentiation according to function may not be completed until the insects are adult, for many bees had well-developed ovaries and scanty yellow fat-body when dissected, and 9 even laid eggs. However, it might be argued that those f-queens which took the f-worker pathway during the experiment were already predestined to do so before pupal emergence; but the significant difference in ovary weights and incidence of egg-laying between treatments B and C on the B. terricola s-queens indicates that imaginal environment must play some part in determining the functional fate of s-queens of this species. Though the quantity of fat-body on dissection was not measured, there was an obvious association between (a) copious white fat-body and undeveloped ovaries and (b) sparse yellowish fat-body and well developed ovaries. Furthermore, in all the groups where much ovary

development had occurred (i.e., B. ternarius, B. perplexus and B. terricola - treatment C) except for B. terricola - treatment A, a significant negative correlation was found between ovary weight and weight gain, suggesting that if the weight gain can be taken as an acceptable measure of the quantity of fat body which a bee has accumulated, those bees which lay down the least fat body undergo the most ovary development by the twentieth day. But it is important to stress that no significant difference was found between the mean maximum weights of the three groups of B. terricola s-queens, nor were the regression coefficients for maximum weight upon radial cell length in the bees of each group significantly different from each other. Thus, though environmental factors (presence of other bees, length of confinement) clearly influenced ovary development and probably affected the breakdown of fat body, there is no evidence that these factors had any effect upon the accumulation of food reserves. Moreover, it is likely that the inverse relationship between weight gain and ovary development is not the whole story, for in the single B. terricola s-queens kept in boxes for thirty-five days (Treatment A) ovary development was largely uncorrelated with weight gain.

The presence of food reserves is probably associated with a "diapause" state during which ovary development is

impossible (supported by domestication experiments with non-overwintered queens - see Section 2.311) and the termination of diapause occurs (under the abnormal conditions imposed by these experiments) earliest in those bees which have laid down the least fat body. Thus, the moderate amounts of slightly yellow fat body found in many of the B. terricola s-queens which had only slightly developed ovaries would indicate a transitional stage in the process of diapause termination.

In the wild state it is possible that except under abnormal conditions (see CUMBER 1963) any s-queen which laid down fat body would enter hibernation; hence the experimental results described above may not by themselves justify the conclusion that caste function remains plastic until after pupal emergence, since it could be argued that a bee which accumulated food reserves must qualify as an f-queen irrespective of whether or not subsequent fat body degeneration and/or ovary development took place without prior hibernation under abnormal experimental conditions. To retain the concept of a qualitative distinction between the two castes, this argument would require that f-workers accumulated no fat body during the first few days of adult life. The method of sequential weighing is probably subject to too much error to be used to detect slight changes in the fat body status of s-workers, but such changes should be readily apparent on dissection.

To summarise: the response of newly-emerged s-queens of the three bumblebee species exposed to unlimited pollen and honey at 32°C has been shown to involve both fat body changes and ovary development. Though significant negative correlations were found between weight gain and ovary development in all groups of bees in which much ovary development had occurred after twenty days, no such correlation was found in the B. terricola group dissected after thirty-five days. Though further experiments will be needed to show whether or not (a) all s-queens lay down some fat body early in imaginal life and (b) any workers lay down any fat body during the same period, we may conclude that the apparent qualitative distinction between castes which Cumber claimed to have demonstrated by his weight gain experiments may have been at least in part due to bias in his selection of experimental material (in that the smaller bees were on the whole older than the larger ones) and that under certain conditions s-queens may show some of the functional characteristics associated with the worker caste.

3.4. The distribution of size among adult females in bumblebee colonies

It has already been remarked (Section 1.3) that there exists a more pronounced bimodality in the size range of females in colonies of "pollen-storing" species of Bombus than in those of "pocket-makers", though even in the latter

the size distribution was fairly bimodal in the colonies measured by CUMBER (1949).

As noted by FREE (1955 a), it was the opinion of FRISON (1927a) that the average size of the worker bees produced by a bumblebee colony increased as the season progressed. Attempts to verify this by RICHARDS (1946), CUMBER (1949) and BRIAN (1951) produced rather conflicting results, probably because of the reasons suggested by FREE (1955 a) and FREE & BUTLER (1959). The matter is of some importance, for if it could be shown that there is a progressive increase in the average size of female bees emerging during colony development, then it would be possible to explain caste determination, at least in those species which show a continuous size range of female bees ("pocket-makers"), quite simply on the basis of a steadily increasing worker/larva ratio. For three North American "pocket-makers" (B. auricomus, B. griseocollis and B. fervidus) a progressive increase in the size of female bees over weekly intervals during at least the latter part of the season has been demonstrated by KNEE & MEDLER (1965).

3.41 Comparison of the degree of bimodality in female size distribution of colony populations of seven species of Bombus

Estimates of the degree of bimodality of female size characteristics of B. terricola, B. borealis, B. fervidus,

B. rufocinctus, B. perplexus and B. ternarius were obtained by measuring the entire female population of colonies which had been started in the laboratory (except for the B. fervidus colony) and then kept in complete captivity in darkness at 29°C with abundant food until all female bees had emerged. The colony of B. fervidus was started naturally in a rolled-up carpet in a garage near Winnipeg; it was brought into the laboratory when three of the first-brood workers had emerged, and kept thereafter under the same conditions as the colonies of the other five species.

As CUMBER (1949) notes, weight is rather an unsatisfactory measure of size in adult bumblebees; MEDLER (1962) discussed various other methods for determining size, and suggested that the length of the radial cell of the forewing was one of the most useful morphometric characters for this purpose. I used both the lengths of the radial cells and the distance between the lateral margins of the two eyes (see Fig. 11). The female bees of each colony were placed individually in numbered receptacles, after which the heads and wings were excised and the thoraces and abdomina discarded. The wings were arranged serially along the sticky surfaces of strips of cellulose tape which were then held between two microscope slides and the radial cell lengths were measured under a microscope with a graduated eyepiece.

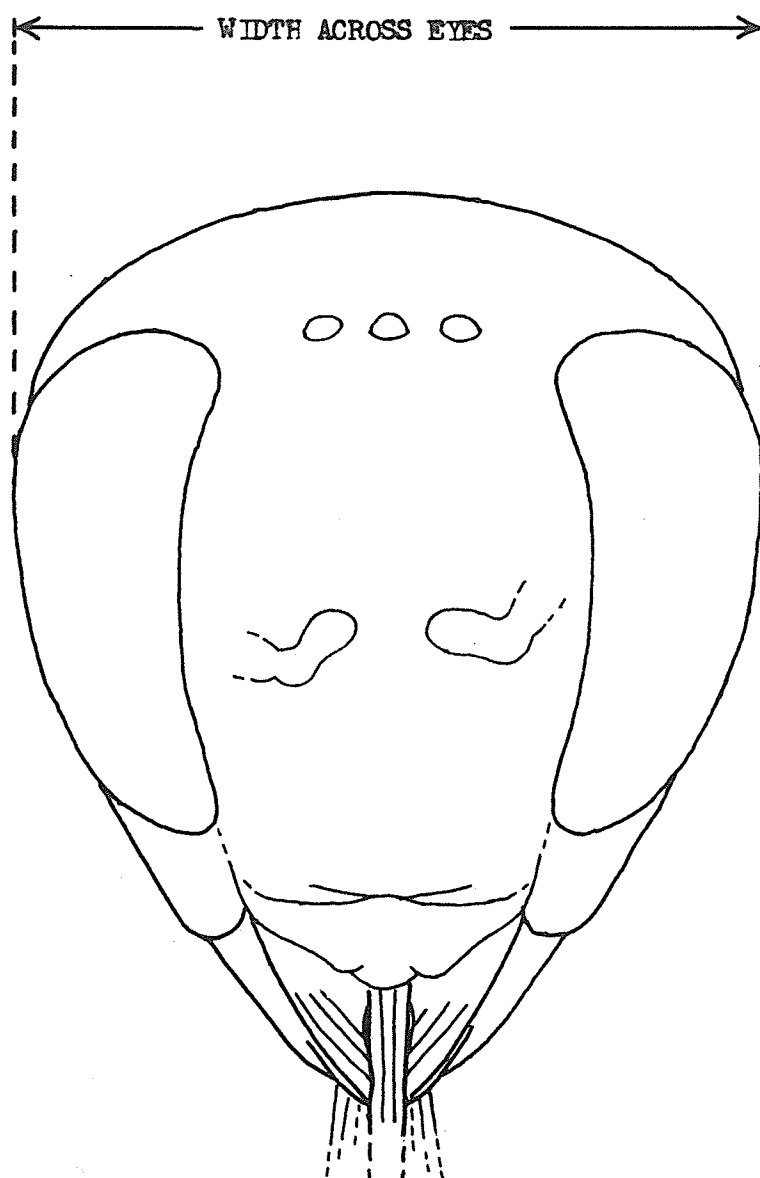


Fig. 11.- Anterior (dorsal) view of head of *B. terricola* queen showing eye-width measurement.

In order to measure the facial width, the tongue was removed and the head placed on an inclined plane ($\text{ca. } 15^\circ$) upon the microscope slide. By rotating the plane relative to the head, it was possible to ensure that the outer margins of both eyes were simultaneously in focus, thus allowing consistent measurements to be taken.

The results are shown graphically in Figs. 12 - 17, in which the ordinal values constitute the mean of both radial cell measurements for each bee (1 unit = 0.0513mm) and the abscissal values the width across the eyes, measured to the nearest $\frac{1}{2}$ unit (1 unit = 0.063mm). Inspection of the scatter-diagrams shows that for each species, the two characters are highly correlated and the relationship between them does not depart significantly from isometry, though there is a slight suggestion of allometry in some of the colonies in favour of proportionately longer radial cells in larger individuals. There is no evidence from the data to suggest that small bees differ from large bees in the underlying functional relationship between the two characters such that a classificatory separation could be used to distinguish a queen-type from a worker-type.

In Vespula, BLACKITH (1958) working with seven morphometric characters, was able to show that caste-differentiation involved differences of form, as well as of size, between queens and workers; for example, the width

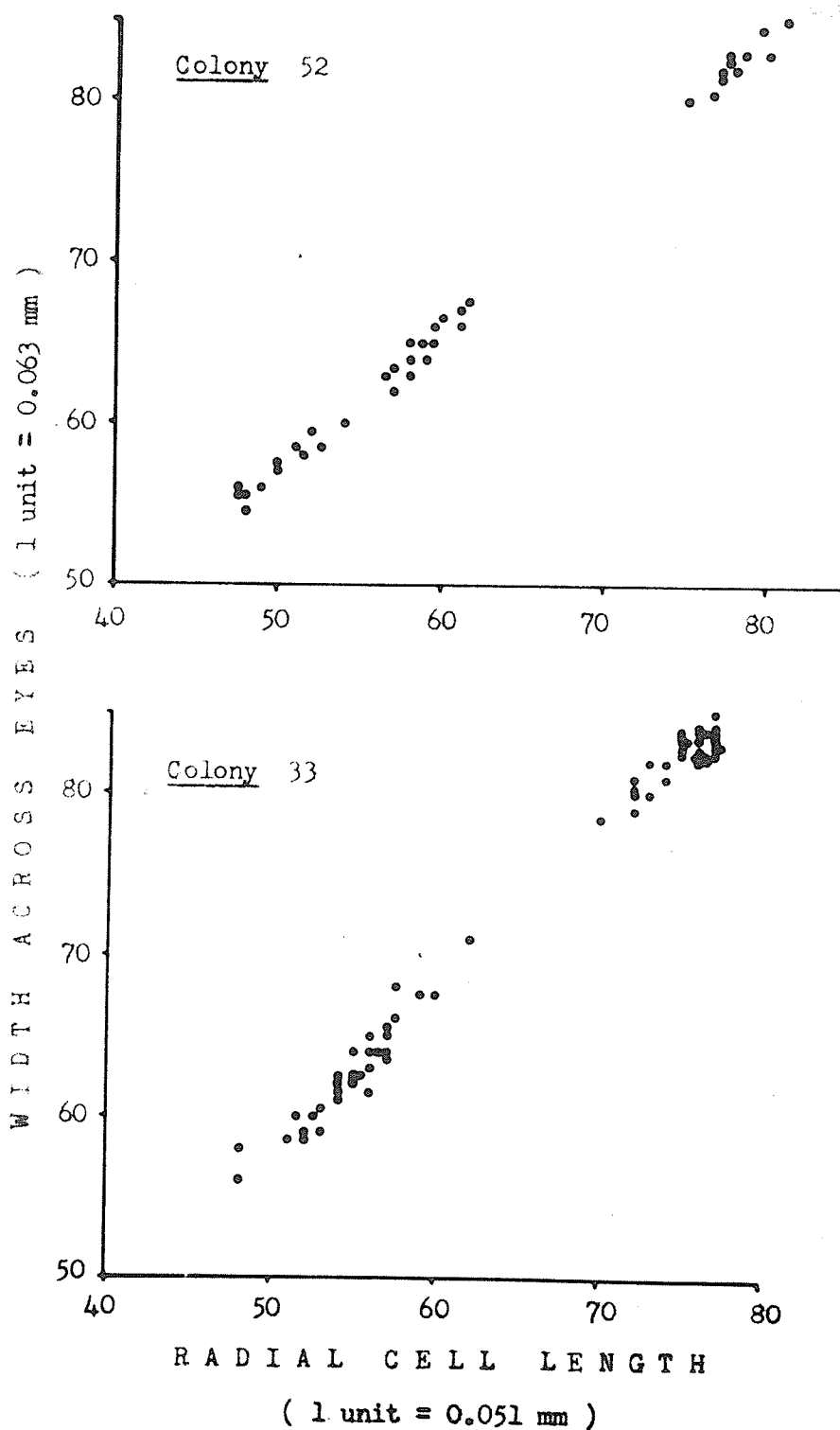


Fig. 12.- Scatter-diagrams of radial cell length and width across eyes of female bees from two colonies of B. terricola.

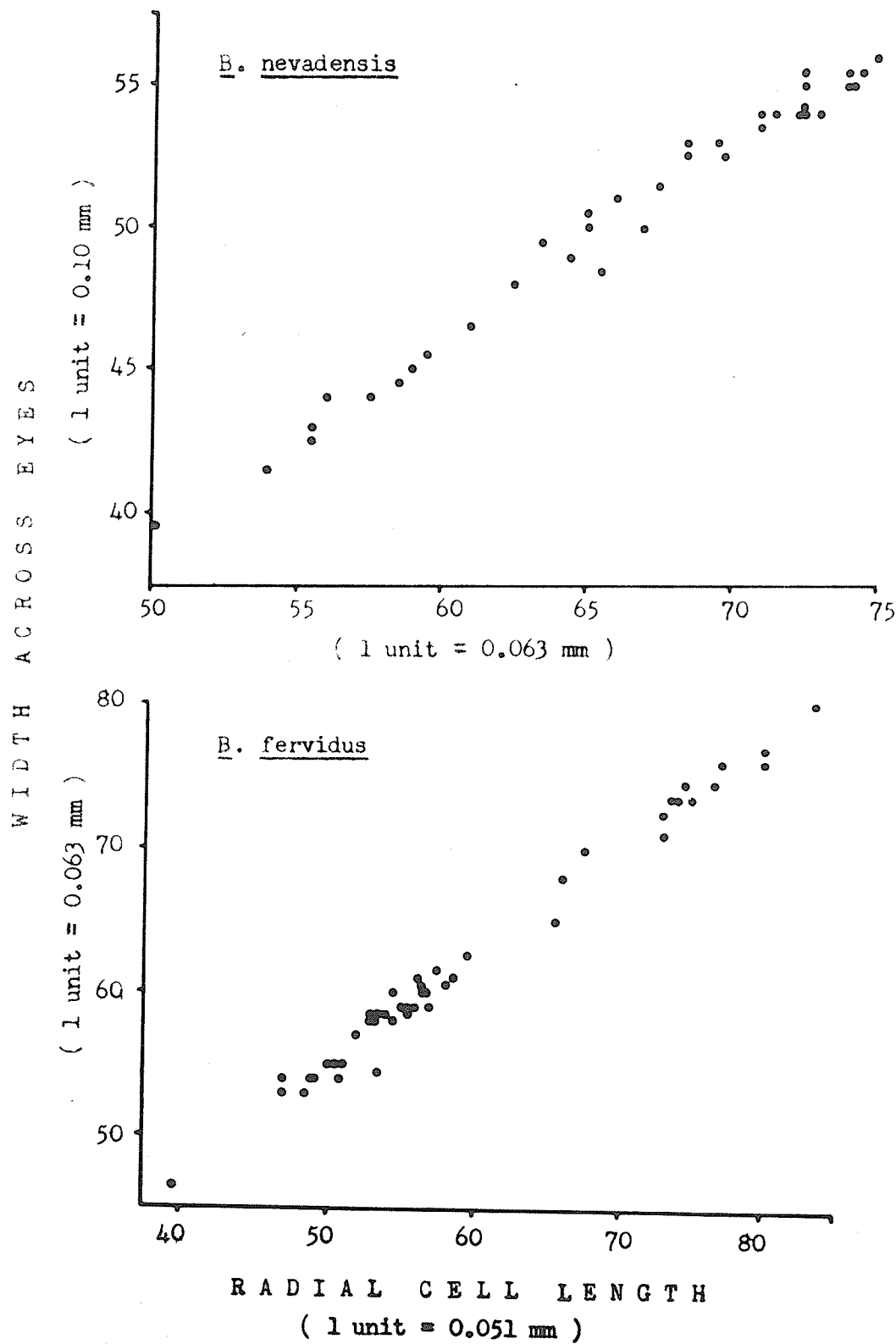


Fig. 13.- Scatter-diagrams of radial cell length and width across eyes of female bees from colonies of *B. nevadensis* and *B. fervidus*.

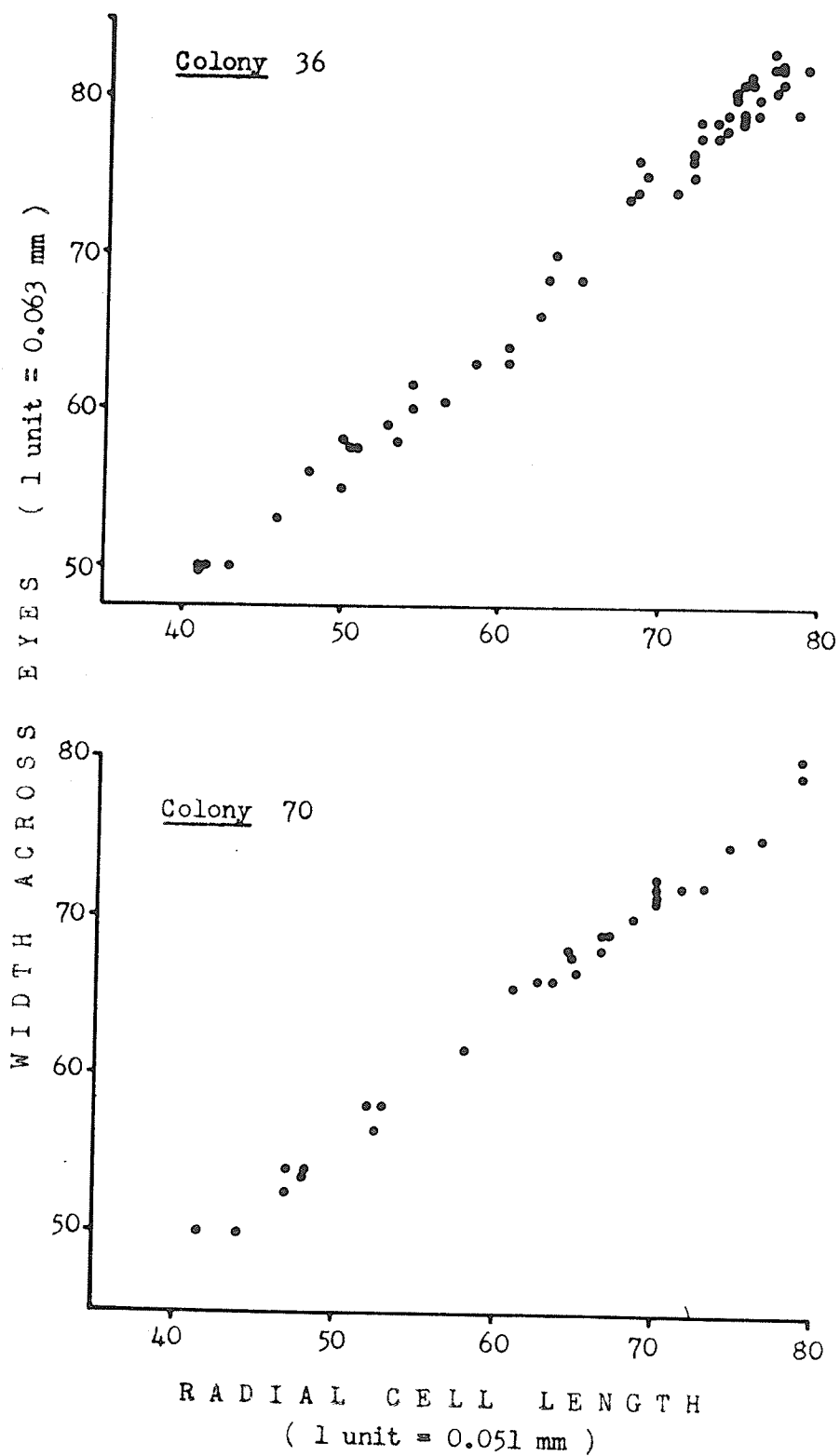


Fig. 14.- Scatter-diagrams of radial cell length and width across eyes of female bees from two colonies of B. borealis.

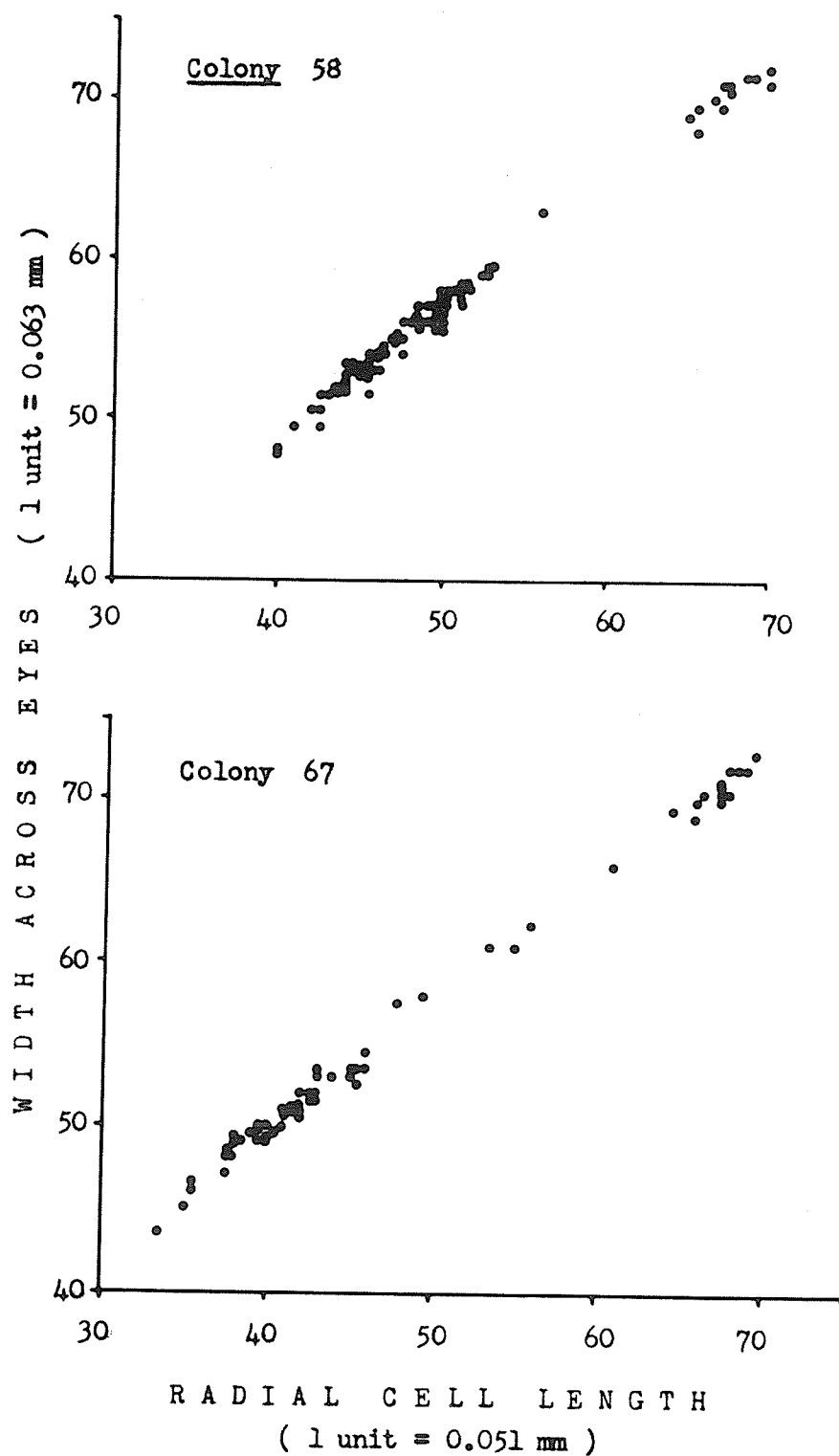


Fig. 15.- Scatter-diagrams of radial cell length and width across eyes of female bees from two colonies of B. rufocinctus.

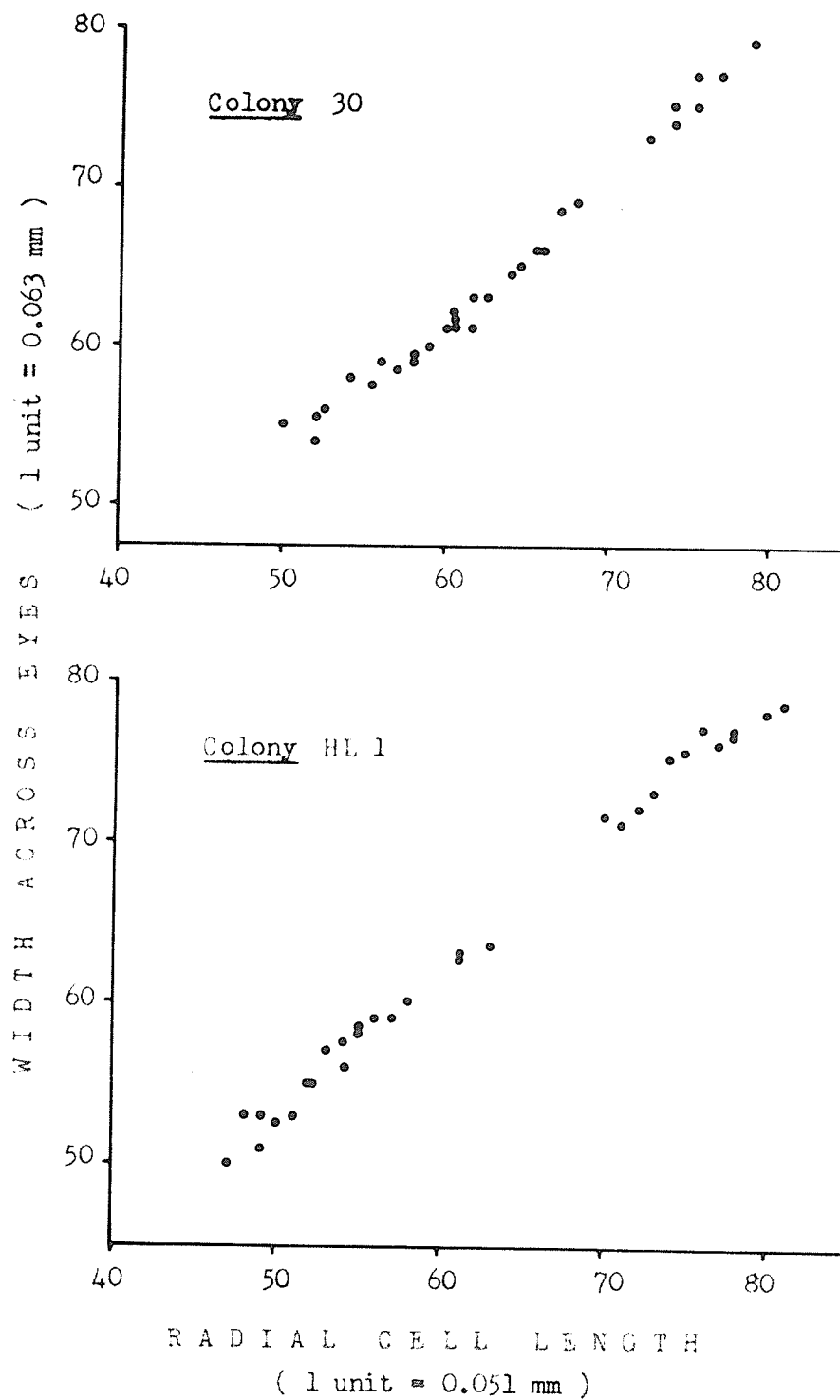


Fig. 16.- Scatter-diagrams of radial cell length and width across eyes of female bees from two colonies of B. perplexus.

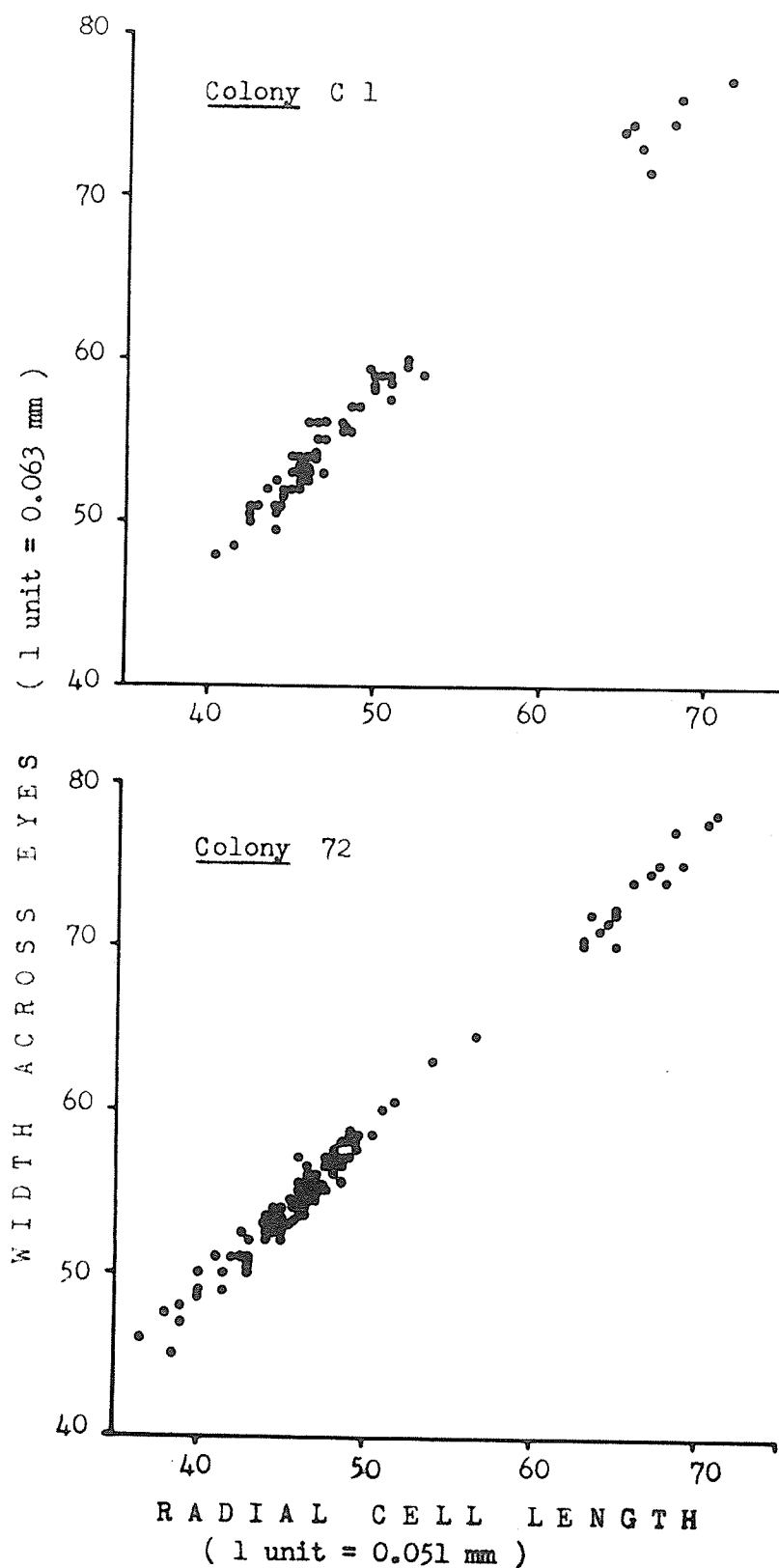


Fig. 17.- Scatter-diagrams of radial cell length and width across eyes of female bees from two colonies of B. ternarius.

of the body posteriorly far more in queens than it does in workers, even taking into account their different sizes - i.e., an allometric relationship exists here. However, as Blackith points out, such differences in form are in no way inconsistent with a purely quantitative distinction between castes. If more characters in Bombus were submitted to morphometric analysis, differences in form between large bees and small bees might be detected; but to demonstrate a "qualitative" distinction between castes, it would, I think, be necessary to show that the allometric index for workers differed significantly from that for queens. Even in Apis, where caste polymorphism is so pronounced, such a difference does not seem to have been found so far for any pair of characters.

Some degree of bimodality is apparent in each species, except possibly B. borealis and B. nevadensis. The "pollen-storing" species, B. terricola, B. rufocinctus and B. ternarius, show the most marked size dimorphism, but in the first colony (No. 58) of B. rufocinctus, several bees of intermediate size were produced. It is interesting to note that the main cluster of workers in this colony were of smaller size than those in the second colony measured (No. 67). BLACKITH (1958) found that the extent of the queen wing-length/worker wing-length dimorphism in Vespula seemed to differ as much from nest to nest as from species to species, and

MEDLER (1962 b) demonstrated that within various species of Bombus considerable variation in the ratio of the radial-cell lengths of queens and workers occurs. Unfortunately, however, Medler does not state what criteria were used to assign the bees (museum specimens) to the worker category or the queen category, so one does not know how bees of intermediate size were treated, if, indeed, any were represented in the 84 female specimens of each species which Medler measured.

The limited data presented here indicates that the occurrence of bees of intermediate size is much more pronounced in the three "pocket-making" species (B. nevadensis, B. fervidus, B. borealis) than in the "pollen-storers", with B. perplexus (a species which appears to possess characteristics of both groups) holding a position closer to that of the "pocket-makers" than to the "pollen-storers". These results are in agreement with CUMBER's (1949) work on English species, except on one important point: none of the colonies of "pocket-making" species which I measured had been able to feed its larvae by means of pollen placed in wax pockets on the larval clumps, since the colony pollen-intake was in the form of lumps of pollen-dough rather than pellets carried in the corbiculae of the workers. I observed all colonies of pocket-making species frequently and found that although wax pockets were often constructed on their larval clumps, these were never used. That such

colonies were able to develop in an apparently normal manner and to yield a more continuous size range of female bees than in "pollen-storing" species under similar conditions makes it unlikely that competition for pollen by the larvae during their fourth instar can be the only factor associated with greater size variation in the workers of "pocket-makers" than in "pollen-storers".

3.42 Variation in size among bees produced during colony development

That a progressive seasonal increase in the size of female bumblebees occurs in at least some species was confirmed during the summer of 1965, when it was noticed that such a progressive increase in size occurred among the female bees emerging in captive colonies of the "pocket-making" species B. nevadensis, B. fervidus, B. borealis and the "pollen-storing" species B. perplexus, but no measurements were taken. Unfortunately, of these species, only B. perplexus colonies were available in 1966.

Eight colonies of B. terricola, 7 of B. perplexus and one of B. ternarius were started in the laboratory by naturally overwintered queens caught near Winnipeg during May 1966. Fresh pollen was supplied every day and honey solution as required, but the bees were not allowed to fly outdoors. Since the laboratory temperature was unusually warm, nest material was removed shortly after the first second-brood

workers had emerged. The number of cocoons in each brood-batch was noted, and photographic records were kept where successive brood-batches might otherwise have been difficult to distinguish.

The numbers of bees emerging on each day were recorded, and one forewing was clipped from each bee about one day after it had emerged. The lengths of the radial cells of the wings thus obtained were measured. The bees appeared to suffer no ill effects as a result of their clipping, though in six instances they were attacked and stung immediately after reintroduction to their colonies. However, I found that this could be avoided by either (a) handling them during clipping with forceps only, or (b) in cases where a finger had to be brought into contact with a bee during its slipping, reintroducing the bee directly onto the comb after having transferred the entire adult population to the antechamber.

In B. terricola and B. perplexus, no workers were produced after the second brood - all subsequent bees were males or queens (B. terricola) or males only (B. perplexus). In both species, therefore, the experiment was discontinued as soon as all second-brood adults had emerged; but for the B. ternarius colony, the clipping was continued until the first queens emerged in the third brood. The results are given in Tables XVI - XVIII and are presented graphically in Figs. 18 - 20.

RADIAL CELL LENGTHS OF FEMALES EMERGING FROM FIRST- AND
SECOND-BROODS OF EIGHT CAPTIVE B. terricola COLONIES

Colony 2

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
19.6.66	First	56 50 53 49 56	52.8
2.7.66	Second	57	57.0
3.7.66	"	59	59.0
4.7.66	"	61 57 57 57	58.0
5.7.66	"	60 60 57	59.0
6.7.66	"	56 53	54.5
8.7.66	"	55 55 56 49	53.8
9.7.66	"	56 54 54	54.7
10.7.66	"	56 50 52 54	53.0
11.7.66	"	43 56 57	52.0
12.7.66	"	52 56 51 48 37	48.8
14.7.66	"	54 50	52.0

Colony 3

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
18.6.66	First	54 50 55 47 54	52.0
2.7.66	Second	72	72.0

Colony 5

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
10.6.66	First	51 55 59 49 54 50 57 49	53.0
23.6.66	Second	39	39.0
24.6.66	"	57 60 60	59.0
26.6.66	"	54 55	54.5
27.6.66	"	49 43 50	47.3
30.6.66	"	50 51	50.5
1.7.66	"	50 55 55 57	54.3
3.7.66	"	43 49	46.0
4.7.66	"	49 47	48.0
5.7.66	"	74	74.0
6.7.66	"	59 72 74	68.3
7.7.66	"	63 71	67.0
10.7.66	"	59 73	66.0

Colony 6

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
10.6.66	First	42 57 57 48 49 52 55 55 54	52.1
24.6.66	Second	56 57	56.5
25.6.66	"	56 53 52 53 56	54.0
26.6.66	"	52 55 54 56 51	53.6
28.6.66	"	50	50.0
1.7.66	"	58 54 54 58	56.0
2.7.66	"	56 57	56.5
4.7.66	"	60 52	56.0
5.7.66	"	56	56.0
6.7.66	"	57 57 59 66	59.8

Colony 7

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
15.6.66	First	59 56 61 56	58.0
30.6.66	Second	56 57 60	57.7
3.7.66	"	54	54.0
5.7.66	"	62	62.0
8.7.66	"	75 71	73.0

Colony 9

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
9.6.66	First	61 66	63.5
20.6.66	Second	49	49.0
23.6.66	"	51 46 47 52 51	49.4
24.6.66	"	49 48 53 51	50.3
25.6.66	"	45 47 46 46	46.0
26.6.66	"	55	55.0
27.6.66	"	49	49.0
28.6.66	"	51	51.0
29.6.66	"	48	48.0
30.6.66	"	48	48.0
4.7.66	"	74	74.0
5.7.66	"	50 74	62.0
10.7.66	"	59 75 76	70.0
14.7.66	"	75	75.0

Colony 10

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
10.6.66	First	57 56 53 56 56 56 56	55.7
24.6.66	Second	58	58.0
25.6.66	"	53 51 58	54.0
26.6.66	"	52 59 57	56.0
28.6.66	"	48 55 57 46	51.5
2.7.66	"	52 56 49	52.3
4.7.66	"	51 47 58	52.0
5.7.66	"	49	49.0
11.7.66	"	73 75 73 75	74.0

Colony 11

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
22.6.66	First	50 47 50	49.0
23.6.66	"	49	49.0
28.6.66	"	49	49.0
30.6.66	"	70	70.0
3.7.66	Second	73	73.0
5.7.66	"	55	55.0
6.7.66	"	57 55	56.0
7.7.66	"	54 51 54	53.0
10.7.66	"	56 48 58	54.0
14.7.66	"	76	76.0
15.7.66	"	71 73	72.0
16.7.66	"	70 72 77	73.0
18.7.66	"	78 72	75.0

RADIAL CELL LENGTHS OF FEMALES EMERGING FROM FIRST- AND
SECOND-BROODS OF SEVEN CAPTIVE B. perplexus
COLONIES

Colony 1

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
4.6.66	First	50 49 53 52 48 50 49 46	49.6
15.6.66	Second	51 55	53.0
16.6.66	"	54 54	54.0
17.6.66	"	57 54 57	56.0
18.6.66	"	57 60	58.5
20.6.66	"	62	62.0
21.6.66	"	62	62.0
23.6.66	"	63	63.0
26.6.66	"	75	75.0
28.6.66	"	80 77 70 76	75.8
29.6.66	"	80 76 71 78 70	75.0
30.6.66	"	75	75.0

Colony 2

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
9.6.66	First	54 53 52 50 52 52	52.2
20.6.66	Second	54 52	53.0
22.6.66	"	53	53.0
24.6.66	"	59 59	59.0
25.6.66	"	58 54	56.0
26.6.66	"	61	61.0
29.6.66	"	52	52.0
2.7.66	"	59 57 53	56.3
3.7.66	"	59	59.0
4.7.66	"	61 61 58	60.0

Colony 3

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
10.6.66	First	53 44 44 42 47	46.0
16.6.66	"	50	50.0
18.6.66	"	60	60.0
20.6.66	Second	52	52.0
21.6.66	"	64	64.0
22.6.66	"	51 58 51	53.3
23.6.66	"	54 57	55.5
29.6.66	"	73 76	74.5
30.6.66	"	75	75.0
1.7.66	"	77	77.0
2.7.66	"	74	74.0
3.7.66	"	81	81.0
5.7.66	"	67	67.0
7.7.66	"	80	80.0

Colony 4

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
9.6.66	First	45 51 52 49 55 47	49.8
15.6.66	"	51	51.0
25.6.66	Second	61 62	61.5
26.6.66	"	63	63.0
27.6.66	"	62 69	65.5
28.6.66	"	78 66 54 59 50 67 62	62.3
29.6.66	"	54 73	63.5
30.6.66	"	61 81 82	74.7
1.7.66	"	81	81.0
2.7.66	"	64 60 53 76	63.3
3.7.66	"	81 82 64	75.7
4.7.66	"	68 70 73 73	71.0
6.7.66	"	71	71.0

Colony 5

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
9.6.66	First	50 55 48 50 49 50 51	50.4
16.6.66	"	54	54.0
22.6.66	"	69	69.0
22.6.66	Second	57	57.0
23.6.66	"	58 50 54 57	54.8
24.6.66	"	60	60.0
25.6.66	"	59	59.0
30.6.66	"	61	61.0

Colony 6

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
9.6.66	First	53 51 52 54 54 48	52.0
20.6.66	Second	54	54.0
21.6.66	"	58	58.0
23.6.66	"	61 63	62.0
24.6.66	"	59 57	58.0
25.6.66	"	60 59 57 59	58.8
27.6.66	"	55	55.0
28.6.66	"	59 61	60.0
29.6.66	"	59 80 67 78	71.0
30.6.66	"	51 54 53	52.7
1.7.66	"	53 50 58	53.7
2.7.66	"	64 66	65.0
3.7.66	"	66	66.0
7.7.66	"	73 76	74.5

Colony 7

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
17.6.66	First	50 49 52 52 50	50.6
30.6.66	Second	49	49.0
2.7.66	"	56	56.0
4.7.66	"	56 58 54	56.0
5.7.66	"	55 58 56 54 56	55.8
6.7.66	"	56 56 62 59	58.3
7.7.66	"	60	60.0
8.7.66	"	53 62	57.5
9.7.66	"	60 62	61.0
10.7.66	"	62 62 64	62.7
13.7.66	"	68	68.0

RADIAL CELL LENGTHS OF FEMALES EMERGING FROM FIRST-,
SECOND- AND THIRD-BROODS OF A CAPTIVE B. ternarius COLONY

Date	Brood	Radial cell lengths 1 unit = 0.051 mm	Mean radial cell length
25.6.66	First	50 45 52 52 48 45	48.7
7.7.66	Second	46.5 47	46.8
8.7.66	"	49 46	47.5
9.7.66	"	50	50.0
10.7.66	"	44.5 49 48 48 45.5 46	46.8
11.7.66	"	51 46 53	50.0
13.7.66	"	52 50 45	49.0
14.7.66	"	53	53.0
15.7.66	"	46	46.0
16.7.66	"	46 47 49 46	47.0
17.7.66	"	36 47 50 48	45.3
18.7.66	"	48 49 50 50	49.3
19.7.66	"	47 48 46 44 44 39 43 43	44.3
24.7.66	Third	48 49	48.5
25.7.66	"	49 48 50 50 47 49	48.8
26.7.66	"	48 47 48 48 49 46 48 49 50 49	48.2
27.7.66	"	49 48 49 49 52 46 51 48	49.0
28.7.66	"	45 49 50 47 49 50 42 49 48 51 49 43 50 51	48.1
29.7.66	"	42 47 46 45 46 53 52 48 42 48 50 47	47.2
30.7.66	"	42	42.0
31.7.66	"	44 54 49	49.0
2.8.66	"	45	45.0
4.8.66	"	65	65.0
5.8.66	"	65 68	66.5

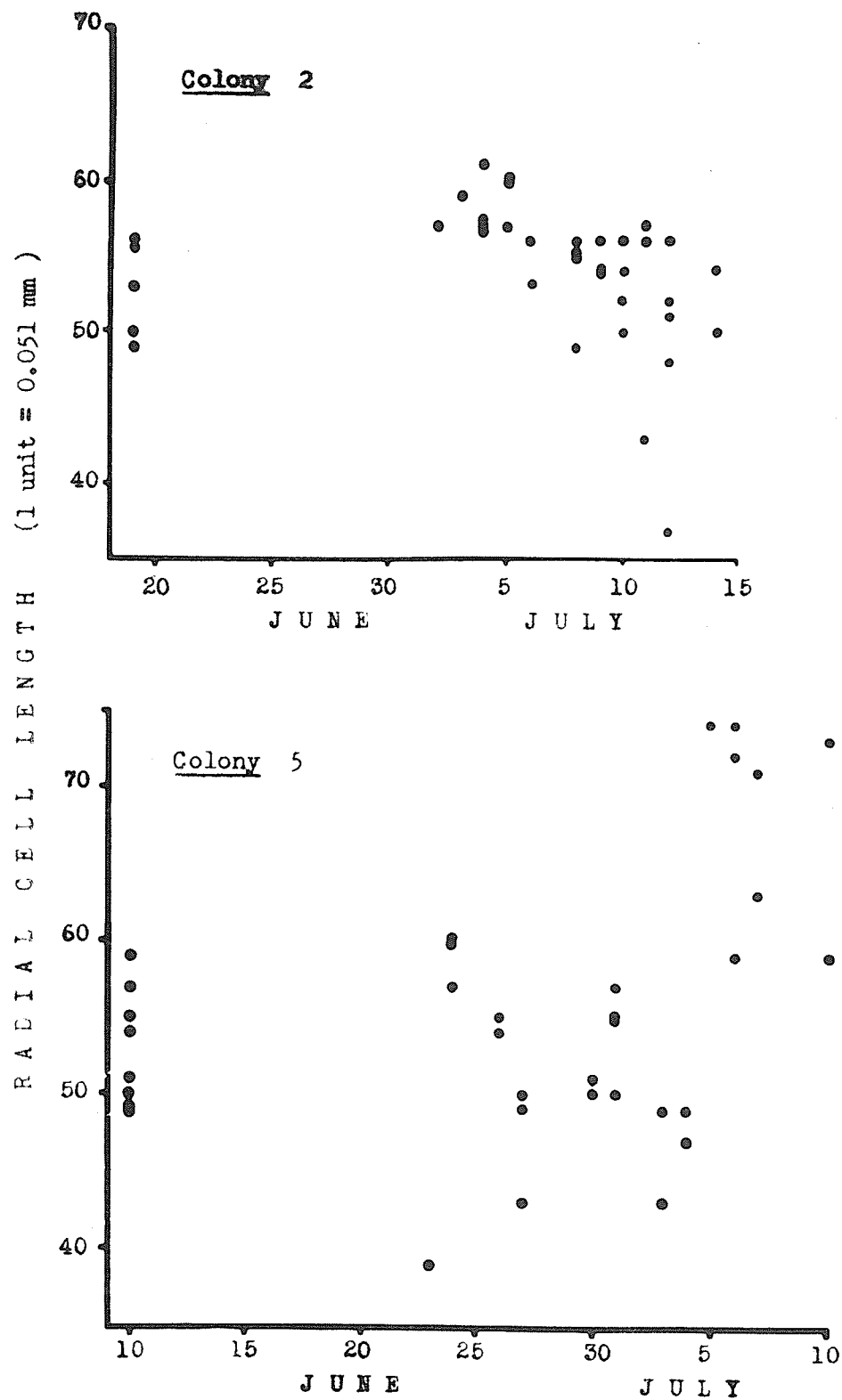


Fig. 18.- Radial cell lengths and dates of emergence of first- and second-brood females in seven captive colonies B. terricola.

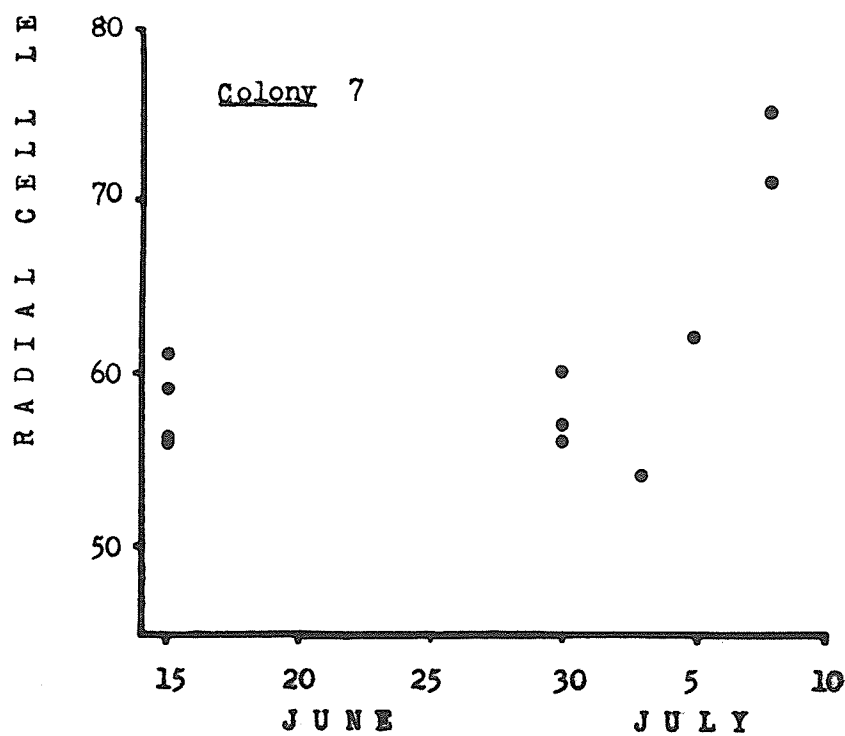
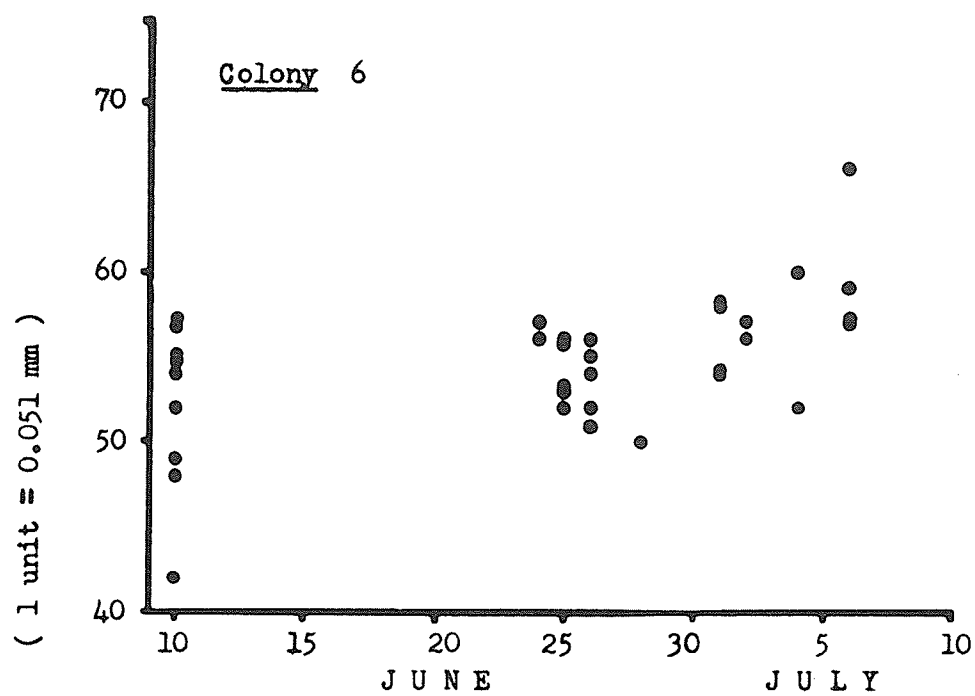


Fig. 18.- continued.

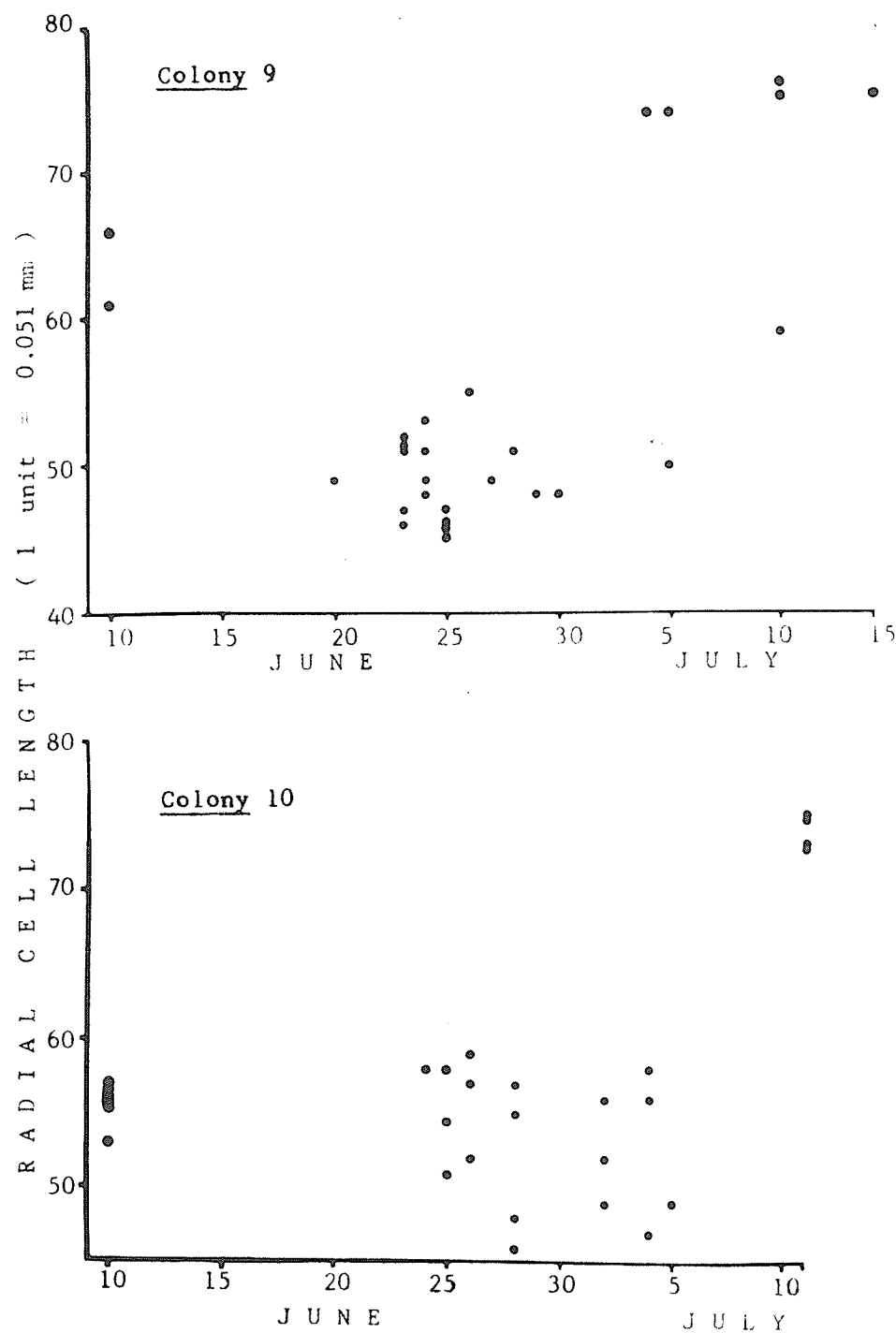


Fig. 18.- continued.

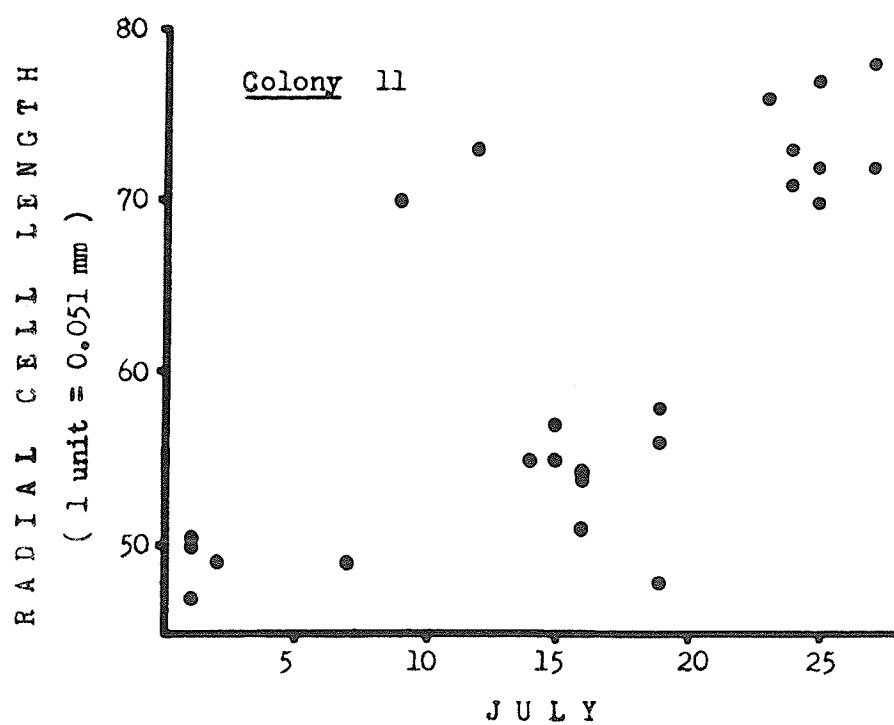


Fig. 18.- continued.

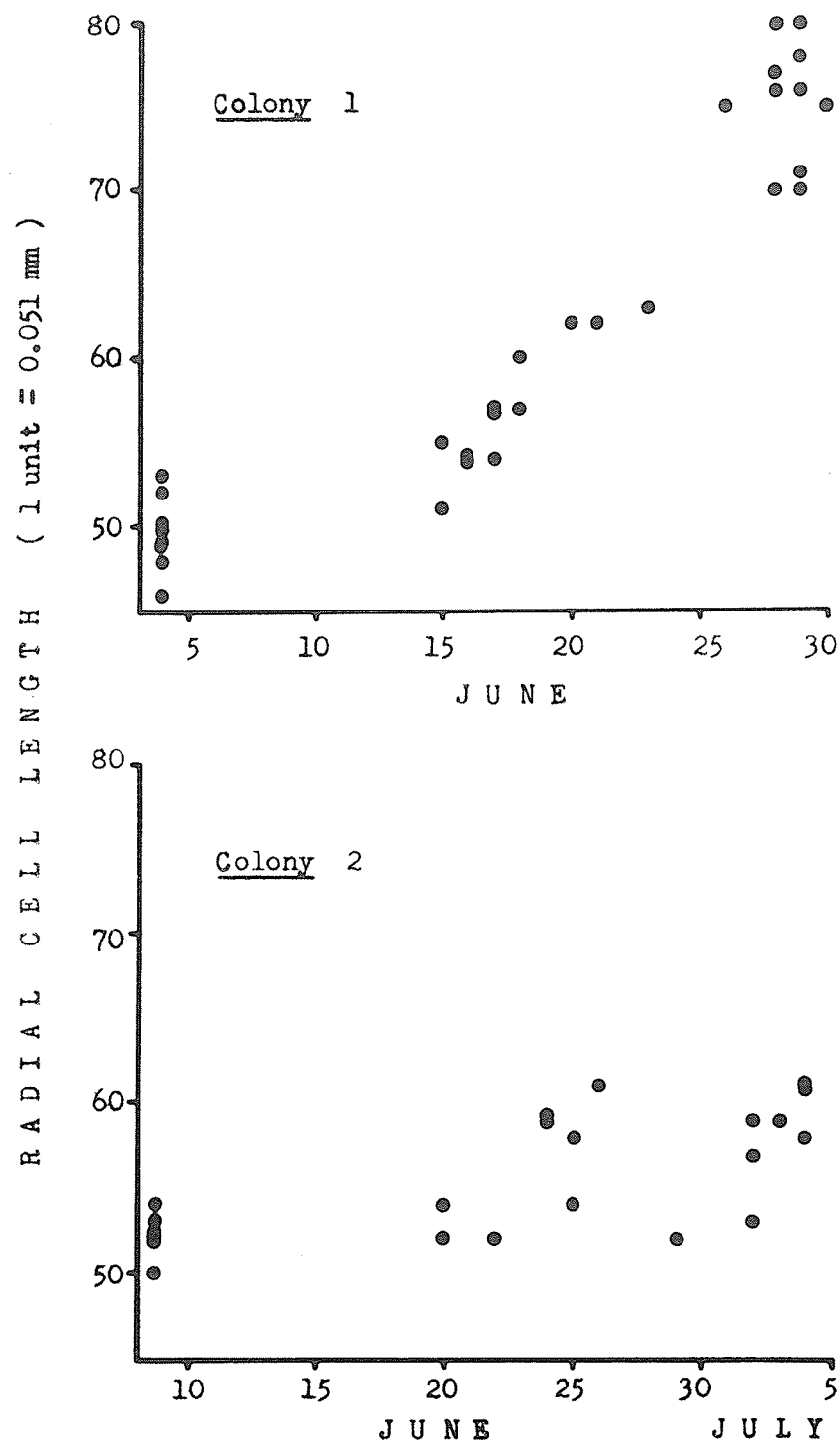


Fig. 19.- Radial cell lengths and dates of emergence of first- and second-brood females in seven captive colonies of B. perplexus.

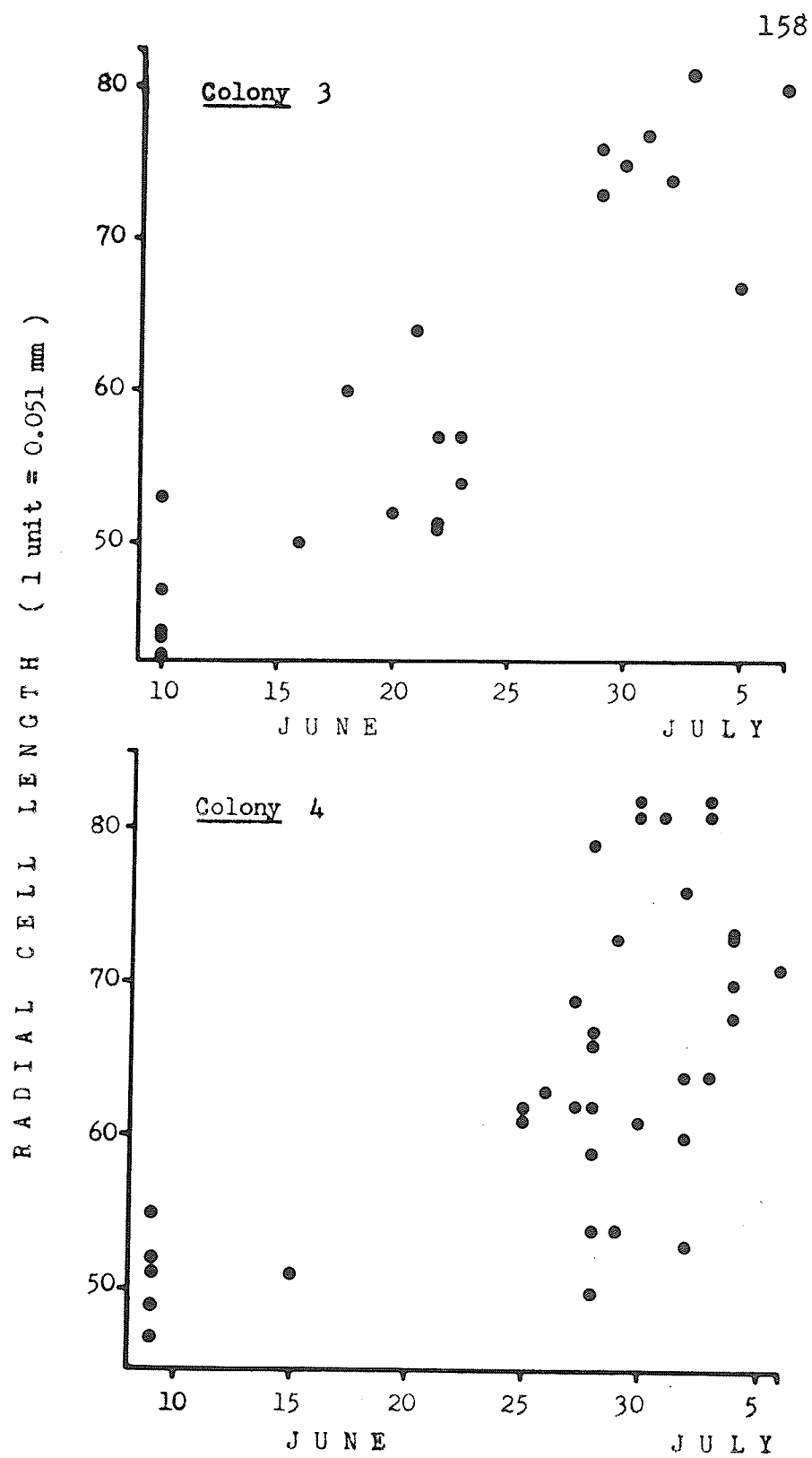


Fig. 19.- continued.

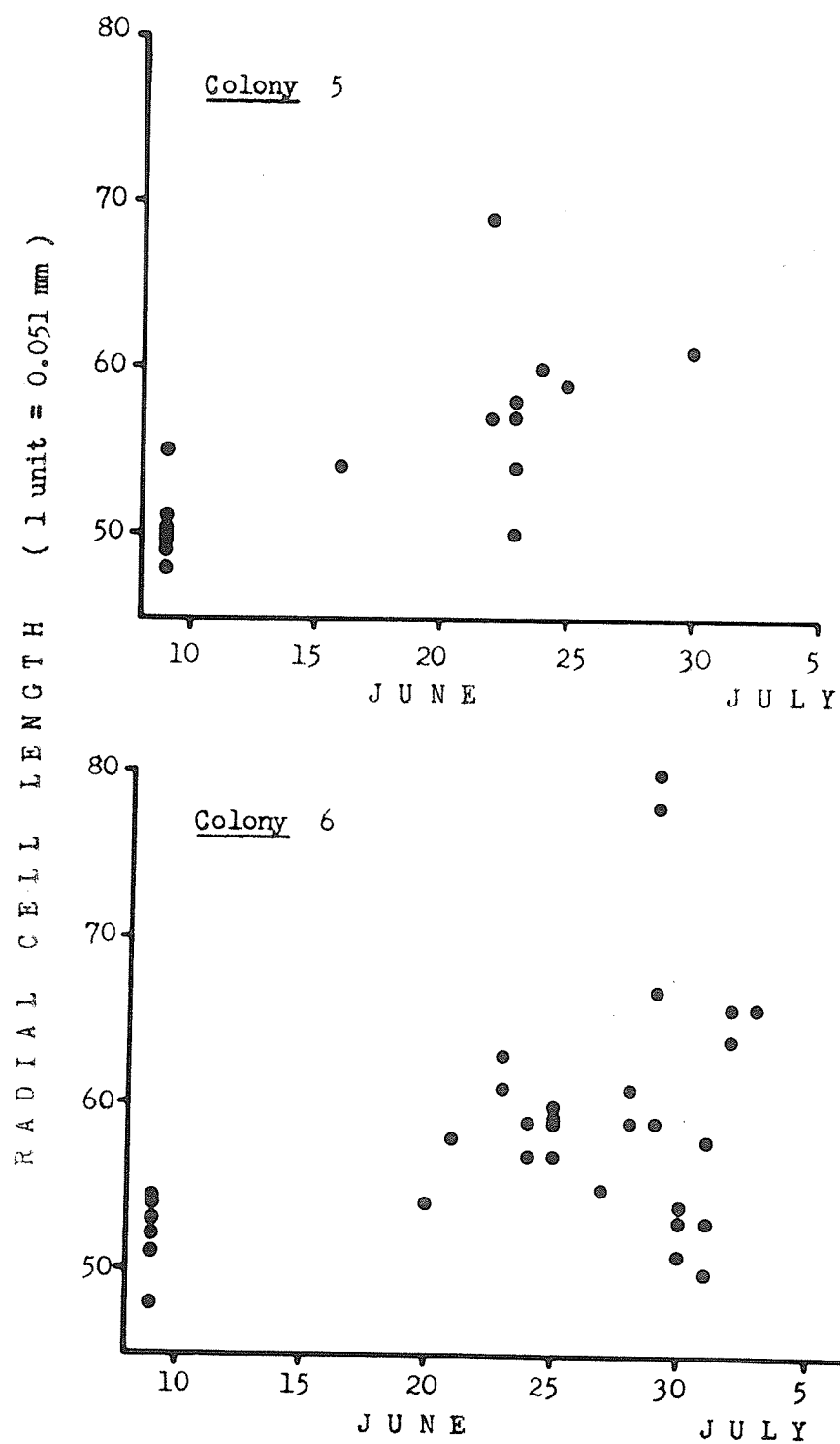


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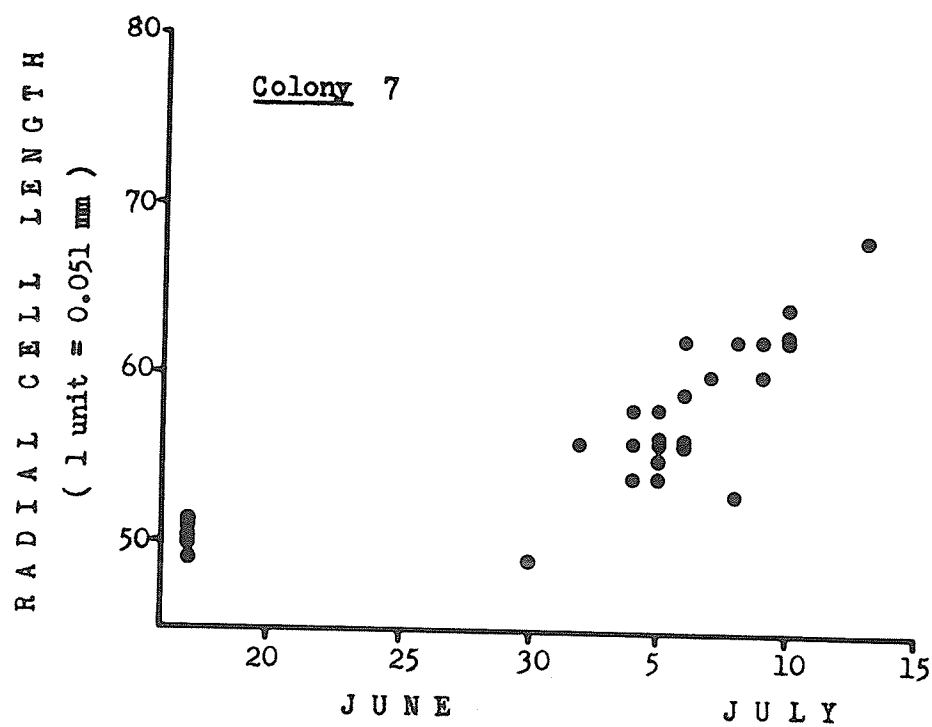


Fig. 19.- continued.

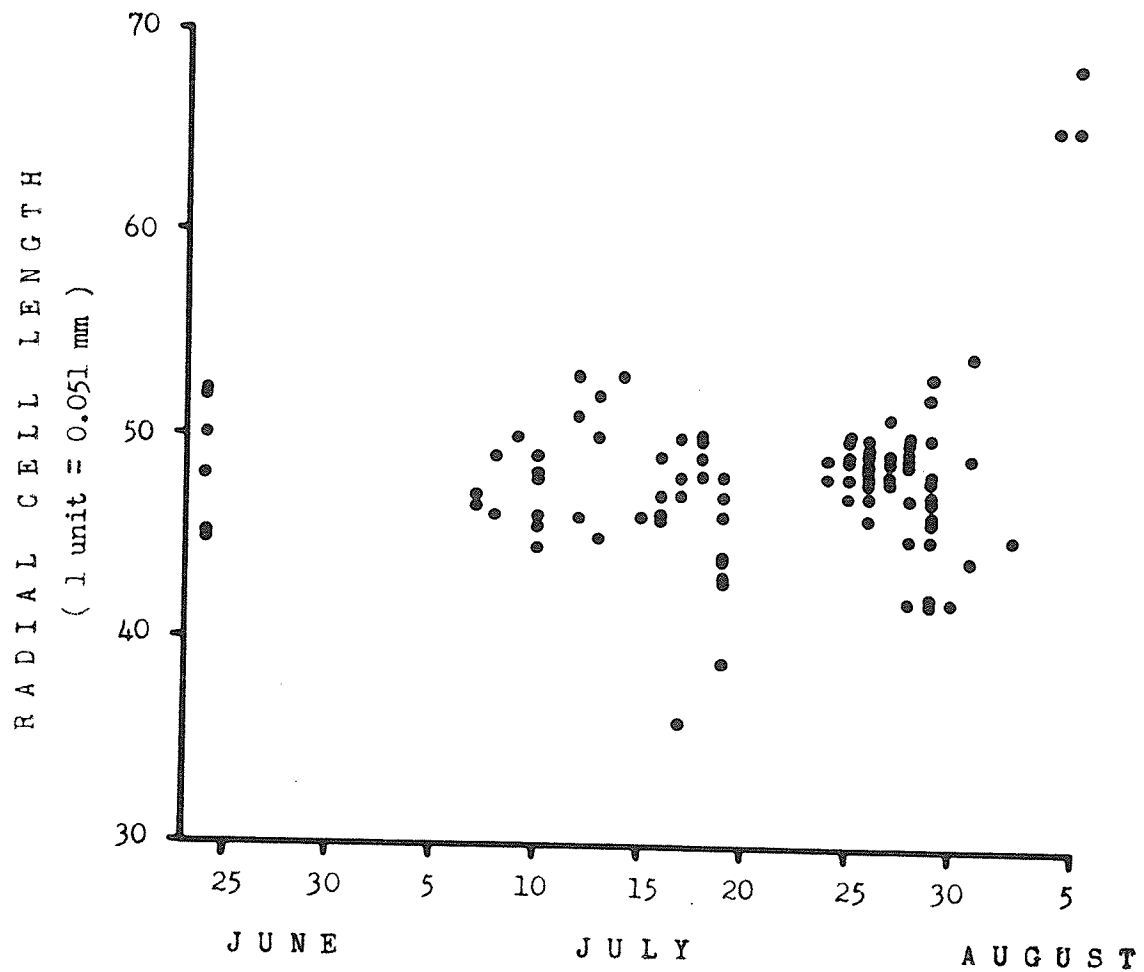


Fig. 20.- Radial cell lengths and dates of emergence of first-, second- and third-brood females in a captive colony of B. ternarius.

KNEE & MEDLER (1965) analysed their results by applying Duncan's multiple-range test to the means of wing measurements ¹ on successive weekly batches of newly emerged females, but it would seem better to work with the correlation between size and date of emergence, since in this way more degrees of freedom are available than when pairs of means are compared. I therefore designated the date on which the first worker emerged as Day 1 and computed the correlation coefficients of mean radial cell length on day of emergence for (a) all the female bees of each colony and (b) all the female bees whose radial cell lengths were less than 70 units (1 unit = 0.051 mm) for all colonies of B. terricola and B. perplexus and less than 65 units for the B. ternarius colony. In this way it was hoped to (a) include and (b) eliminate the distorting effect produced by the emergence of young queens. The correlation coefficients are given as follows:

¹ They claim to have measured the lengths of marginal cells, justifying this procedure by reference to an earlier paper by MEDLER (1962). However, in his earlier paper, Medler advocates use of radial cell measurements ! Probably "radial" cell should be read for "marginal" cell wherever the latter occurs in the later paper.

Species	Colony #	Correlation coefficient for all females		Correlation coefficient for females other than s-queens	
		r	d.f.	r	d.f.
<u>B. terricola</u>	2	-0.1105 ^{NS}	10	-0.1105 ^{NS}	10
	5	0.4816 ^{NS}	11	0.1021 ^{NS}	10
	6	0.5988 ^{NS}	8	0.5988 ^{NS}	8
	7	0.4759 ^{NS}	3	0.0843 ^{NS}	2
	9	0.5199 ^{NS}	12	-0.2582 ^{NS}	10
	10	0.2900 ^{NS}	7	-0.6703 ^{NS}	6
	11	0.7010 ^{**}	11	0.8726 [*]	5
<u>B. perplexus</u>	1	0.9191 ^{***}	10	0.9155 ^{**}	6
	2	0.6935 [*]	8	0.6935 [*]	8
	3	0.8932 ^{***}	12	0.7824 [*]	12
	4	0.5248 ^{NS}	11	0.7741 ^{**}	9
	5	0.8007 [*]	6	0.8007 [*]	6
	6	0.6128 [*]	12	0.5107 ^{NS}	11
	7	0.8366 ^{**}	9	0.8366 ^{**}	9
<u>B. ternarius</u>	1	0.3302 ^{NS}	22	0.2730 ^{NS}	20

Two colonies of B. terricola (Nos. 2 and 6) and three of B. perplexus (Nos. 2, 5 and 7) failed to rear any females with radial-cell lengths exceeding the arbitrarily chosen value (70 units) in their second brood batches.

While there is a significant positive correlation between mean radial cell length and date of emergence in all but one of the seven colonies of B. perplexus, even when s-queens are excluded from the results, in B. terricola, evidence for a progressive increase in worker size was obtained in only one colony (No. 11). In the B. ternarius colony, no female size increase occurred until s-queens were produced (Fig.20).

By comparing the variance due to deviations from regression, with that within the daily samples, it was possible to determine whether or not the regression of radial cell length on date of emergence was linear. In Colony 1 of B. perplexus (the only one thus examined) the mean-square of the deviations of the radial cell lengths from regression (queens excluded) was significantly ($p < 0.01$) greater than the variance within the daily samples, indicating a non-linear regression. Further experiments, using colonies which produce more workers than those studied here, are planned in order to investigate in more detail the functional relationship between female size and date of emergence.

These results, then, suggest that worker size generally increases (though not necessarily in a linear fashion) during the development of B. perplexus, while indicating that progressive increase is by no means the general rule in colonies of B. terricola and B. ternarius. However, none

of the colonies of B. perplexus or B. terricola produced many workers and no workers at all were reared in the third broods by either species. It is quite possible that under natural conditions, the seasonal pattern of size change might not correspond with that occurring under the highly artificial conditions imposed by this experiment, though the combs from four wild B. perplexus colonies found near Winnipeg showed an apparent gradual size increase in the dimensions of the empty cocoons from the earliest and lowest to the more recent in the upper layers of the comb.

It should be quite easy to follow the progression in size of female bees emerging in colonies which are allowed to forage naturally. However, the method used by KNEE & MEDLER (1965) in which workers were marked with paint spots and those still surviving measured at the end of colony development, suffers from the obvious disadvantage that bees which die while away from the colony cannot be measured. I developed a modification of their technique in the summer of 1965 (though shortage of time did not allow its use), in which workers were either chilled or anaesthetised by carbon dioxide so that their radial cell lengths could be measured without the necessity of removing their forewings. After measurement, each bee was marked with a paint spot to indicate on future occasions that it had already been measured. I also found it possible to measure the radial cell lengths of bees which were not

anaesthetised, though this was much more tedious since it was necessary to confine each bee in a specially constructed wire cage so that it could be rendered immobile. Nevertheless, because chilling or anaesthesia might weaken those bees subjected to such treatment, and hence exert an indirect effect upon larval nutrition, I suggest that for at least some colonies the use of chilling or anaesthesia should be avoided.

To summarise: in agreement with other investigators (see Section 1.3), a greater bimodality in the distribution of female size was found in "pollen-storing" species than in "pocket-makers"; but one species -- B. perplexus -- which, by virtue of its systematic position (Subgenus Pyrobombus D.T.), would be expected to show pollen-storing characteristics, possessed a rather weaker size-dimorphism than in other "pollen-storing" species. Moreover, captive colonies of B. perplexus generally reared progressively larger females as they developed, in contrast to most colonies of two other "pollen-storing" species -- B. terricola and B. ternarius.

3.5 The determination of imaginal size in female bumblebees

3.51 Interaction between adult and larval populations during colony development

CUMBER (1949) used an extensive method (i.e. one in which a number of colonies were taken and killed at different

stages of development) to study changes in the worker/larva ratio in bumblebee colonies. He found that in colonies which were rearing queens this ratio was higher than in those at earlier stages in their development. BRIAN (1951) failed to demonstrate any correlation between the worker/larva ratio and the size of adults during an intensive study of the colony development of B. agrorum F., but both her colonies remained small, and neither succeeded in rearing queens.

SLADEN (1912), using B. subterraneus L., and FREE (1955 a), using B. pratorum L., have both provided experimental evidence that queen production in bumblebee colonies is governed by the number of adults available per larva, by demonstrating that if sufficient workers and food are provided experimentally, even the first batch of eggs will develop into queens, whereas under normal circumstances, when the queen rears her first brood unaided, only workers are produced.

In the present work, study of the worker/larva ratio has been used to investigate the determination of size in both first and second brood females.

3.511 The size of females produced in the first broods of B. terricola and B. perplexus

Data on the radial cell lengths of first brood females of both species reared in Series III boxes in 1965 and 1966 have been given in Tables VII and XII. Significant negative

correlative coefficients for radial cell length on number of larvae per first brood were obtained for both species:

Species	r	d.f.
<u>B. terricola</u>	-0.3496 ***	123
<u>B. perplexus</u>	-0.3593 **	73

We may conclude that the fewer larvae a queen has to care for in her first-brood batch, the larger tend to be the resulting adults. Since the food available for use by the queen was not a limiting factor in the early stages of these colonies (all brooding queens were kept supplied with a surplus of pollen and honey), it must be the demand on the nursing activities of the queen which limits the size to which the larvae can grow under these experimental conditions. In nature, of course, the need for the queen to forage for her brood would impose a further constraint upon the system; it would be interesting, therefore, to compare the number and size of the first-brood workers reared in captivity with those produced by queens in the wild state.

Queens were frequently reared in first broods of B. terricola and occasionally of other species. As one might expect, queens usually appeared in boxes where the number of first-brood larvae was small, but in a few instances B. terricola queens reared a single queen in addition to a normal complement of first-brood workers. In each case the solitary queen remained as a larva for a few days after her

sisters had pupated, during which time her phase of maximum growth took place. This situation is easy to understand in terms of the concentration of nursing attention which the mother queen would be able to give to a single larva remaining after all the worker larvae had pupated, but since it will be shown later (Section 3.523) that presumptive queen larvae of B. terricola are probably already determined by the time they reach the fourth instar, we still have to explain how these first-brood larvae succeed in becoming queen differentiated in the presence of presumptive worker larvae.

Possibly these queen larvae result from eggs laid subsequently to those which give rise to the worker complement of the first brood. If this were so, the later-hatched larvae might benefit from more liberal nutritive environment than would have been experienced by their older sisters at a comparable stage of development. Though bumblebee queens of most species do not normally oviposit during the time interval between completion of the first-brood egg cells and the formation of first-brood cocoons, SLADEN (1912) found that in B. subterraneus L. (= B. latreillelulus Kirby) an additional batch of eggs was commonly deposited alongside the first brood.

An alternative explanation, adopting the premise that presumptive queens undergo a longer larval stadium than presumptive workers (see Section 3.521), would be that due to

random inequality in the sharing of available food, one larva in the first-brood batch could feed so voraciously as to enable it to become queen-determined, even though all the larvae were approximately of the same age.

The problems thus raised are clearly similar to those posed by the occurrence of mixed clumps of queen and worker larvae in later brood batches, and will be discussed further below (Section 3.522).

3.512 The size of females produced in second broods of
B. terricola, B. perplexus and B. ternarius

Data on the number of bees in the first- and second-brood batches of B. terricola, B. perplexus and B. ternarius colonies, reared and allowed to develop in captivity with unlimited food supply, are given in Table XIX. The radial cell lengths of all the second-brood females were measured in each of the colonies of the first two species (Tables XVI and XVII), but in most B. ternarius colonies, records were kept only of the number of queens and workers in each batch, without any attempt to measure their size.

Correlation coefficients relating the number of first-brood workers (x_1), the number of second-brood bees (x_2), and the size of the second-brood females (x_3) were calculated for the colonies of B. terricola and B. perplexus:

Species	$r_{x_1x_2}$	$r_{x_2x_3}$	$r_{x_1x_3}$	d.f.	$r_{x_1x_3 \cdot x_2}$	d.f.
<u>B. perplexus</u>	0.5197***	0.1455	0.1843	141	0.3637***	140
<u>B. terricola</u>	0.2505*	0.02964	0.2007*	157	0.2064*	156

NUMBER, SEX AND CASTE OF BEES IN FIRST AND SECOND BROODS
 OF CAPTIVE COLONIES STARTED BY NATURALLY OVERWINTERED
 QUEENS OF B. terricola, B. perplexus AND B. ternarius
 DURING 1966

Species	No. of colony	No. of bees in first brood	No. of bees in second brood batches		
			queens ¹	workers	males
<u>B. perplexus</u>	1	8	11	12	11
	2	6		16	32
	3	6	7	8	4
	4	7	12	18	1
	5	9		8	35
	6	6	4	24	3
	7	5		23	
<u>B. terricola</u>	2	5		32	
	3	5	1		14
	5	8	5	22	
	6	9		26	7
	7	4	2	5	2
	9	2	5	21	
	10	7	4	18	
	11	6	9	9	
<u>B. ternarius</u>	1	7		95	
	2	8		82	
	3	6	3	32	4
	4	8	1	47	
	5	8		54	
	6	3		32	
	7	5	1	33	5
	8	4		61	
	9	10	2	51	9
	10	6	1	45	1
	11	8	3	40	8

¹ All females of B. terricola and B. perplexus with radial cell lengths of 70 units (1 unit = 0.051 mm) or over are classed as queens in this table.

For B. ternarius a more insensitive test had to be used, since the bees were not individually measured. Correlation coefficients relating the number of first-brood workers (x_1), the number of second-brood bees (x_2), and the number of queens reared in the second-brood batches (x_3) were calculated:

$r_{x_1x_2} = 0.4042$	d.f. 9
$r_{x_2x_3} = -0.3985$	9
$r_{x_1x_3} = 0.3260$	9
$r_{x_1x_3 \cdot x_2} = 0.7286 *$	8
$= p < 0.02$	

Thus, in all three species there is evidence that (under the experimental conditions used here) the size of the female produced in the second-brood batches is positively correlated with the number of first-brood workers and negatively correlated with the number of bees in the second-brood batches.

In some of the B. terricola colonies, due to the separation of the fourth instar larvae, which obscures their relationship with others of the same egg batch, it was difficult to be certain of the precise number of second-brood bees, even with the help of photographs. (This difficulty was also experienced by CUMBER 1949, who made an error, however, in supposing that it applied to all pollen-storing bumblebee species; for in B. lapidarius L., the arrangement of the brood batches shows up quite well in the cocoon

clusters.) In cases of doubt, the most "unfavourable" of the possible values for the number of second-brood bees was taken, so the true value of the partial correlation coefficient ($r_{x_1x_3.x_2}$) is probably greater than that quoted.

The relationship between the numbers of first-brood bees (x_1) and the second-brood bees (x_2) is to some extent a measure of changes in the egg-laying rate of the queens. Thus, the significant positive correlation coefficients of x_1 on x_2 obtained for all three species are in agreement with the expectation of an exponential increase in egg-laying rate during the early part of colony development (see Section 3.513). However, pre-imaginal mortality ensures that the number of adult bees emerging in a brood batch is not a reliable measure of the number of eggs laid in the cells from which the batch resulted. Though eating of third-brood eggs by workers of B. terricola and B. perplexus was frequently observed, egg-eating by workers probably could not contribute to the second-brood mortality, since by the time the earliest first-brood workers emerge from their cocoons, all, or almost all, of the second-brood eggs have hatched. Larval mortality due to ejection of larvae, on the other hand, certainly occurred in several colonies of both B. terricola and B. perplexus and in one colony of B. ternarius. Though accurate counts were not made of the

numbers of larvae thrown out by all colonies, it is interesting that in B. perplexus, Colony 3, which reared 19 adult second-brood bees, ejected at least seven second-brood larvae, while in Colony 2 (48 adult second-brood bees) no second-brood larvae were ejected. BRIAN (1951) recorded considerable brood mortality in B. agrorum, chiefly in the egg or early larval stage, which she attributed to cannibalism. In my colonies, if such early mortality occurred, it was undetected; very young larvae were seldom ejected by any species - most were thrown out in the late third or early fourth instar.

Ejection of larvae may help to explain why the numbers of second-brood bees were so much fewer in B. terricola and B. perplexus colonies than in those of B. ternarius, though it seems that queens of the latter species lay more prolifically than those of B. terricola and B. perplexus, at least under the experimental conditions obtaining here.

Ejection of larvae invariably occurred in B. perplexus and B. terricola when colonies were accidentally allowed to run short of pollen (5 instances in B. perplexus and 4 in B. terricola) but in the present experiment pollen-shortage was never permitted to occur. In many cases, especially in B. perplexus, larvae which were ejected were observed on the previous day to be incompletely covered by wax, so it is possible that shortage of wax may bring about the ejection of larvae.

Since all the colonies used in the present experiment were kept liberally supplied with food and their workers not permitted to forage, one might expect that the division of labour among the workers would be heavily biassed in favour of a predominance of nurse-bees and hence that queens would be produced earlier in colony development than would be the case in wild colonies. This was probably so, at least in B. terricola and B. perplexus, but since no controlled experiments were conducted on colonies with restricted food supply and very little information was available as to how many workers are reared in wild colonies of these species before the changeover to queen-rearing occurs, the matter cannot be conclusively settled at the present time.

The results described above provide grounds for the hope that it will soon be possible, by using colonies allowed to develop under controlled conditions, to arrive at a mathematical model by which the onset of queen production may be related to the egg-laying rate of the queen, colony-intake, brood mortality, etc. However, any technique which seeks to estimate the worker/larva ratio in terms of the number of bees emerging from successive brood batches suffers from the disadvantage that it fails to take into account the continuous nature of colony development in bumblebees. For though there is a pause in egg-laying between the

completion of the first-brood egg-cells and the spinning of the first-brood cocoons, egg-laying proceeds more or less continuously thereafter; thus the second brood normally consists of at least two groups of egg cells (often four groups in captive B. ternarius colonies) located at opposite sides of the first-brood cocoon clump. The queen requires several days to complete her second-brood egg-cells, so that, while the larvae hatching from the earliest-laid second-brood eggs are fed by the queen alone during their early development, those hatching later are cared for by the first-brood workers. Similarly, the earliest second-brood adults usually emerge before the last second-brood larvae have ceased to feed. Thus, (a) the influence of the first brood is not distributed evenly among the second brood, and (b) the second brood may be partially capable of influencing its own differentiation.

No doubt some gain in precision would be made by subdividing the second brood into groups corresponding to the several batches of egg cells of which it is comprised, but even this procedure would fail to take into consideration the temporal differences in development between the earliest and latest members of each batch. Such differences may amount to several days, especially in B. ternarius, whose queens often construct up to six egg-cells at one location (in one colony - No. 54 - eight cells comprised one of the two second-brood batches).

The most accurate method for estimating the worker/larva ratio is by taking actual counts of the numbers of adult workers and larvae present at successive points in time during colony development, as was done for B. agrorum by BRIAN (1951). I took such counts for 6 colonies of B. terricola and 5 of B. ternarius over a two week period during which the changeover to queen production occurred, but could find no satisfactory way by which the production of the first queen larvae could be related to changes in the worker to larva ratio. It may not be out of place, therefore, to consider the extent to which the worker/larva ratio constitutes a satisfactory measure of the availability of larval food.

It would seem that the capacity for food intake by the brood in a bumblebee colony should be related not only to the number, but also to the size of the larvae present - larger larvae being capable of greater food consumption than smaller ones; this is confirmed by the generally exponential pattern of the area increase of larval clumps of B. ternarius during the early part of their development (see Section 3.522). Presumably, a female larva at a stage earlier than that at which its caste becomes determined is capable of some maximum rate of food consumption which, if fulfilled, permits it to become queen-determined; but larvae which are past the point at which queen determination is possible will not necessarily show a capacity for food

consumption related to their size. The most extreme disparity would be expected to occur among larvae which have reached the size at which (a) those destined to become workers cease feeding in preparation for pupation, and (b) those which are queen-determined continue to display a capacity for food intake proportional to their size. However, such error due to the presence of larvae of both castes could not arise until after the first queens become differentiated.

The age, and hence the size and capacity for food intake, of the larvae present in a colony at any particular time must be dependent upon the temporal pattern of oviposition over an earlier time interval. The number of adult workers depends upon the oviposition rate at a still earlier time. Assuming that other factors (colony food-intake, nursing behaviour and brood mortality) can be accounted for, it should be possible to relate the two variables (adult population and capacity of brood for food-intake) to a "single" one - the form of the temporal pattern of oviposition.

To summarise: the size (as measured by the length of the radial cell of the forewing) of first-brood females from captive colonies of B. terricola and B. perplexus was negatively correlated with their number, presumably owing to competition for food supplied by the queen. The size of second-brood females in these species and the number of

second-brood queens produced per colony in B. ternarius was significantly correlated with the ratio of number of first-brood workers to number of second-brood bees, thus providing statistical confirmation that the worker/larva ratio is a factor in determining caste. However, since colony development in bumblebees is a continuous process, the worker/larva ratio at any time is only approximately described by comparing numbers of bees in successive broods. An attempt to express the worker/larva ratio as a continuous function of the queen's egg-laying rate is described in the following section.

3.513 The effect of the queen's oviposition rate upon the worker/larva ratio

Of the two factors which have been suggested to explain increase of the worker/larva ratio (and hence the onset of queen production) during colony development - increased brood mortality and decrease in the queen's egg-laying rate - only the latter was investigated during the present study. B. ternarius was chosen for this purpose, since (a) queens of this species are exceptionally prolific in contrast to B. terricola and B. perplexus, (b) egg-eating was never observed in any B. ternarius colony in captivity, and (c) once the first brood has emerged, larval mortality appears to be very low.

Five colonies of B. ternarius were selected from those started by naturally overwintered queens in Series II boxes and were kept in darkness at 29°C with abundant honey and pollen. A record was kept of the number and position of all new egg-clumps and of the date on which workers emerged from each batch of cocoons. To facilitate this as the colonies grew populous, daily photographs were taken of each colony with a Polaroid-Land camera; larval clumps and pupae were identified by labelling the photographs with a ball-point pen (see Fig. 21). At the end of colony development, the comb was carefully taken apart so that the accuracy of the photographic records could be checked by counting the number of cocoons resulting from each larval batch.

Though the number of eggs in each cell was not counted, the number of cocoons formed from each larval clump was taken to be closely related to the number of eggs laid in each batch of egg cells - i.e., a constant mortality rate was assumed. Cumulative frequency diagrams of the number of eggs laid which resulted in pupae in each colony are given in Fig. 22. It should be noted that in all colonies the queens were still laying when the experiment had to be discontinued, though in all but one colony (No. 28) the oviposition rate had begun to fall off by this time. Most of the cumulative frequency curves in Fig. 22 have a generally logistic form, indicating that the queen's egg-laying rate builds up to a



Fig. 21.- Successive daily photographs of the comb of a *B. terrestris* colony, showing the system of labelling used in order to follow colony development.

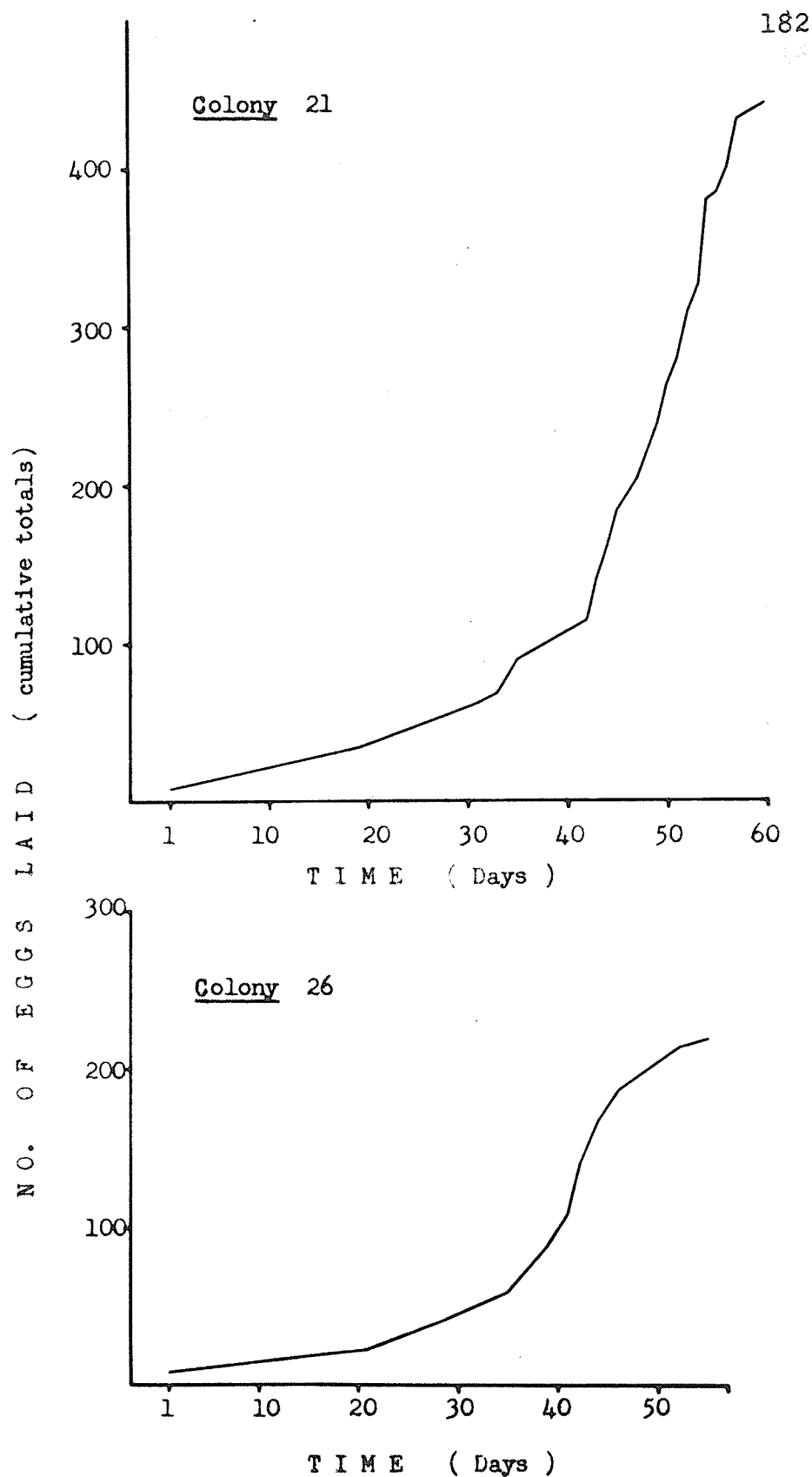


Fig. 22.- Cumulative totals of numbers of adult-producing eggs laid during the first sixty days of development of five B. ternarius colonies.

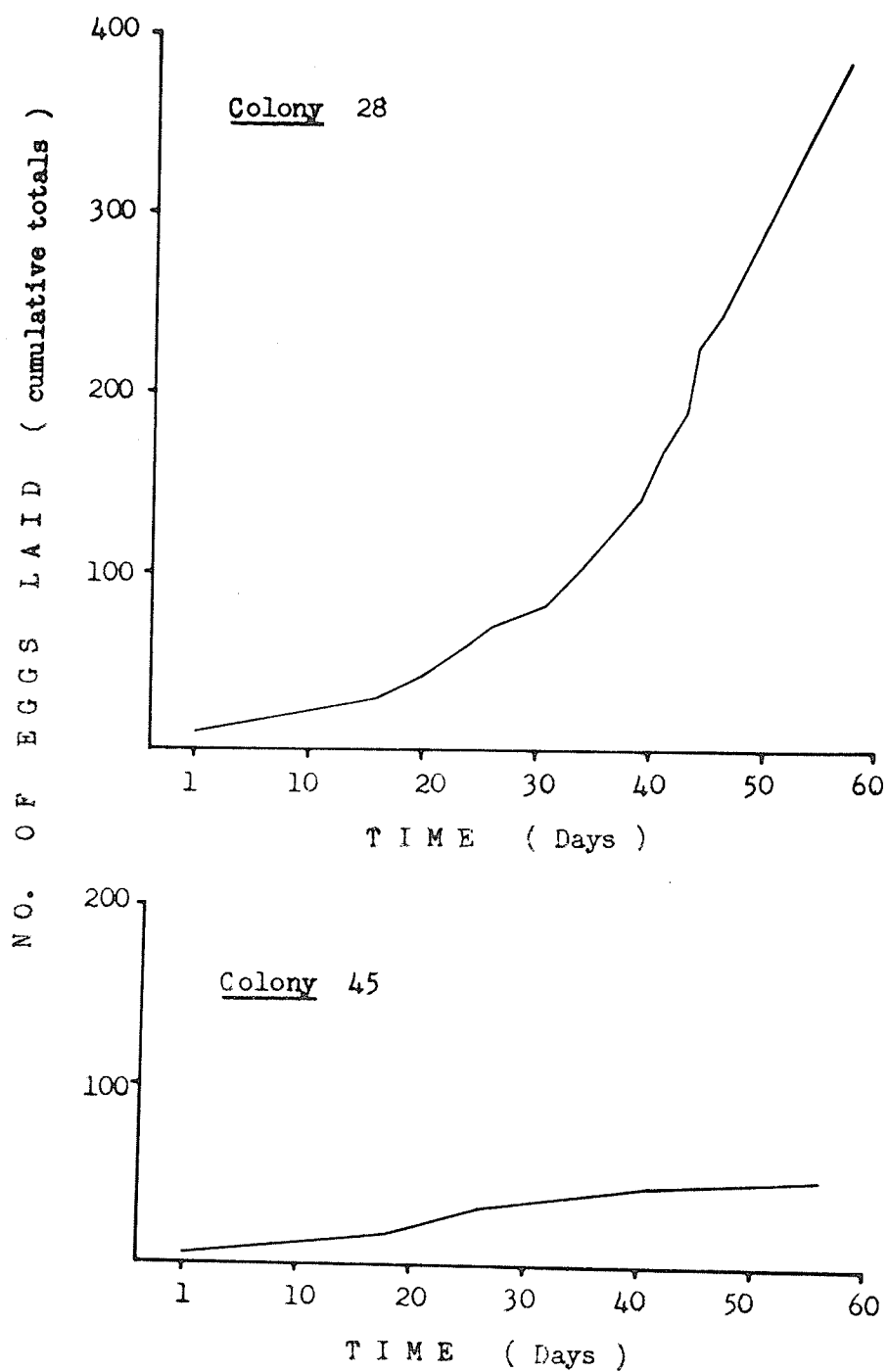


Fig. 22.- continued

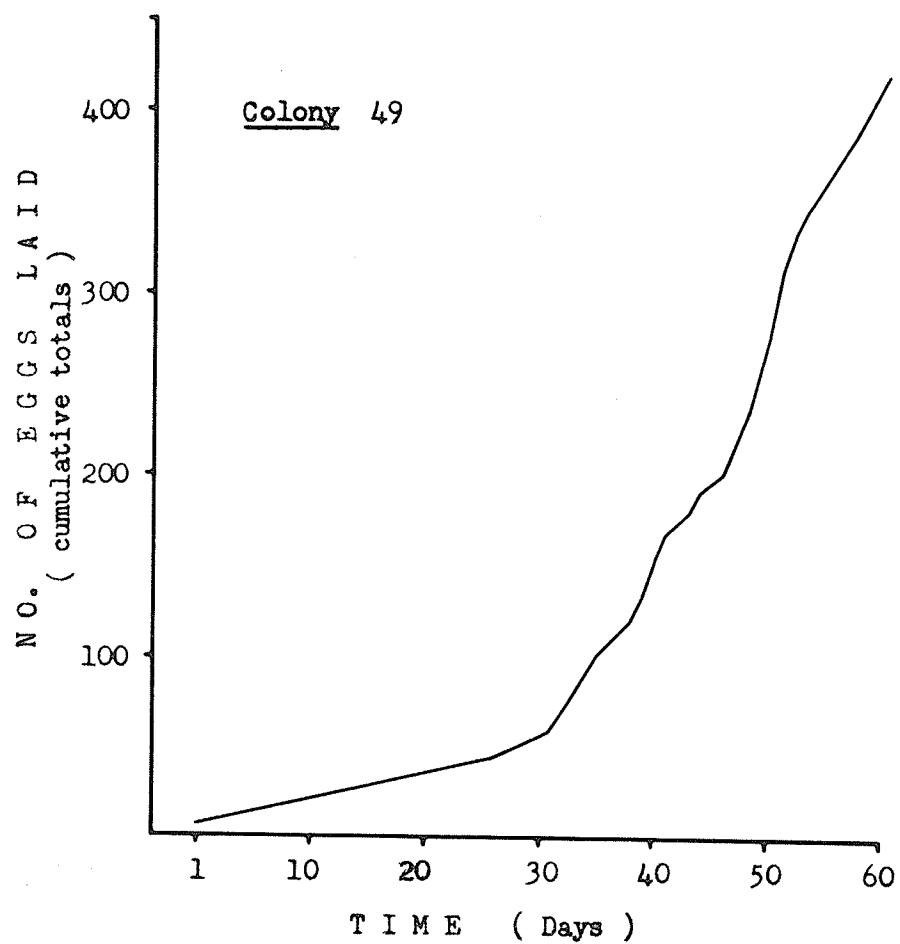


Fig. 22.- continued

maximum and then gradually falls off, as found (using extensive methods) by CUMBER (1949).

Up to the point of inflection, the logistic curve is approximately exponential in form so that $\log y = a + bt$ is approximately linear. Fig 23 reproduces the data of Fig. 22 using the logarithmic values of the egg numbers; as expected, the relationship between the variables is satisfactorily linear over the earlier part of the range. The coefficients of linear regression for \log_{10} number of eggs on time were calculated for the first 19 points in Colony 21, the first 8 in Colony 26, the first 12 in Colony 28, the first 3 in Colony 45, and the first 9 in Colony 49. The following values of a and b in the equation

$$\log_{10} y = a + bt$$

(where y = the cumulative total of adult-producing eggs and t = the time in days since the first eggs were laid) were obtained for each colony:

Colony no.	a	b
21	0.7991	0.0322
26	0.8008	0.0301
28	0.9365	0.0310
45	0.7430	0.0281
49	0.7678	0.3370

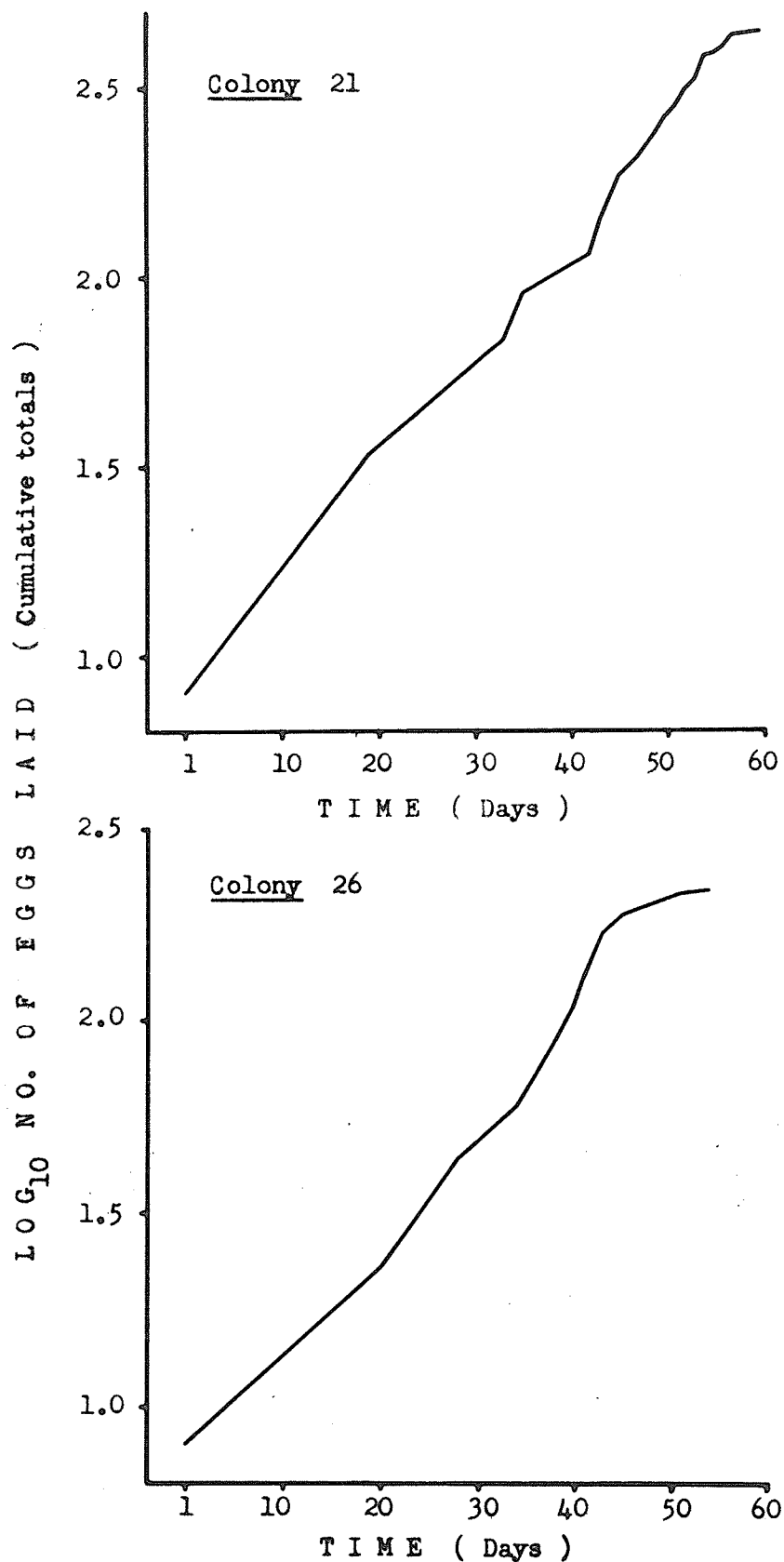


Fig. 23.- Logarithms of the cumulative totals of numbers of adult-producing eggs given in Fig. 22.

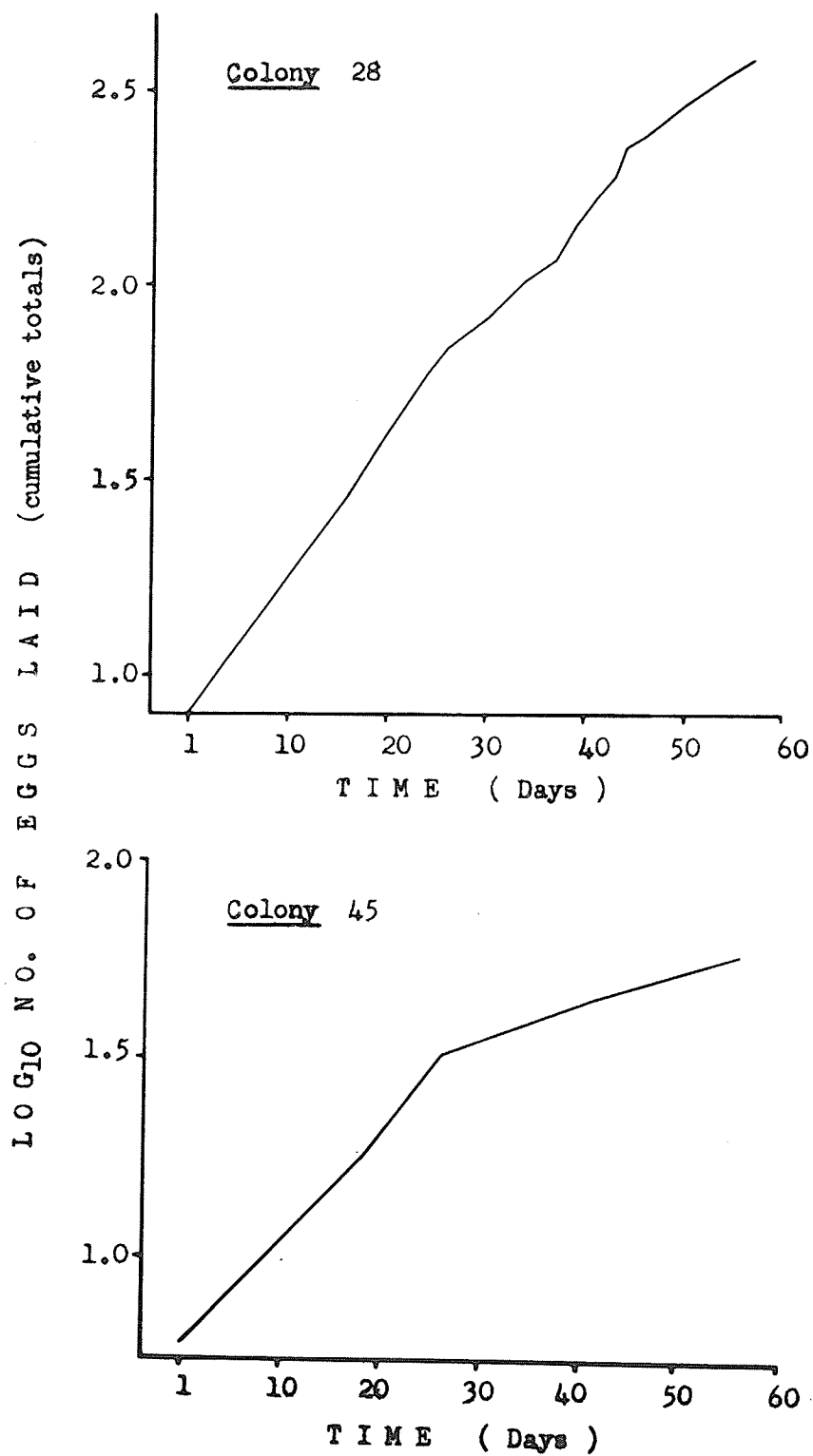


Fig. 23.- continued

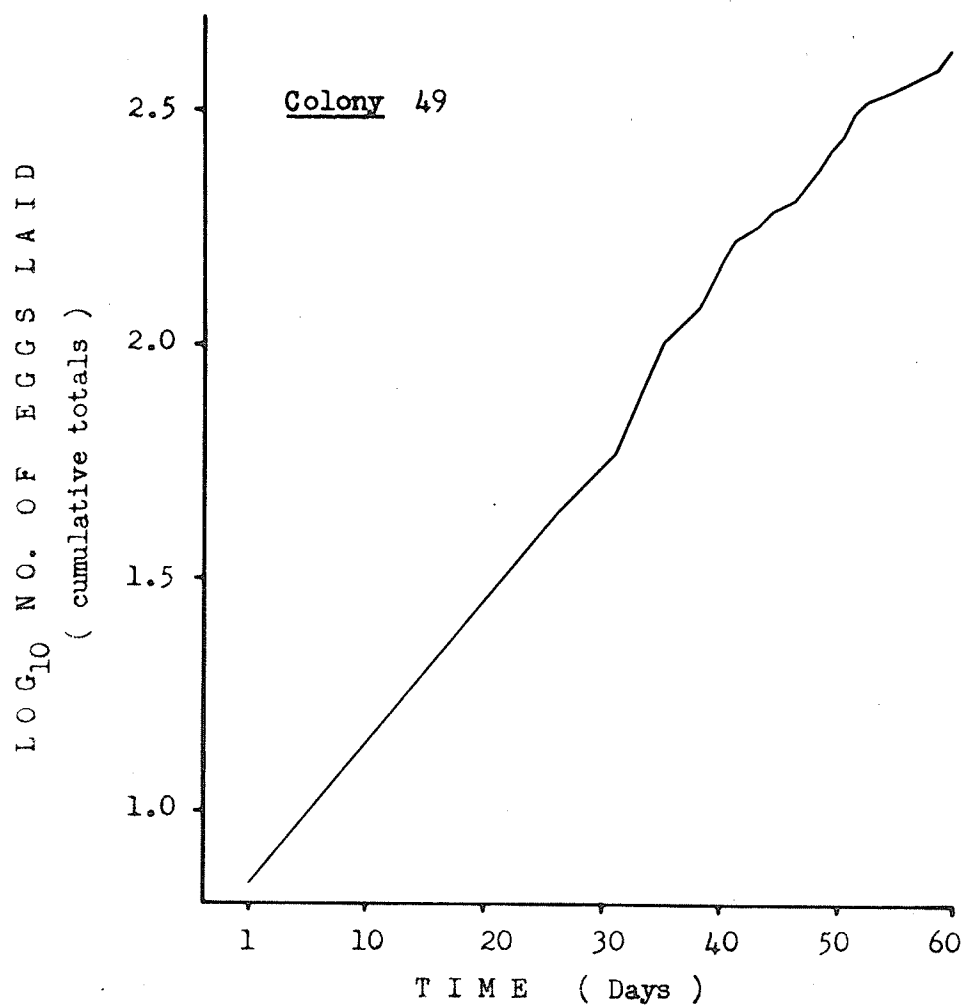


Fig. 23.- continued

from which the equations appropriate to the lower parts of the curves in Fig. 22 can be obtained as:

$$y = 10^a (10^b)^t$$

Since bumblebee colonies do not normally produce any more workers once they have embarked upon queen production (FREE & BUTLER 1959) we may take the total number of workers produced by a colony as being related to the duration of its development elapsing before changeover to queen production. The total number of workers reared by each of the five B. ternarius colonies was:

No. of colony	Total no. of workers
21	221
26	57
28	134
45	49
49	234

The total number of workers produced by each colony is significantly positively correlated ($p < 0.05$) with the value of the constant b , thus providing further confirmation that the changeover from worker to queen production is determined by the egg-laying rate of the queen. However, it should be re-emphasised that the method used here to assess the oviposition rate takes no account of brood mortality.

CUMBER (1949), BRIAN (1951) and FREE (1955 a) all discuss the relation of oviposition rate to change in the worker/larva ratio. On the basis of data obtained by using an extensive (see Section 3.51) method on colonies of B. agrorum, Cumber concluded that the egg-laying cycle of the queen would account for an increasing worker/larva ratio towards the time of queen production, but since he found that about twice as many eggs were laid as developed into adults, he concluded that the egg-laying cycle was probably irrelevant and that the change in ratio was brought about by egg destruction effected by the workers. Cumber considered that the rate of such egg destruction must increase suddenly, and tentatively suggested that this might be triggered by the laying of haploid eggs.

Brian, who found no egg-eating by the workers of her two colonies of B. agrorum, pointed out that there was no need to postulate an increase in the proportion of eggs eaten, since a steady rate of egg destruction, in conjunction with the queen's normal egg laying cycle, would be sufficient to produce the change in ratio.

It is surprising that none of these authors discussed the mathematical implications of the various possible types of oviposition curves. Free suggested that, by analogy with other population studies, it is likely that the egg laying cycle of queen bumblebees assumes the form of a

sigmoid curve. He notes that a curve of this form would account for the sharp difference which Cumber observed in the larva/worker ratio between bumblebee colonies which were and were not producing queens. However, he does not indicate at which point on the curve the ratio will start to change.

It must be noted that the relationship of the oviposition curve to changes in the worker/larva ratio will be modified by the rate of adult mortality and that the operative effect of the worker/larva ratio on larval nutrition will in turn be modified by the pattern of division of labour among the adult workers. In the absence of evidence to the contrary, I shall assume that adult mortality is insignificant (or, if significant, that the mortality rate shows no proportional increase over the relevant period of colony development) and that the proportion of adult bees which take up nursing duties remains constant. At least the first of these assumptions seems justified in the case of my captive B. ternarius colonies, for very few bees had died in any of them by the time that the first queens were produced.

We must also distinguish between curves which describe the oviposition rate of the queen and those which represent the cumulative total of eggs laid. The diagram given by CUMBER (1949: Fig. 9) is of the former type, whereas the sigmoid curve mentioned by FREE (1955 a) must presumably (by analogy, as he says, with the results from other

population studies) describe the cumulative total of eggs laid. In any case, since the former curve merely describes the slope of the latter, it can be obtained by differentiating the appropriate function. Since it is mathematically simpler to derive the worker/larva ratio from a cumulative egg-total curve, the following discussion is confined to a consideration of this type.

Given a functional relationship between the cumulative total of adult-producing eggs laid (y) and time (t):

$$y = f(t)$$

the worker/larva ratio can be derived in the following way. Assuming insignificant imaginal mortality, the number of workers present in the colony at any time (t_n) - at least during the earlier part of colony development (during which no male eggs are laid) - is given by the value of y at an earlier time ($t_n - d$) such that d = the duration of pre-imaginal development, which may provisionally be assumed to be constant. The number of larvae present at t_n is given by the difference between the values of y at ($t - e$) and ($t - e - l$) where e = the duration of the egg stage and l = the duration of the larval stage, both of which are assumed to be constant.

Thus the worker/larva ratio (R) at t_n is expressed as follows:

$$R = \frac{f(t_n - d)}{f(t_n - e) - f(t_n - e - l)}$$

constant, resulting in a linear rate of increase of the worker/larva ratio; but before the point of inflexion is reached, the oviposition rate increases, hence the worker/larva ratio must increase less rapidly than at the point of inflexion or, if the rate of increase of the oviposition rate is sufficiently great, not at all. Beyond the point of inflexion the oviposition rate decreases, resulting in a more rapid rate of increase of the worker/larva ratio since, while the number of adult workers converges upon a maximum value, the number of larvae present at any one time decreases. Thus we may infer that the worker/larva ratio increases slowly, if at all, during the early part of colony development, but as time progresses the rate of increase of the ratio accelerates.

Such a pattern of increase in the rate of change of the worker/larva ratio would satisfactorily account for the continuous size increase observed in females of pocket-making species, without the need to hypothesise that egg-destruction is required as an additional factor. Obviously, however, if brood mortality should increase during colony development, the worker/larva ratio would be even further increased and the production of bees of intermediate size more completely suppressed. Nor is it necessary to postulate

If the oviposition rate remains constant, the functional relationship between the cumulative total of eggs and time is linear in form:

$$y = a + bt$$

so that we may write

$$R = \frac{a + b(t_n - d)}{[a + b(t_n - e)] - [a + b(t_n - e - 1)]}$$

$$\text{therefore } R = \frac{a + b(t_n - d)}{b(t_n - e) - b(t_n - e - 1)}$$

$$\text{therefore } R = \frac{a}{b(t_n - e) - b(t_n - e - 1)} + \frac{(t_n - d)}{(t_n - e) - (t_n - e - 1)}$$

$$\text{therefore } R = \frac{a}{bl} + \frac{t_n - d}{1}$$

Since a , b , l and d are constants, R varies directly as t , i.e. if the oviposition rate remains constant, a linear increase in the worker/larva ratio occurs with respect to time. Thus we may conclude that provided adult mortality is insignificant, it is not necessary that the queen's oviposition rate should decrease in order that an increase in the worker/larva ratio shall occur - a steady oviposition rate produces this effect.

The curves in Fig. 22 suggest the logistic function (or some other function which describes an s-shaped curve) as an appropriate mathematical model for the relationship between the cumulative egg-total and time. Around the point of inflexion the oviposition rate must become approximately

the existence of a developmental switching mechanism which could segregate female larvae into one or other morphogenetic pathway.

But in pollen-storing species, such as B. ternarius, though the data discussed above show that the worker-larva ratio increases, there is no evidence that the increase is sufficiently sudden that it could account for the rarity of female bees of intermediate size which is characteristic of the species (see Section 3.41). As mentioned earlier (Section 3.2) the changeover from worker to queen production in B. terricola and B. ternarius is normally marked by the occurrence of cocoon clumps containing both s-queens and s-workers. Though an increased worker/larva ratio probably provides the conditions under which such mixed clumps can occur, some other factor(s) must surely be responsible for the striking dimorphism in the cocoon sizes within them. Attempts to identify these factors are described in the next section.

3.52 Characteristics of larval development

To find out how the availability of larval food could influence the course of development of female larvae in pollen-storing species so as to result in the production of two clearly differentiated size groups, three aspects of development were investigated - (a) stadial duration, (b) rate of larval growth and (c) prepupal size.

3.521 The length of larval and pupal stadia in *B. terricola*
and *B. ternarius*

Using a Polaroid-Land camera, daily photographs were taken of the combs of 6 colonies of *B. ternarius* and 6 of *B. terricola* during part of their development. Each egg-batch, larval clump, fourth instar larva and cocoon was given a code number so that its progress could be followed by inspection of the photographs which were labelled immediately after completion (see Fig. 21).

Of these colonies, 2 *B. ternarius* and all 6 *B. terricola* changed over from worker to queen production during the 16 day period over which the daily photographs were taken. Following this period the colonies were examined from time to time to determine the sex and caste of bees emerging from "mixed" clumps.

Altogether, 3 mixed clumps yielding queens and workers of *B. ternarius* and 6 of *B. terricola* passed through enough of their development during the 16 day photographic period to allow the lengths of either the larval or the cocoon period of their occupants to be estimated.

Since all that was required was comparative information for the two castes, no attempt was made to determine the precise point in time when the larval stadium began or ended. The first day of the larval stadium was taken as that on which some or all of the egg cells in the batch were observed

to have swelled, and the first day of the cocoon period was designated as that on which, after feeding had ceased, the change in shape from oblate-spheroid to ellipsoid was completed (even though the pupal ecdysis does not take place for some time after this). Both of these events were easy to recognise from the photographs - the former because the new wax which is added to cover the newly hatched larvae is of a rougher texture and lighter in colour than that of which cells containing unhatched eggs are composed, and the latter because the adult bees remove all wax from the cocoon as soon as it has changed shape, resulting in a pronounced lightening in colour which shows up well even on black and white photographs. Furthermore, in B. ternarius, the faecal smears in the walls of the cocoon become very apparent following the removal of the wax.

In some cases, usually because the cocoon was located on the side of the cocoon clump and hence partially obscured from view, it was difficult to decide with certainty whether or not a larva had ceased feeding. Such individuals were ignored, as also those (mostly in B. ternarius) which were entirely hidden beneath the brood clump.

Table XX gives the values for the larval periods of both species, and in Table XXI the durations of cocoon periods are given for B. terricola. (Though data on the lengths of cocoon periods of B. ternarius show that the queens spend about 2 - 3

TABLE XX

THE DURATIONS OF THE LARVAL STADIA OF FEMALES FROM
MIXED CLUMPS OF B. terricola AND B. ternarius

Species	Colony & clump	Duration of larval stadium (days)	
		workers	queens
<u>B. terricola</u>	60 F	7 7 7 7	9 9 8 10 8 8 10
	62 G	10 7 8 8 8 7 8 9	10 11
	13 H	9 7 8	10 10 11 9 10 10 9 9 9
	I	8 8	11 9 12 12
<u>B. ternarius</u>	26 D	7 7 8 7 7 7 7 7 8	11
	F	6 8 7 8 7 7	8
	28 H	7 7 7 7 7 7 7 7 7	8 10 10 10

TABLE XXI

THE DURATIONS OF THE COCOON STAGE IN FEMALES
FROM MIXED CLUMPS OF A B. terricola COLONY (NO. 33)

Clump	Duration of cocoon stage (days)	
	workers	queens
D	10 10 9 9 9	12 12
E	9	12 12 12 12 12 11

days longer as pupae before emerging than do workers, the bees concerned were not from the same cocoon clumps, so I have not included these results in Table XXI. In each case, the queens spent significantly longer ($P < 0.001$) in the relevant development stage than workers in the same brood batches, in contrast to the situation found in honeybees.

It must be stressed that the figures given in Table XX for larval development of the bees in the same brood clumps differ only insofar as some larvae ceased feeding earlier than others. No account has been taken of the differences in time of hatching from the egg. Thus it is quite possible that the longer larval development apparently shown by the queens is merely a result of the fact that the larvae which subsequently developed into queens hatched later than those destined to be workers and that no real difference in duration of larval development exists; or that a real difference does exist but is not so great as would appear from Table XX. However, this ambiguity is not important here, since the argument which will be developed in Sections 3.522 and 3.533 is concerned mainly with the time at which larvae cease feeding, irrespective of when they hatched from the egg.

It may be noted that FRISON (1928) gave detailed information on the development of 4 B. bimaculatus colonies from which he concluded that the larval and pupal stadia of queens were of longer duration than those of workers or males,

though he did not subject his data to statistical analysis.

Table XXII gives the lengths of larval stadia (in hours) for two of Frison's colonies, from which the following analysis of variance was obtained:

Source	S.o.S.	d.f.	EV	F
Total	124,570	57		
Colonies	104	1	104	
Castes	38,957	2	19,478	1.34 ^{NS}
Colonies x castes	29,044	2	14,522	13.37 ***
Residual	56,465	52	1,086	

LSD₉₉ =

LSD_{1%} =

Thus, a highly significant interaction between colonies and castes was obtained, the meaning of which is evident by inspection of the mean stadia lengths for the three castes of the two colonies:

Caste	Queens	Workers	Males
Colony 5	332.5	236.0	215.4
11	280.7	256.7	265.3

The mean stadia length for males in Colony 5 was shorter than that of the workers, whereas in Colony 11 the reverse was true. Significant differences between the means are shown in Fig. 24.

Thus, though the mean duration of the larval stadia of queens in Colony 5 significantly exceeds that of both males

THE DURATIONS OF THE LARVAL STADIA OF QUEENS, WORKERS
AND MALES OF B. bimaculatus GRESSON ¹

Colony	Clump	Length of larval stadium (hours)			
		workers		queens	males
5	G	218	218	241	241
	J			359	391
	L	262			
	N			311	311 344
				370	381
	O			286	286 286
	P				199 199 199 199
	Q				249 249
	R				214
11	F	240			240 240
	G	240			
	L				311 311 311
	M	290		290 290	312 312
	N				190
	O			240	216
	R			312	240 240 312
	S			290	263
	T			315 289	289
	U			289 264 289 264	264
	V			289 291	264
	X				243

¹ from FRISON (1928)

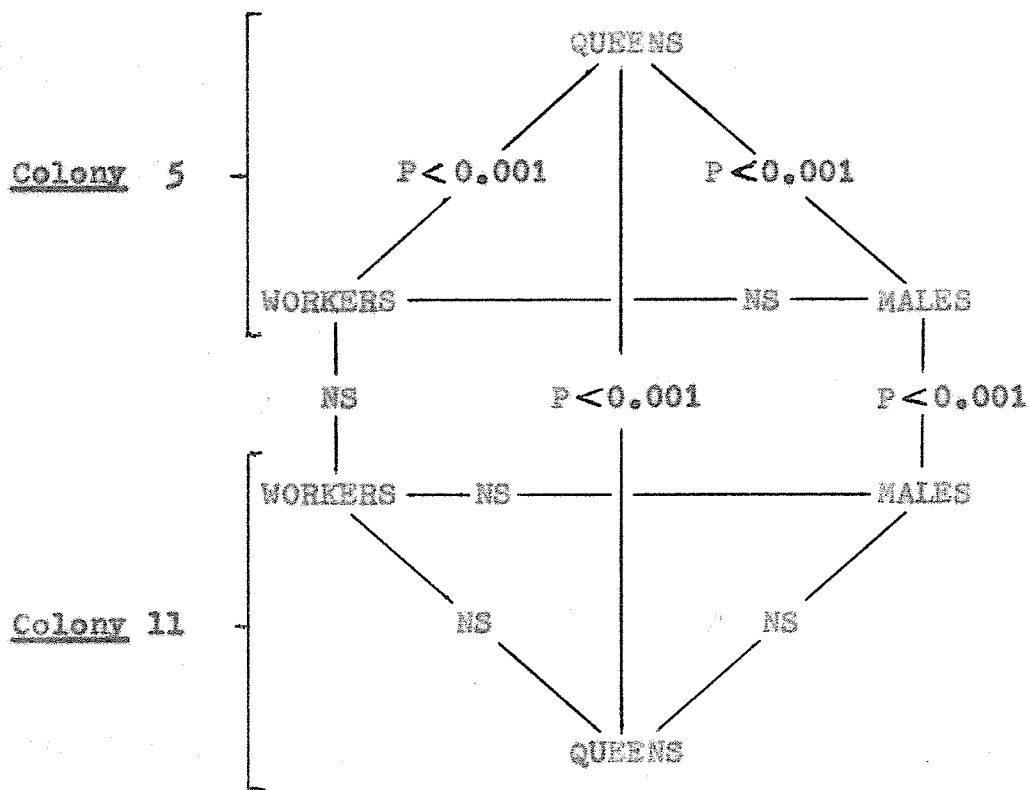


Fig. 24.- Comparisons between mean durations of larval stadia of castes in two colonies of *B. bimaculatus* Cresson.

and workers, no differences between the castes were evident in Colony 11. Moreover, the mean duration of the larval stadia of queens and males differed significantly between the two colonies. It is not surprising that such a situation should arise in colonies which are allowed (as these were) to forage naturally, for FRISON (1928) noted that the larval stadia of workers reared in the early stages of colony development were often much prolonged, probably because of poor nutrition. Furthermore, BRIAN (1951), working with B. agrorum F., found great variability in the rates at which larvae in different brood batches developed. On several occasions, the adults from one batch of eggs emerged well in advance of those from a batch laid previously, even though both groups were of the same sex and caste. Unfortunately queens were not produced in either of Brian's colonies, so she was unable to compare their developmental periods with those of workers and males.

Though insufficient data are at present available to permit statistical analysis, it is probable that the duration of the larval stadia of queens and males in B. ternarius colonies kept in complete captivity at constant temperature with abundant food becomes shorter as colony development progresses, presumably because of the increased availability of larval food. On two occasions, also, fourth instar larvae intermediate in size between worker and queen were

neglected by B. ternarius queens which had reared them in their first broods. Both larvae remained as such for over a week without pupating. WHEELER (1928) considered the ability of larvae to survive long periods of sub-optimal nutrition to be important in the origin of polymorphism in colonies of social insects.

Although SLADEN (1912) had reported that the developmental stages of queens were of longer duration than those of males and workers, FREE (1955a) did not find that the duration of the developmental stages of queens of B. pratorum L. were greater than those of workers. Free suggested that the duration of the pre-imaginal stages does not in itself play an important part in the production of queen bumblebees. However, Free's colonies were arranged to test several worker/larva ratios, so that the availability of larval food would have varied from colony to colony. In my opinion, useful comparisons between developmental rates of the different castes can only be made between individuals in the same colony, and preferably in the same brood clump. In any case, the relevance of the lengths of the larval stadia to the changeover from worker to queen production chiefly centers around the fate of mixed clumps of worker and queen larvae, as will be shown in the next section.

Nevertheless, even within the same colony (No. 8), Free found that the six queens in the first brood emerged

before the three workers reared in the same brood clump. I have obtained mixed queen and worker first broods in 6 colonies of B. rufocinctus, 7 of B. ternarius, 3 of B. perplexus and numerous (> 20) of B. terricola during the past two years. (In fact, the rearing of queens in first broods has been something of a problem when attempting to rear vigorous colonies in captivity.) In all but two cases the workers emerged before the queens and in the exceptions (both B. terricola) the single workers which emerged after the first of the queens were from cocoons hidden beneath the brood clumps and were of diminutive size.

To summarise: in two North American bumblebee species (B. terricola and B. ternarius) and probably in a third (B. bimaculatus), larvae destined to become queens cease feeding later than presumptive worker larvae in the same brood clump, at least in captive colonies kept supplied with a perpetual food surplus. Though this may to some extent be due to age differences within the brood clump (see FREE & BUTLER 1959) in that some larvae result from eggs laid later than others, it is likely that the durations of the larval stadia of queens exceed those of workers (and probably males also). The relevance of these results to the process of caste determination will be discussed in the following sections.

3.522 The development of mixed larval clumps in *B. terricola*
and *B. ternarius*

From a study of daily photographs of *B. ternarius* and *B. terricola* colonies as they approached and passed through the period of changeover from worker to queen production, a detailed comparison of the mode of origin of mixed cocoon clumps (i.e. those containing both queen and worker pupae) was made. Colonies of both species usually produced such mixed clumps, though two *B. terricola* colonies began queen production by rearing clumps containing only queens and males, and one of *B. ternarius*, after a period during which only males were produced, later reared a single queen among a batch otherwise composed entirely of males.

As previously mentioned (Section 3.2), larvae of *B. terricola*, as in other species of its subgenus, separate from each other early in the fourth instar, so that the compact form of the brood clump is lost. Though the caste fates of the larvae are probably already determined by this stage (see following Section), it is not always possible to distinguish the castes with certainty, but after a day or two the queens can be distinguished from males or workers by their greater size and the less advanced state of their cocoons. The queen larvae continue to grow after the worker and male larvae in their brood batch have ceased feeding and have completed their cocoons in readiness for pupation.

Those larvae which became queens were almost always in the most exposed (but not necessarily the most central) parts of their brood clumps; and even in colonies which were well past the changeover from worker to queen production, occasional worker cocoons were found concealed underneath those of males and queens, suggesting that their position had prevented them from receiving enough food for them to develop into queens.

In B. ternarius, the larvae remain enclosed within a communal wax envelope until their cocoons have changed shape after the cessation of feeding, though as in B. agrorum (CUMBER 1949) their relative positions become fixed by their partially spun cocoons well before they cease to feed. There are no external signs by which presumptive queen larvae may be distinguished from workers until the latter have ceased feeding.

Fig. 25 comprises nine photographs taken at 24 hour intervals of part of the comb of a B. ternarius colony (No. 45) reared and maintained in complete captivity during the summer of 1965. The brood clump "C" was the first "mixed" clump (fifteen workers and three queens) to be produced by this colony; its development was similar to that of the earliest mixed clumps in all the other B. ternarius colonies studied. It will be seen that the presumptive queen larvae (C₆, C₇, and C₁₄) were not distinguishable from the workers

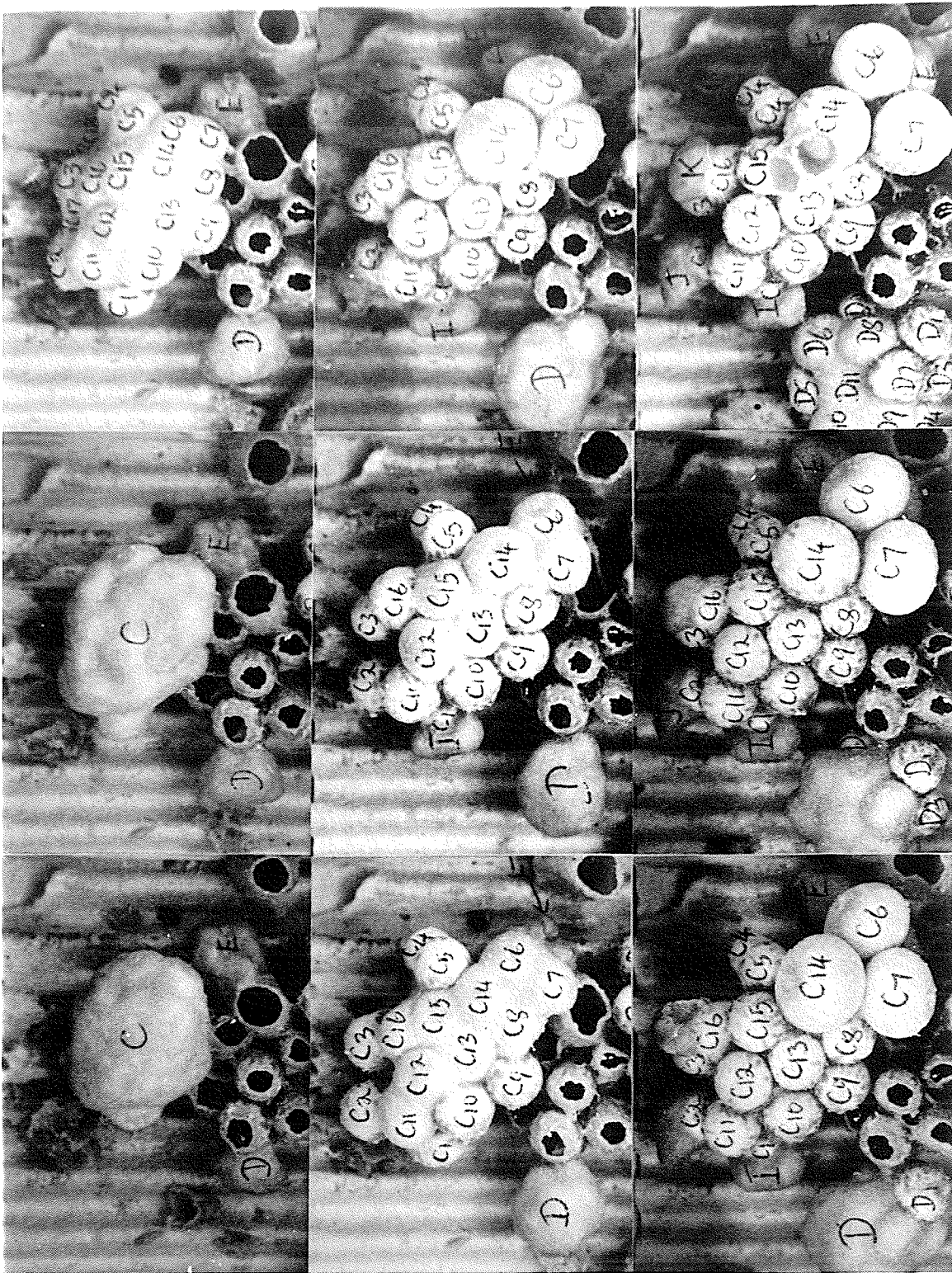


Fig. 25.- Successive daily photographs of part of the comb of a captive *B. ternarius* colony, showing the development of a mixed clump.

until most of the latter had ceased feeding and had had the wax removed from their completed cocoons (5th day). By the sixth day, all the worker cocoons had been completed and the three queen larvae were distinctly larger than any of the worker cocoons, and by the ninth day all three queen larvae had ceased feeding.

Since all the mixed (i.e. worker and queen) clumps in B. ternarius colonies (24 were studied) at the point of changeover originated in the same way, we may assume provisionally that this mode of development is characteristic of the species, in which case the interesting question arises: are the larvae which develop into queens already caste-determined by the time that their worker clump-mates cease feeding, or is the latter event causally connected with their differentiation into queens? An attempt to provide a partial answer to this question is described in the following section; at this point the results of a procedure for estimation of the rate of growth of B. ternarius are discussed.

Since the daily photographs of B. ternarius colonies were taken at a fixed distance from the surface of the comb, the daily increase in area of the larval clumps may be taken as a measure of their rate of growth (though because the clumps were not equidistant from the lens of the camera, comparison between the areas of different clumps would be

misleading). Areas were measured by projecting the photographs with an epidⁱascope and tracing the outlines onto paper, from which area measurements were taken using a planimeter. The daily area increase in two mixed clumps from Colony 26 and two from Colony 28 are shown in Fig. 26. An arrow on each plot marks the point at which all the worker members of the clump had ceased feeding.

The curves in Fig. 26 are obviously sigmoid. The cessation of feeding of the worker larvae does not appear to have much effect upon the shape of the curve, i.e. the increase in size of the remaining (queen) larvae allows the general sigmoid form to be continued until these queen larvae have ceased to feed. This seems to support the hypothesis (developed in the following section) that the rate at which food is brought to a mixed larval clump does not sharply decrease when its worker members cease feeding; from which it follows that the remaining queen larvae must receive an increased rate of food supply when their worker clump-mates cease to feed.

3.523 Caste determination in female larvae of *B. terricola* and *B. ternarius*

To find out whether queen determination occurs during or prior to the fourth instar, a study was made of the relation between the width of the last larval instar (fourth) head capsule and the weight of prepupae.

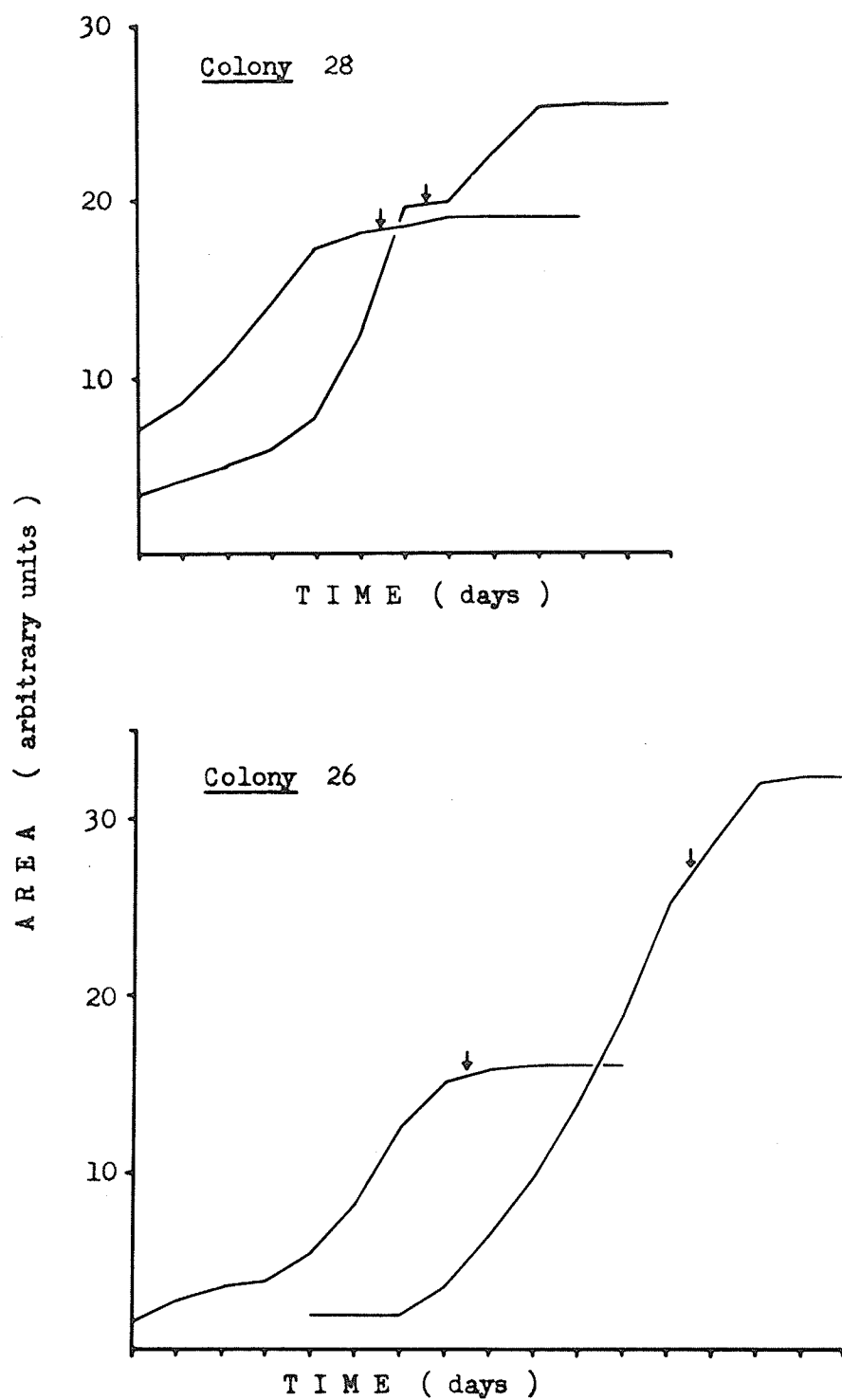


Fig. 26.- Area increase in four mixed larval clumps of B. ternarius.

Two colonies of B. terricola and two of B. ternarius were reared from naturally overwintered queens in the summer of 1966 and allowed to develop in captivity at 30°C with abundant honey and pollen. Prepupae were removed from the colonies by cutting open recently completed cocoons, and after weighing, were chilled for about 5 minutes at 1°C to render them temporarily immobile. After positioning the prepupae in the grooves of a piece of corrugated cardboard, their head capsule widths were measured under a microscope with a graduated eyepiece.

After they had been measured, and in order that their sex might be determined, the prepupae were held at 32°C (RH=50% approx.) until they had pupated. The sex of bumblebee pupae is easily ascertained by inspection of the genital and sting rudiments (Fig. 27). Adults from the pupae of B. terricola thus obtained were allowed to emerge and their radial cell lengths were measured, but shortage of time did not allow this to be done for B. ternarius.

Data for both species are given in Tables XXIII and XXIV and in Figs. 28-30. A significant positive linear correlation ($p < 0.01$) between prepupal weight and head capsule width was obtained for B. terricola, but inspection of Fig. 28 shows that we are evidently dealing with two populations which do not show an overlap of values of either character. Furthermore, two cocoon clumps (C and D) from the second

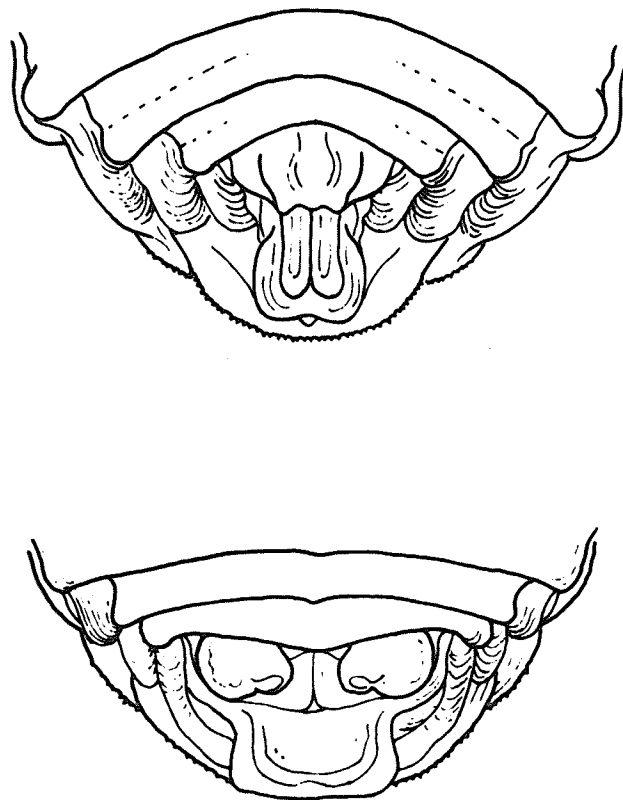


Fig. 27.- Ventral views of the posterior abdominal segments of B. ternarius pupae (upper - female, lower - male).

PREPUPAL WEIGHTS, FOURTH-INSTAR HEAD-CAPSULE WIDTHS AND RADIAL
CELL LENGTHS OF FEMALES REARED IN TWO B. terricola COLONIES

Colony	Brood	Clump	Prepupal weight mgm	Head-capsule width 1 unit = 0.025 mm	Radial cell length 1 unit = 0.051 mm
1	First	A	215 232 171 209 205 199	72 74 75 75 72 75	
8	First	A	139 131 171 146	72 72 71 73	47 45 51 50
	Second	B	247 223 206 212 240 222	73 74 70 71 72 75	56 53 50 55 55 55
		C	213 747 152 67 148 692 761 195 228 597 740 798 705 714 738 822	73 81 72 69 73 78 82 76 76 83 79 82 81 78 83 80	55 70 46 36 47 73 74 51 53 70 73 75 74 74 75
		D	753 291 735 755 752 753 793 762	78 74 77 78 78 83 83 82	73 59 75 74 76 75 76 76

PREPUPAL WEIGHTS AND FOURTH-INSTAR HEAD-CAPSULE WIDTHS
OF FEMALES REARED IN A B. ternarius COLONY

Brood	Clump	Prepupal weight mgm	Width of fourth-instar head-capsule 1 unit = 0.025 mm
First	A	98	70
		115	68
		88	69
		110	71
		119	67
Third	G	550	70
		618	70
		148	64
		204	70
		140	70
		135	66
		219	75
		180	68
		122	63
		179	65
		178	65
		169	69
		147	67
		184	65
	H	219	72
		228	72
		202	75
		140	71
		760	71
		197	74
		205	74
		168	71
		169	69
		200	73
		497	70
	I	158	70
		166	68
		166	71
		655	77
		597	76
		642	78
		569	70
		621	74
		601	74
		650	69
		156	72
		169	67

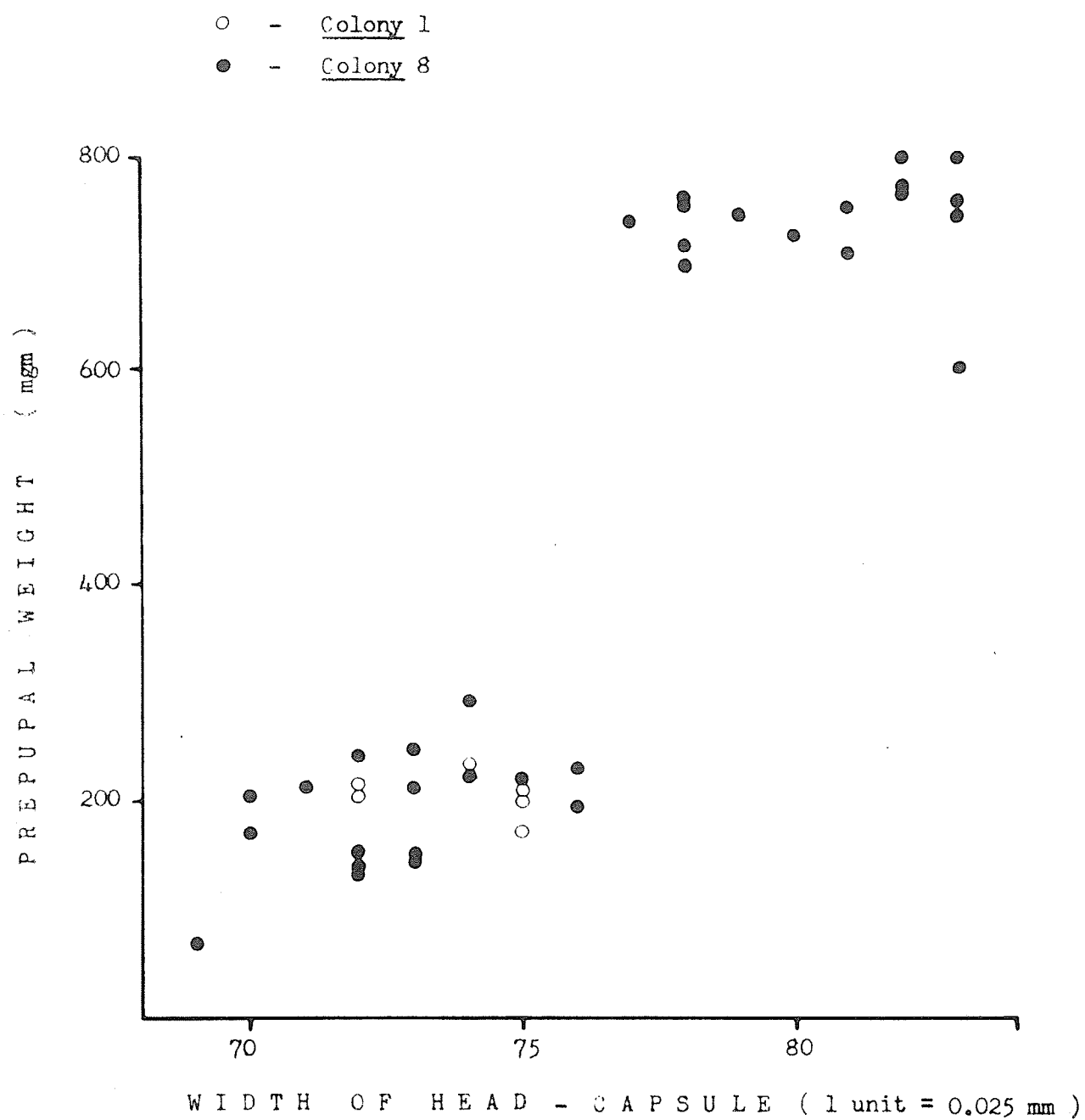


Fig. 28.- Scatter-diagram of prepupal weights and fourth-instar head-capsule widths of females reared in two B. terricola colonies.

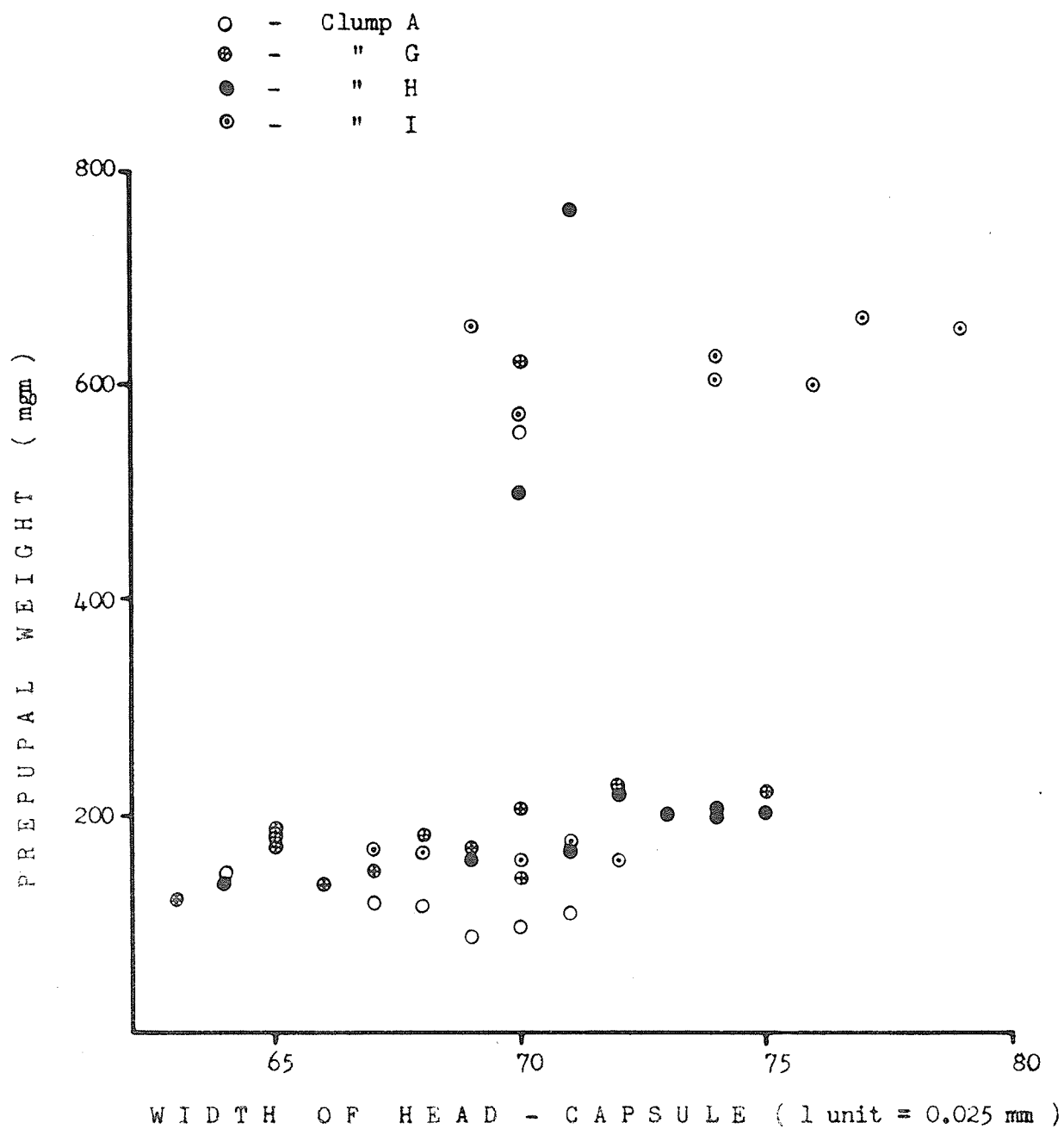


Fig. 29.- Scatter-diagram of prepupal weights and fourth-instar head-capsule widths of females reared in a B. ternarius colony.

brood of Colony 8, contained members of both of these populations.

It may be noted that within Colony 8, the radial cell lengths of the adults are highly correlated with prepupal weight ($r = 0.9773^{***}$ for 32 d.f.). The width of the fourth instar head capsule is also highly significantly correlated ($r = 0.8857^{***}$ for 32 d.f.) with radial cell length. Since the partial correlation coefficient for head capsule width on radial cell length, keeping prepupal weight constant, is also highly significant ($r = 0.6333^{***}$ for 31 d.f.), it seems that adult size is influenced by the size of the larvae as they begin the fourth instar, independently of the size attained by the prepupal stage.

The regression of radial cell length on prepupal weight is obviously non-linear (Fig. 30a). Physical considerations would suggest a relationship of the form:

$$\text{radial cell length} = (\text{weight})^{1/3}$$

Accordingly the logarithms of the prepupal weights were plotted against the logarithms of the radial cell lengths (Fig. 30b). The correlation coefficient for log prepupal weight on log head capsule width is 0.9916^{***} for 32 d.f., indicating a better fit than was obtained with the untransformed data. The appropriate regression equation is:

$$x = 1.0784 + 0.276y$$

where $x = \log_{10}$ radial cell length and $y = \log_{10}$ prepupal

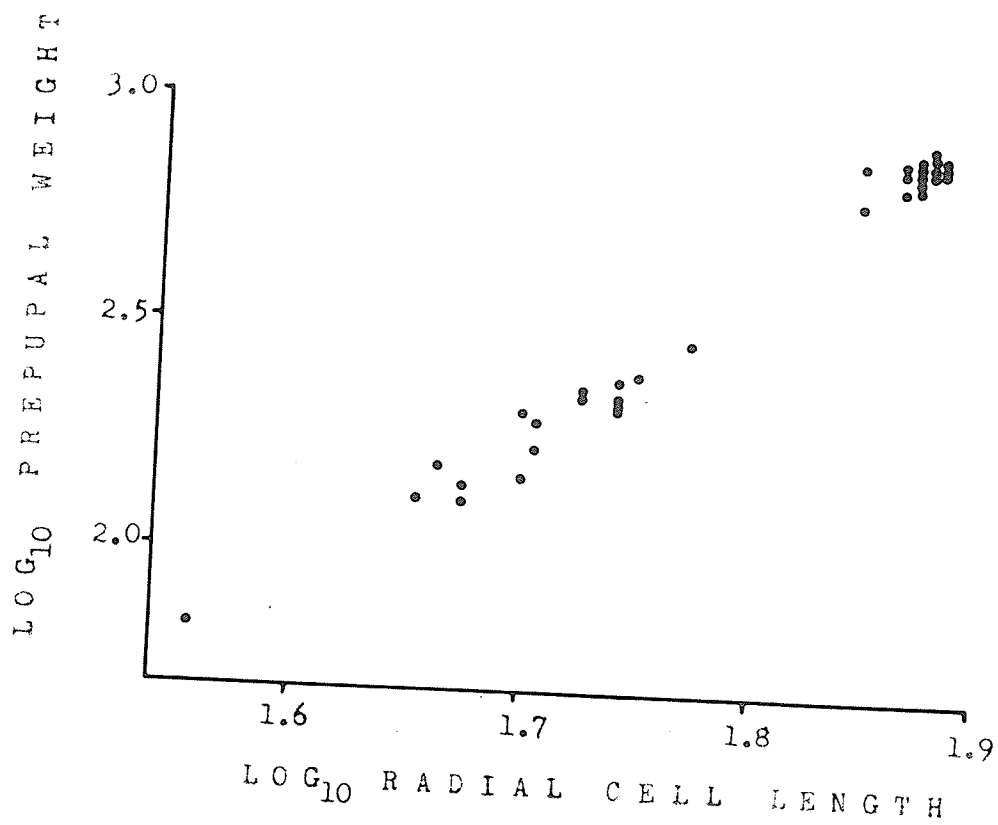
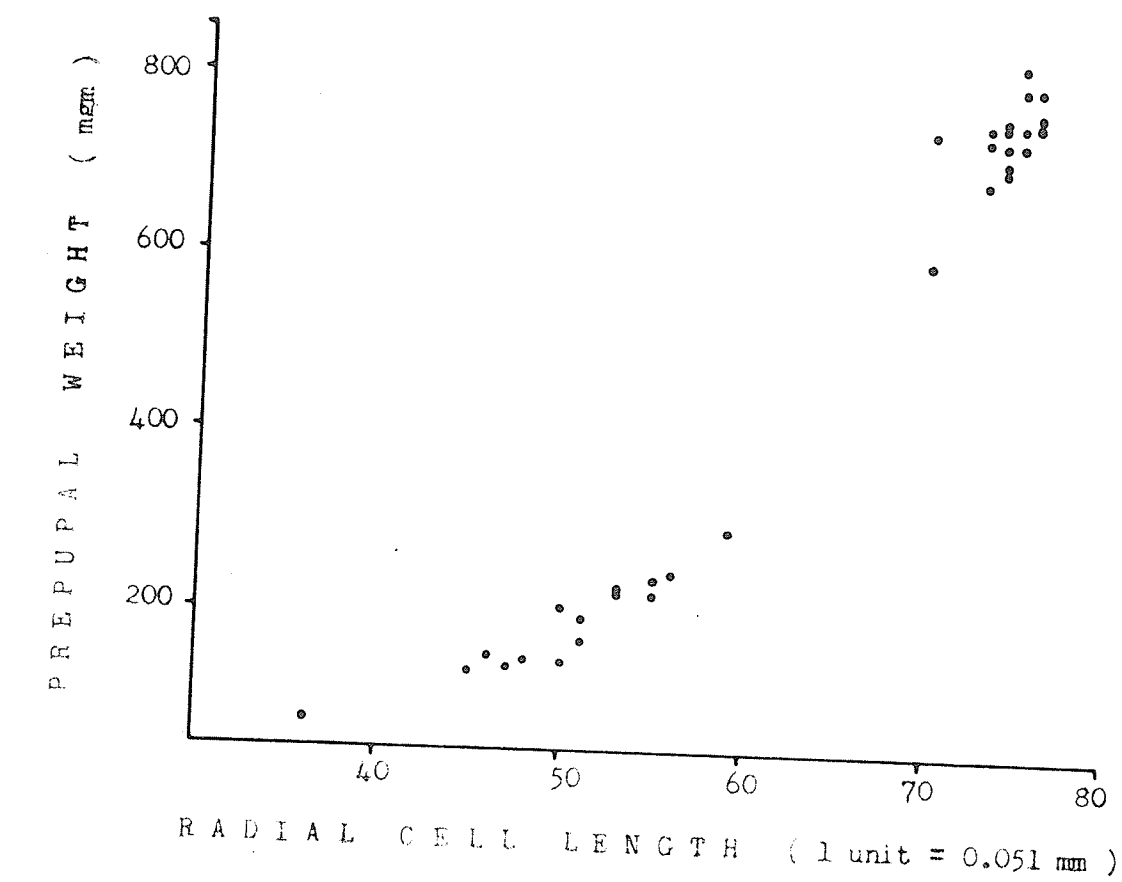


Fig. 30.- Scatter-diagrams of (a) prepupal weights and radial cell lengths and (b) \log_{10} prepupal weights and \log_{10} radial cell lengths of *R. terreus*.

weight. The slope (0.276) is significantly ($p < 0.001$) less than the expected value of 0.3 but no explanation has been suggested for this.

In B. ternarius, though the distribution of prepupal weights is again heterogeneous with no overlap (Fig. 29), the range of head capsule widths of s-queens overlaps that of s-workers. Furthermore, though the head capsule widths of the s-queen prepupae in the final mixed clump which was produced in the colony (Clump I) are mostly greater than those of the s-workers in the same clump, there is no evidence that within the earlier mixed clumps (G and H) the s-queens have larger head capsules than the s-workers.

It follows, therefore, that if the existence of a difference in the width of the fourth instar head capsule of the two castes can be taken as evidence that the caste fate of larvae is determined by the end of the third instar, we may conclude that in B. terricola at least, such an early determination does occur. The only alternative explanation for the data which has so far been suggested is that the head capsule might be somewhat extensible, so accounting for the greater widths in B. terricola s-queen larvae, but this explanation would require as a sub-hypothesis that the head capsules of B. terricola behaved differently from those of B. ternarius. In any case, if the variability in head capsule width were attributable to expansion during fourth

instar larval growth, one expect an overall regression (not necessarily linear) to be continued within as well as between the two size groups. This is clearly not the case in Fig. 28.

Assuming, then, that the width of the fourth instar head capsule as measured on prepupae is a reliable indication of its size at the start of the instar, we may presume that the size of the larvae as they enter ecdysis at the close of the third instar is related to their caste fate. Whether large larvae at this stage of development inevitably become queens is not known, but should provide an interesting topic for further study. In any event, it is unlikely that queen determination in B. terricola is a matter of simple correlation between size (e.g. at the beginning of the fourth instar) and the potentiality for subsequent growth, if only because the distribution of the head capsule widths is so much more continuous than that of prepupal weight. Some sort of "threshold" concept seems unavoidable at present in our approach to caste determination of B. terricola and allied species, and in the absence of any detectable external event which could be linked with the origin of the very striking difference between the sizes of mature larvae of the two castes, often even within the same brood clump, it must be supposed that the mechanism which so effectively separates the developmental pathways is of an internal

physiological nature. How it operates is unknown.

The situation in B. ternarius may be rather simpler. Though the absence of any clear difference between the head-capsule widths of s-queen and s-worker prepupae is not by itself evidence that the larvae remain undetermined until the fourth instar, the observations discussed in the previous section, taken together with the head capsule data, are consistent with the following tentative hypothesis.

At the point of changeover from worker to queen production, the capacity of the colony for brood feeding is presumably inadequate to allow the rearing of more than a few queens from all the available female larvae. Thus most of the larvae in each brood clump cease feeding and complete their cocoons when they have reached worker size. However, because the larvae in a clump vary somewhat in age (see Sections 3.1 and 3.52) it will seldom happen that all of them cease feeding simultaneously. We may suppose that those still feeding after their sisters have ceased receive a sudden surplus of nursing attention which causes the onset of pupation to be delayed until the larvae have attained queen size. This hypothesis, which is consistent with the facts as they are known at the present time, suggests that the "discontinuity mechanism" (i.e. the isolation of larvae by the pupation of their clumpmates) is at least in part external to the larva. However, at least one physiological mechanism is

required to explain the absence (or great rarity) of intermediate size bees; why do not some larvae cease feeding when they are larger than worker- yet still smaller than queen-size? It is hoped that future experiments will help to provide an answer to this problem.

3.6 Conclusions

The two aspects of the caste phenomenon which have been investigated in this thesis--the origin of size dimorphism and the relationship between adult size, environment and caste fate--though obviously interrelated, may nevertheless deserve to be considered separately as the manifestations of two stages in the evolution of caste in Bombus.

In claiming advanced status for the subgenus Bombias on the grounds that only one egg is laid per cell, HOBBS (1964a) gives a very misleading impression. In that they rest upon a supposed homology between bumblebees and honeybees, Hobbs' arguments do not seem very convincing, but even if it were accepted that the oviposition behaviour of Bombias queens is of an advanced and specialised type, there are good reasons for considering that so far as the evolution of caste is concerned, the species of Bombias and other pocket-making subgenera are representative of a more primitive state than that reached by the pollen-storing species. I see no reason, therefore, to doubt that the discontinuous female size distributions achieved in the latter group had its evolutionary origin in the continuous distribution of female size which is characteristic of the pocket-makers.

CUMBER (1949) suggested that the method of larval feeding used by pocket-makers could be a sufficient cause of the greater variability in the size of their adults, but as

mentioned earlier, the fact that continuous size distributions resulted even from colonies unable to practise their normal method of larval feeding, throws doubt upon Cumber's suggestion. Probably the continuous distribution of female size in pocket-making species is related to the physiological mechanisms which control growth and ecdysis in their larvae.

Although the pocket-makers have not evolved a mechanism which so efficiently suppresses the production of female bees of intermediate size, as has been developed in pollen-storing species, they do show a well developed functional caste differentiation related in a general way to adult size. It is for this reason that I have considered it justifiable to separate caste differentiation from size determination. BRIAN (1957) expresses the two concepts by stating that in Bombus caste is a matter of size and diapause.

More work of the sort described in Section 3.3 will be needed before the relationships between caste function, adult size and imaginal experience can be known in detail. It should be possible to extend such studies to include mating and hibernation behaviour in females of both pollen-storing and pocket-making species. The negative correlation between weight-gain and ovary development that was demonstrated in females of B. ternarius and B. perplexus (see Section 3.3) supports the hypothesis that diapause as a physiological

state in Bombus is connected with fat body status, at least in these two species. It was also demonstrated that if one accepts that oviposition without prior hibernation is a defining characteristic of worker caste-function, the caste fate of B. terricola s-queens appears to be modifiable by the imaginal environment. In default of reliable techniques for the estimation of small changes in fat body status, it was not possible to be certain that s-workers experienced analogous changes in fat body status during early imaginal life to those experienced by queens, but such results as were obtained suggested that CUMBER's (1949) claim that queens and workers represent two physiologically distinct types may need some critical re-examination. The validity of the experimental results upon which Cumber based his claim may be open to question. I do not consider that imaginal caste differentiation can be discounted, at least for pocket-making species, on the basis of the information which we have available at present.

Determination of adult female size was studied by examining colony and larval development in captive colonies of B. terricola, B. perplexus and B. ternarius. A progressive increase in the size of females emerging as colonies developed was found in B. perplexus, in some colonies of B. terricola, but not in the single colony of B. ternarius which was studied. Unfortunately, it was not possible to

obtain detailed information on the seasonal variation of female size in either of the two pocket-making species common near Winnipeg (B. borealis and B. fervidus) but observations on captive colonies of these species suggested that a progressive size increase occurs throughout their colony development, as in B. perplexus.

The size of the females of first broods of both B. terricola and B. perplexus (as measured by the lengths of the radial cells of their forewings) was shown to be inversely proportional to the number of bees in each brood. Since excess food was always present while the first-brood larvae were being fed, it was inferred that the size of the first-brood adults must be limited by "competition" for nursing attention from the queen.

The size of second-brood females of B. terricola and B. perplexus was found to be significantly correlated with the ratio of number of first-brood workers/ number of second-brood larvae. Also correlated with this ratio was the number of queens produced in second broods of B. ternarius colonies. It was also shown that the total number of workers produced in B. ternarius colonies was highly correlated with the constant b in the function: $\log_{10} y = a + bt$ which satisfactorily describes the early part of the cumulative adult-producing egg-total curve. Since egg destruction was at no time observed in B. ternarius colonies, it was assumed that the

ratio of number of eggs laid to number of resulting adults remained constant during at least the earlier part of colony development.

Insofar as bumblebee colonies which change over to queen production early rear fewer workers than those whose changeover is delayed (but see below) we may claim that in B. ternarius the association between the egg-laying curve and total number of workers produced is evidence for the dependence of the changeover upon the oviposition rate. In Section 3.513, the five B. ternarius colonies whose egg-laying rates were studied gave sigmoid cumulative egg-total curves. Assuming insignificant pre-imaginal mortality, the worker/larva ratio should therefore show an increasing rate of increase as colony development proceeds. In the vicinity of the point of inflexion, the worker/larva ratio must show an approximately linear rate of increase, but above the point of inflexion the ratio should increase more and more rapidly. It may be concluded, therefore, that (making the assumptions specified above) it is not necessary for the queen's oviposition rate to decline in order that the ratio should start to increase; all that is required is

that the increase in oviposition rate should fall off.

Since the colonies used in this study were reared and maintained under highly artificial conditions, it is necessary to consider to what extent the conclusions reached here can be applied to the development of bumblebee colonies in the wild state. Though records of the numbers of bees produced in wild colonies of bumblebees in southern Manitoba are scanty, it has been noted above that those of B. terricola and B. perplexus probably achieve larger worker populations on average than those I reared and kept in captivity. Captive B. ternarius colonies, on the other hand, usually achieved worker populations as large as, or larger than, the worker populations reported in the few wild colonies of the species taken in the Winnipeg area.

It seems difficult to explain these results on the basis of CUMBER's (1953) suggestion that liberal colony food intake tends to hasten the changeover from worker to queen production, for if the B. terricola and B. perplexus colonies started queen production abnormally early because the colonies were liberally fed, why did not the B. ternarius colonies, all of which had surplus honey and pollen present at all times, also undergo premature changeover to queen production?

Perhaps the explanation lies in differential brood mortality. As noted earlier, egg eating and ejection of

larvae were commonly observed in colonies of B. terricola and B. perplexus, but egg eating was never seen (and ejection of larvae only rarely) in B. ternarius colonies. This is reflected in the figures for numbers of second-brood adults in the three species (Table XIX). In any case, the effect of colony food intake upon the onset of queen production is likely to be complex, since both variables are presumably linked to the queen's oviposition rate, brood mortality and forager/house-bee polyethism. For example, though an increased colony food intake may be supposed to result in more liberal larval nutrition both (a) directly, inasmuch as more honey and pollen will be available per larva, and (b) indirectly in that the division of labour among adult workers may be shifted in favour of a greater proportion of house-bees (see FREE 1955b), the effect may be to some extent counteracted by (c) increased oviposition, and (d) lower larval mortality. In species such as B. perplexus, where ejection of larvae may be of regular occurrence (see Sections 3.2 and 3.51), increase of the worker/larva ratio by worker-regulated larval mortality could possibly be of great importance in initiating queen rearing.

Further speculation on the factors responsible for the changeover to queen production in wild colonies must await the results of more research, but at least it is fair to conclude that some of these factors, insofar as they were

inoperative in captive colonies, do not play an essential part in the process. For instance, CUMBER (1949) states that in colonies of pollen-storing species "it is thought that . . . there is a sudden change to all or almost all male brood when the eggs giving rise to the sexual forms are laid, so that a return to female brood occurs at a higher level of economy in the colony." By this he seeks to explain the rarity of females of intermediate size. Though I do not question Cumber's suggestion that a preponderance of male brood exerts a stabilising effect upon female size, the experimental results presented above (Sections 3.4 and 3.5) refute the hypothesis that the production of the first queens is consequent upon any sudden change to all or almost all male brood. Such a sudden change at about the time that the first queens were reared did occur in some captive colonies, but it was by no means general--the first queens in B. terricola and B. ternarius usually resulted from brood batches which also contained workers, and though males were generally reared shortly thereafter, in at least three colonies of B. ternarius a series of two or more mixed queen/worker brood clumps were produced.

Similarly, though failure to observe the occurrence of egg eating in B. ternarius colonies is not proof that it never occurred, it seems unlikely that destruction of eggs by workers could have contributed much to the changeover

from worker to queen production.

In B. perplexus, which exhibits a fairly continuous range of female size, the progressive increase in the size of female bees produced throughout the course of colony development is probably explicable in terms of a gradually increasing worker/larva ratio, coupled with random differences in the nursing attention given to larvae within the same brood batch. Perhaps, as suggested by CUMBER (1949), differences in age among the larvae in each brood clump contribute to the variability in size, in that those larvae which are youngest may receive food at a rate appropriate to the size of their older companions. The data given in Section 3.41 indicates that where males are reared at the same time as the first s-queens, their presence may help to produce discontinuity in size between s-workers and s-queens.

The measurements on prepupae of B. terricola and B. ternarius appear to show that the two species differ in the point at which the caste fates of female larvae become determined. Determination in B. terricola occurs before the fourth instar, whereas B. ternarius larvae remain undetermined (at least as far as their head-capsule widths are concerned) until in the fourth instar. Observations on the way in which mixed queen/worker clumps originate suggests that a possible mechanism for the production of s-queens in B. ternarius may involve the "isolation" of presumptive

queen larvae by the pupation of the other bees in their clumps. Such isolation could result in extra lavish feeding which perhaps has the effect of postponing the onset of pupation. This hypothesis should be tested experimentally.

I feel that in the present state of our knowledge, it is impossible to avoid the conclusion that caste differentiation in B. terricola is effected by means of some internal physiological mechanism which switches the larvae into one pathway or another (see BRIAN 1957), of which the differing head capsule widths are the earliest obvious external manifestation. Because female bees of intermediate size, though rare, do occur in B. terricola colonies, I suggest that if such a mechanism exists, it is likely that while worker larvae are determined (in the sense that however liberally they are fed subsequently, they are unable to exceed a certain size before they cease to feed in preparation for pupation), presumptive queen larvae enter the fourth instar only as queen-potential in the sense that if undernourished during the fourth instar they may nevertheless survive to become adults of intermediate size. The elucidation of the mechanism of caste determination of larvae of B. terricola and allied species presents a fascinating field for further study.

CHAPTER IV

SUMMARY

1. In this study some aspects of domestication and caste differentiation in bumblebees (genus Bombus Latr., Hymenoptera) were investigated. The need to increase wild populations of these insects has been recognised by numerous authors, some of whom have maintained that domestication should constitute an essential part of this process. The phenomenon of caste also has some bearing upon the economic usefulness of bumblebees, in that pollinating efficacy of bumblebee colonies depends upon the synchronisation of their maximum foraging capacity (coincident with the attainment of peak worker population) with the time of flowering of the crop to be pollinated.

2. Domestication experiments were restricted to an attempt to circumvent the problems of inducing captive bumblebee queens to found colonies in the laboratory, with the eventual aim of developing techniques for maintaining laboratory cultures on a year-round basis. Colony founding trials were run on eight of the species occurring in and around Winnipeg, using (a) naturally overwintered (b) artificially overwintered and (c) non-overwintered queens.

3. Two patterns of laboratory nest box, incorporating features from the designs employed by previous investigators

as well as a number of original features, were tested on naturally overwintered queens. In the first type (Series II), run at 29-30 C in total darkness, each nest box consisted of a pair of small glass-covered wooden boxes with corrugated cardboard floors and with or without nest material. The second pattern of nest box (Series III), held at room temperature on the laboratory bench, comprised a larger glass-covered wooden box for feeding and defaecation and a smaller box, lined with upholsterer's cotton, in which a central honey-smear cavity contained a lump of pollen dough. Both types of nest box were effective in stimulating colony initiation by most of the species tested, though B. fervidus (F.) could not be induced to start colonies in any type of nest box. Of the species in which twenty or more queens were tested, very successful results, both in incidence of oviposition and rearing of first broods, were obtained for B. terricola Kirby (an important alfalfa pollinator) and B. perplexus Cresson using Series III boxes in both 1965 and 1966, while B. ternarius Say appeared to start colonies most readily in Series II boxes. Of the other species, in which only small numbers of queens were available, encouraging results were obtained in Series II for B. nevadensis Cresson and B. vagans F. Smith, and in Series III for B. borealis Kirby and B. rufocinctus Cresson.

Oviposition occurred more rapidly in Series II boxes

containing pairs of B. ternarius queens than in those in which queens were confined individually, but it was shown that this does not necessarily mean that queens confined in pairs show an inter-stimulatory effect. In Series II boxes containing pairs of queens, one queen almost invariably died at about the time that the first eggs were laid, but the survivor generally succeeded in rearing the brood. The presence of nest material in Series II boxes was not found to encourage colony initiation and probably actually inhibited successful colony founding in some cases. Even at room temperature queens of several species constructed their first egg cells in unenclosed situations, confirming the suggestion of HOLM (1966a) that the presence of nesting material is not an essential factor for eliciting oviposition in queen bumblebees.

4. Attempts were made to get colonies started by artificially overwintered queens of B. terricola, B. nevadensis, B. borealis, B. rufocinctus, B. perplexus and B. ternarius, using a variety of experimental methods. Best results were had with B. terricola, but some success was obtained for all species. Though large colonies of B. rufocinctus and B. ternarius were reared, premature changeover to the rearing of sexual forms was found to be a frequently occurring problem with colonies started by artificially overwintered queens. Thus, though the number of first-brood eggs

and the radial cell lengths of first-brood females from colonies of B. terricola started by artificially overwintered queens did not differ significantly from similar data for colonies started by naturally overwintered queens, the incidence of queens and males in second broods was higher in the former group than in the latter.

5. Oviposition by non-overwintered queens occurred in B. terricola, B. nevadensis, B. rufocinctus, B. perplexus and B. ternarius. Though some vigorous colonies were obtained, and a culture of B. rufocinctus was maintained over four generations, a number of technical problems remain to be solved before colony founding by non-overwintered queens can be used as the basis of a reliable method of bumblebee domestication.

6. To determine whether caste status is irrevocably fixed by the beginning of imaginal life, female bees were removed from colonies of B. terricola, B. perplexus and B. ternarius either just before or just after they emerged from their cocoons and were held at 32°C with abundant honey and pollen. An estimate of changes in the fat-body status of these bees was obtained by daily weighings, and at the end of the experimental period each bee was dissected and the state of its ovaries and fat body noted. In queen-size bees of B. perplexus and B. ternarius, ovary development was significantly negatively correlated with weight gain,

indicating that those bees which laid down least fat body underwent the greatest ovary development during the experimental period. Many queen-size bees of all three species, as well as most worker-size bees of B. ternarius, laid eggs during the experiment, although none had mated. The incidence of egg-laying in B. terricola was significantly greater when queens were confined in pairs than when they were in solitary confinement. If egg-laying without mating or prior hibernation is taken as a defining characteristic of the worker caste, then it may be concluded that (a) not all queen-size imaginal females are irrevocably determined as functional queens, and (b) the caste fate of queen-size bees, at least in B. terricola, can be modified by imaginal experience. Further investigation will be needed to discover whether a classificatory physiological separation on the basis of fat-body metabolism can be used to distinguish queens from workers.

7. Colonies started by queens in laboratory nest boxes and maintained in total captivity with abundant food were found to show a pattern of development similar to those in the wild state, though in some species the changeover to queen production may have occurred earlier in captive than in wild colonies. Bimodality in the distribution of female size was more pronounced in captive colonies of "pollen-storing" species than in those of "pocket-makers" even

though captive colonies of the latter group were not permitted to feed their larvae in the usual manner due to the abnormal way in which pollen was supplied to the colonies. It was concluded, therefore, that the feeble dimorphism in size between queens and workers in pocket-making than in pollen-storing bumblebees cannot be fully explained by the difference in mode of larval nutrition normally existing between the two groups. In captive colonies of B. perplexus, a species which seems to have affinities with both groups, a progressive increase in the size of females reared during colony development was usually found, in contrast to two characteristic pollen-storing species, B. terricola and B. ternarius, in which the changeover from worker to queen production is generally marked by a sudden increase in the size of females produced.

8. The radial cell lengths of first-brood females of B. terricola and B. perplexus were negatively correlated with the number of bees per brood and the radial cell lengths of second-brood females of the same species were negatively correlated with the number of bees in each second brood and positively correlated with the number of first-brood workers. In B. ternarius, the number of queens produced in the second brood was also negatively correlated with the number of second-brood bees and positively correlated with the number of first-brood workers. These results

support the claim of previous investigators that the worker/larva ratio is an important factor in the changeover from worker to queen production in bumblebee colonies. An increase in this ratio could result either from increased brood mortality or from changes in the egg-laying rate of the queen. No attempt was made in this study to obtain quantitative data on brood mortality, but in both B. perplexus and B. terricola eggs were eaten by workers and apparently healthy larvae were ejected from colonies. Egg eating was not observed in captive B. ternarius colonies and larval ejection seldom occurred from second and succeeding broods of this species. Accordingly, an attempt was made to account for the changeover from worker to queen production in B. ternarius in terms of the queen's oviposition rate, by recording the number of adult-producing eggs laid over the first 60 days of colony development in five colonies. The cumulative total of number of adult-producing eggs plotted against time gave a sigmoid curve for each colony, and the constants of the exponential equations which fitted the lower parts of the curves were significantly correlated with the total number of workers produced by each colony. A theoretical investigation of the mathematical implications of the forms of oviposition curves on changes in the worker/larva ratio showed that a sigmoid cumulative egg-total curve implies an increasing rate of increase of the worker/larva ratio as colony develop-

ment proceeds. Even were the queen's oviposition rate to remain constant the worker/larva ratio would show a linear rate of increase provided that adult mortality remained insignificant.

9. To investigate the mechanism of queen determination in larvae of pollen-storing species, detailed observations on the process of changeover from worker to queen production in captive colonies of B. terricola and B. ternarius were made. In most colonies of both species, the first queen larvae developed in brood clumps which also contained worker larvae. In such mixed clumps, the presumptive queen larvae ceased feeding later than their presumptive worker companions. In B. terricola, the fourth-instar head-capsule widths of presumptive queen larvae were wider than those of presumptive worker larvae in the same brood batch, suggesting that in this species caste is already determined by the close of the third instar. But in B. ternarius, though a marked discontinuity between the prepupal weights of the two castes was found, and though the fourth-instar head-capsule widths of presumptive queen larvae in later batches generally exceeded those of presumptive workers in the same clumps, in the earliest mixed clumps of the colony which was studied the head-capsule widths of fourth-instar larvae of the two castes showed an overlapping distribution, nor was there any evidence that the head-capsule widths of presump-

tive queens tend to exceed those of presumptive workers. Possibly, therefore, the caste fate of B. ternarius larvae remains undetermined until the fourth larval instar; it is suggested that the first queens produced during the development of colonies of this species may result from a sudden increase in rate of food supply to one or more larvae in a clump (brought about by the pupation of all other individuals within that clump) having the effect of postponing the onset of pupation and thus allowing these larvae to continue feeding until they reach queen-size.

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TABLE XXV

1. B. terricola one queen per box

Box no.	No. of days between installation and egg-laying	Remarks
1	12	
2	no eggs laid	queen died on day 13
3	" "	discontinued on day 20
4	" "	" "
5	" "	discontinued on day 15
6	" "	discontinued on day 20
7	" "	discontinued in day 15
8	" "	queen died on day 9
9	14	
10	no eggs laid	discontinued on day 20
11	" "	wax secreted; discontinued on day 20
12	" "	discontinued on day 15
13	" "	" "
14	" "	" "
15	" "	discontinued on day 17
16	8	
17	no eggs laid	discontinued on day 13
18	12	
19	12	
20	9	
21	no eggs laid	discontinued on day 13
22	" "	discontinued on day 21
23	12	
24	no eggs laid	died on day 2
25	" "	egg cells on day 12 but no eggs laid
26	" "	died on day 12
27	" "	discontinued on day 16
28	12	
29	no eggs laid	died on day 8

Boxes in which eggs were laid :	number	=	8
	% of total	=	28

B. terricola contd. two queens per box

Box no.	No. of days between installation and egg-laying	Remarks
1	6	
2	6	
3	18	
4	4	one queen escaped
5	no eggs laid	discontinued on day 22
6	7	
7	12	
8	no eggs laid	one queen died on day 10
9	3	
10	no eggs laid	both queens dead by day 8
11	" "	both queens dead by day 10
12	" "	both queens dead by day 11
13	10	both queens died on day 10
14	no eggs laid	discontinued on day 12

Boxes in which eggs were laid :	number	= 8
	% of total	= 57

2. B. ternarius one queen per box

Box no.	No. of days between installation and egg-laying	Remarks
1	no eggs laid	discontinued on day 24
2	" "	queen died on day 7
3	" "	discontinued on day 18
4	" "	" "
5	" "	" "
6	12	

Boxes in which eggs were laid :	number	=	1
	% of total	=	17

two queens per box

Box no.	No. of days between installation and egg-laying	Remarks
1	12	
2	10	
3	11	

[illegible]

3. B. perplexus one queen per box

Box no.	No. of days between installation and egg-laying	Remarks
1	6	
2	6	queen died on day 12
3	no eggs laid	discontinued on day 18

Boxes in which eggs were laid : number = 2
% of total = 67

two queens per box

1	no eggs laid	discontinued on day 13
2	" "	" "

Boxes in which eggs were laid :	number	= 0
	% of total	= 0

4. B. fervidus one queen per box

Box no.	No. of days between installation and egg-laying	Remarks
1	no eggs laid	discontinued on day 38 ¹
2	" "	discontinued on day 34
3	" "	" "

¹ This queen was provided with comb, workers and nest material

Box no.	No. of days between installation and egg-laying	Remarks
4	no eggs laid	discontinued on day 18
5	" "	" "
6	" "	" "
7	" "	" "
8	" "	" "
9	" "	queen died on day 8

1	no eggs laid	discontinued on day 17
2	" "	" "

COLONY-FOUNDING BY NATURALLY OVERWINTERED QUEENS IN Series II

1. B. terricola One queen per box 1965.

A - no nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	discontinued on day 21
2	18	+	
3	18	+	
4	5	+	
5	14	+	
6	2	+	
7	5	+	
8	6	+	
9	no eggs laid	-	discontinued on day 21

B - with nest material

Box no.	No. of days between installation and egg laying	Adults reared	Remarks
1	no eggs laid	-	successfully reared colony in a later trial
2	" "	-	discontinued on day 21
3	" "	-	discontinued on day 21, but successful in a later trial
4	4	+	considerable larval mortality in first brood

B. terricola contd. Two queens per box 1965

C. - no nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	6	-	one queen died on day 14; egg clumps not on pollen lump
2	5	-	one queen died on day 7; egg clumps not on pollen lump
3	no eggs laid	-	one queen died on day 9; discontinued on day 22
4	3	+	one queen died on day 7
5	3	+	one queen died
6	6	+	one queen died on day 8

D - with nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	one queen died on day 1; discontinued on day 22
2	7	+	eggs laid in antechamber, i.e. not in nest material
3	7	+	all nest material carried to antechamber; one queen died
4	no eggs laid	-	discontinued on day 22

2. B. nevadensis One queen per box 1965
no nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
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1	5	+	
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3. B. perplexus One queen per box 1965
no nest material

Box no.	No of days between installation and egg-laying	Adults reared	Remarks
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1	4	+	all six first brood were queens
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4. B. ternarius One queen per box 1965

A₁ - no nest material, queens installed immediately after capture

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
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1	no eggs laid	-	dissected on day 22; fertile; ovaries well-developed
2	" "	-	dissected on day 22; fertile; ovaries well-developed
3	6	+	first egg batch not reared
4	14	+	
5	7	-	
6	no eggs laid	-	discontinued on day 22
7	3	+	
8	6	+	

B. ternarius contd. One queen per box 1965

A₂ = no nest material, queens confined in a cage for one week prior to installation

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	48	+	
2	6	+	
3	no eggs laid	-	discontinued on day 50
4	" "	-	discontinued on day 50
5	30	+	
6	45	+	
7	30	+	
8	36	-	queen died while brood still larvae

B = with nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	discontinued on day 22
2	" "	-	discontinued on day 22
3	19	+	nest constructed in nest material
4	no eggs laid	-	discontinued on day 22
5	" "	-	discontinued on day 22

B. ternarius contd. Two queens per box 1965

C - no nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	discontinued on day 22
2	6	+	
3	18	+	
4	14	+	
5	8	-	queen died on day 17
6	6	-	brood neglected
7	4	+	
8	5	+	

D - with nest material

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	10	+	eggs laid in antechamber, i.e. not in nest material
2	no eggs laid	-	discontinued on day 22
3	6	+	nest constructed in nest material
4	6	+	eggs laid in antechamber
5	7	-	eggs laid in antechamber; brood not successfully reared
6	8	+	eggs laid in nest compartment but not in nest material

5. B. vagans One queen per box 1965

no nest material

Box no.	No. of days between installation and egg laying	Adults reared	Remarks
1	16	+	
2	not recorded	+	
3	14	-	queen died on day 28

TABLE XXVII

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COLONY-FOUNDING BY NATURALLY OVERWINTERED QUEENS IN Series III

1. B. terricola 1965

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	11	+	set out in the field
2	7	+	
3	11	+	set out in the field
4	17	+	" "
5	9	+	" "
6	2	+	" "
7	7	+	" "
8	6	+	" "
9	no eggs laid	=	queen died on day 6
10	5	+	set out in the field
11	18	+	" "
12	6	+	" "
13	5	+	
14	5	+	
15	13	+	
16	12	+	
17	5	+	
18	3	+	set out in the field
19	4	+	" "
20	4	+	" "
21	4	+	" "
22	7	+	
23	6	+	
24	2	+	
25	6	+	
26	5	+	
27	4	+	
28	3	+	
29	no eggs laid	=	queen died on day 16
30	3	+	

B. terricola contd. 1966

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	3	+	
2	13	+	
3	13	+	
4	11	+	
5	4	+	
6	4	=	eggs laid in tunnel between compartments of nest box
7	9	+	
8	4	+	
9	3	+	
10	5	+	
11	8	+	

2. B. fervidus 1965

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	discontinued on day 20
1966			
1	no eggs laid	-	discontinued on day 30

3. B. borealis 1965

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	5	+	
2	4	+	

1966

1	no eggs laid	-	discontinued on day 30
2	17	-	
3	10	+	

4. B. rufocinctus 1965

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	discontinued on day 30; ovaries well developed
2	3	+	
3	5	-	queen died on day 19
4	4	+	
5	5	+	
6	no eggs laid	-	discontinued on day 30; ovaries well developed
7	11	+	
8	no eggs laid	-	discontinued on day 30; ovaries well developed
9	" "	-	" "

5. B. perplexus 1965

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	16	+	set out in the field
2	2	+	
3	6	+	
4	6	+	
5	2	+	set out in the field
6	8	+	" "
7	6	+	" "
8	12	+	
9	5	+	original queen died; brood reared by a
10	5	+	foster mother

1966

1	2	+
2	2	+
3	2	+
4	2	+
5	3	+
6	2	+
7	1	+

6. B. ternarius 1965

Box no.	No. of days between installation and egg-laying	Adults reared	Remarks
1	no eggs laid	-	discontinued on day 25
2	" "	-	" "
3	" "	-	" "

1966

1	22	+	
2	12	-	queen died on day 18
3	31	+	first-brood mostly male

7. B. vagans 1965

Box no.	No. of days between installation and oviposition	Adults reared	Remarks
1	no eggs laid	-	queen died on day 12
2	" "	-	queen died on day 11