# THE EFFECTS OF THE POSITION AND APPARENT MOVEMENT OF THE SUN AND A COLONY'S QUEEN STATE ON THE ORIENTATION OF DRONE HONEY BEES (*Apis mellifera* L.) TO THEIR HIVES

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© Robert W. Currie

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## ABSTRACT

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The Effects of the Position and Apparent Movement of the Sun and a Colony's Queen State on the Orientation of Drone Honey Bees (*Apis mellifera* L.) to Their Hives. Major Professor: S.C. Jay.

Drones that return from flights often make orientation errors and drift into neighbouring hives. The purpose of this study was to determine if the behaviour of drones orienting to their hives was affected by a colonies queen state.

Drones were marked with individually numbered tags and introduced into colonies. Acceptance of marked drones was highest when drones were introduced into the colonies in the afternoon after being confined within the colony overnight. The proportion of introduced drones accepted by colonies was the highest when 50 drones were introduced into the single storey colonies.

The proportion of drones that drifted between pairs of hives varied according to the colony's queen type and the direction that hives faced. In pairs of hives that faced east or west, drift between queenless colonies did not differ significantly from colonies with virgin queens, but was higher than in queenright colonies.

The direction toward which drift was greater depended upon the direction that the pairs of hives faced. In pairs that faced north or south, a higher proportion of drones tended to drift towards the west than the east, while in pairs that faced east or west a higher proportion of drones tended to drift towards the south than the north. How-

ever, these differences were significant only in south- and east-facing pairs. These trends varied only slightly in colonies with different queen states and in drones that were from 5-25 days old. In south-facing pairs, drones that drifted west did so after longer flights (45 min.) than did drones that drifted east (35 min). The directions that drones drifted were correlated to the position and apparent movement of the sun.

More drones drifted to colonies that contained virgin queens or *trans*-9-oxodec-2-enoic acid than to either queenless or queenright colonies. The attractiveness of virgin queens to drones increased with the age of the drone. More drones were attracted to colonies with virgin queens greater than 7 days old than to colonies with younger virgin queens. The number of drifting drones attracted was correlated with quantitative and qualitative differences in the pheromones produced by different queen types.

Colonies with virgin queens did not retain their own drones but a higher proportion (48%) was attracted back to the colonies with virgin queens on subsequent flights (the same day) than was attracted back to either queenright (10%) or queenless (19%) control colonies. When both hives in a pair had the same queen state tthen the proportion of drones that dirfted between colonies that were queenright , queenless, or had virgin queens was not significantly different. It is proposed that drones that are in colonies with virgin queens may become habituated to the virgin queen's pheromones and this may play a role in preventing inbreeding in feral populations of honey bees.

# CHAPTER I

## INTRODUCTION

Honey bee drones do not forage or participate in colony maintenance or defense. Their only known function is in mating with virgin queens. Virgin queens mate with from 6-17 drones while in flight (Peer 1956; Taber and Wendel 1958; Adams et al. 1977). However, each drone mates only once and then dies shortly after mating (Witherell 1965a).

Virgin queens mate with drones while in flight (Roberts 1944; Peer 1956, 1957; Woyke 1960, 1964). Virgin queens produce a pheromone that attracts drones from distances of up to 60 m (Butler and Fairey 1964). Both mated and virgin queens will attract drones when used in mating lures and mated queens can attract more drones than virgin queens (Boch et al. 1975). There are quantitative and qualitative differences in the pheromone production of mated and virgin queens (Butler and Paton 1962; Boch et al. 1975), but both produce the major component of the sex attractant pheromone 9-oxo-trans-2-decanoic acid. The number of drones attracted by a queen is proportional to the quantity of 9-oxo-trans-2-decanoic acid in the queen's mandibular glands (Boch et al. 1975).

Drones are not attracted to virgin queen pheromone when they are in the hive (Pain 1973) and are not known to mate with virgin queens inside the hive. When virgin queens leave the hive they are not pursued by drones even though the flight activity of drones and virgin queens overlaps (Lensky and Demter 1985). Formerly virgin queens were not thought to attract drones from other colonies or to retain their own drones (Butler 1939; Levents 1951). Recently, however, Currie (1982) observed large numbers of drones drifting to colonies with virgin queens. Therefore colonies with virgin queens may attract drones from other colonies.

The mechanism that prevents drones from pursuing virgin queens from their own colonies as they leave on mating and orientation flights is not known. Formerly it was thought that drones responded to the virgin queen's pheromone only at heights above 10 m (Butler and Fairey 1964), and in some circumstances drones will not chase queens outside of congregation areas (Ruttner and Ruttner 1965a). Recently, however, drones have been observed pursuing lures at 1-2 m (Tribe 1982; Gerig and Gerig 1983), beyond congregation areas (Butler and Fairey 1964; Tribe 1982) and pursuing and mating with queens returning to their colonies (Gary 1961; Ruttner 1966; Dixon 1979).

The position and apparent movement of the sun (Currie 1982) and the absence of a queen (Free 1958) can also influence drone drift and may have significant effects on the amount and direction of drone drift between pairs. Therefore, these factors must be taken into account in experiments designed to test the number of drones attracted to virgin queens from neighboring colonies.

Studies on the drifting of drones are important in the selection of drones for breeding purposes, to determine the potential of drones as disease vectors, to help understand how drones orient and to determine what factors may influence them while in flight. If drones mate with queens from their own colony (i.e. sisters) the inbreeding results in reduced brood viability (Woyke 1963a, b; Page and Laidlaw 1985). If a queen mates with a drone that shares the same sex allele, then the viability of that queen's brood is reduced to 50 percent (Page and Laidlaw 1985). However, queens usually mate with several drones and the sperm mixes in the spermatheca (Laidlaw and Page 1984). If only one of the drones with which the queen mates should possess a sex allele in common with her, then the brood viability of that queen could still approach normal (assuming random mating) (Page and Laidlaw 1985). Therefore, natural selection should favour queens that do not inbreed.

Drones often make orientation errors, when returning to their colony from orientation or mating flights, and drift into other hives (Free 1958, 1961; Witherell 1965b; Currie 1982). Many honey bee breeding programs, such as those that employ top-crossing methods (Page et al. 1985; Page and Laidlaw 1985), require drones of known parental origin. Knowledge of the drifting behaviour of drones is necessary to determine if the drones that are selected from colonies for inseminating queens are the progeny of that colony's queen or if they may be drones that drifted from neighbouring colonies. Drifting drones have also been implicated as vectors of sacbrood (virus), Forest disease (virus), Nosema, acarine mites and varroa mites (Moreaux 1953, 1959; Bailey 1972; Bailey and Fernando 1972; Hanko and Lemkova 1971; De Jong et al. 1982; Tewarson 1983). Transmission of these diseases by drones is of concern because large proportions of drones drift, they frequently drift to more than one hive and they can drift to colonies up to 150 m away (Currie 1982).

Drifting behaviour of bees can also be influenced by the position and apparent movement of the sun (Jay and Warr 1984) and by the type of queen a colony has (Free 1958). Studying the drifting behaviour of drones may provide more information on how drones orient to their hives and how different types of queens can influence their behaviour.

The objectives of this study were: 1. to develop a method for individually marking large numbers of drones; 2. to develop a method for introducing drones that would increase the number of drones accepted and reduce the variability in acceptance between hives with different queen types; 3. to determine if the position and apparent movement of the sun influences the amount or direction of drone drift in pairs of hives facing the four cardinal points of the compass; 4. to determine if drone age or a colony's queen type influences the amount or direction of drone drift in pairs of hives facing the four cardinal points of the compass; 5. to determine if colonies with virgin queens attract drones from neighbouring queenright colonies and if colonies with virgin queens retain their own drones; 6. to determine if the principle component of the queen's sex pheromone, 9-oxo-*trans*-2-decanoic acid, is the major factor that attracts drones; 7. to determine if drone age, and/or queen age, influence the proportion of drones attracted to or retained by colonies with virgin queens.

# CHAPTER II

## LITERATURE REVIEW

Drones are male honey bees (*Apis mellifera* L.) and develop from haploid eggs (Kerr 1974). Drones can develop from diploid eggs (Mackensen 1951), but diploid drone larvae are eaten by workers within a few hours after hatching (Woyke 1965a, b, 1975; Woyke *et al*. 1966; Woyke and Knytel 1966; Woyke 1975). The developmental time from egg laying until adult emergence averages from 24-25 days (Jay 1963; Fukuda and Ohtani 1977).

Reports on the life span of adult drones are highly variable. Averages of 13-14 days (Fukuda and Ohtani 1977; Currie 1982), 21-24 days (Kepena 1963; Drescher 1969; Witherell 1972) and 54 days (Howell and Usinger 1933; Lavrekhin 1947) have been reported. The life span can vary seasonally (Garofalo 1972; Fukuda and Ohtani 1977). Fukuda and Ohtani (1977) found that the average life span of drones ranges from 13 days in the summer to 38 days in the autumn. The mortality rates increase sharply with the beginning of flight activity (Witherell 1972; Fukuda and Ohtani 1977; Currie 1982). Survival decreases with the number of flights made (Witherell 1972). Neukirch (1982) found that the life span of honey bees is dependent upon the flight performance and energy consumption during flight. Therefore the discrepancies between the mean longevity of adult drones in the various studies may reflect differences in environmental conditions in the different geographical regions at different times of the year. Any conditions that reduce flight activity would result in increased longevity of drones. Witherell (1972) feels that predation is an important factor affecting drone longevity and the numbers and types of predators in different regions may also vary.

## Acceptance of Drones

Honey bees regulate the numbers of drones present in the colony by limiting the amount of drone comb constructed, regulating the production of drone brood and by evicting adult drones from the colony. The age and fecundity of the queen influences the construction of drone cells or their conversion into worker cells (Darchen *et al*. 1957), through a pheromone from the queen's mandibular glands (Darchen 1960; Chauvin *et al*. 1961). The presence of queen or worker larvae can also increase the amount of drone comb built (Free 1967).

Drone comb construction is limited by colony size, the time of year and the total number of drone cells already present (Allen 1963; Free and Williams 1975; Free 1977). The presence of drone comb stimulates the rearing of drone brood (Allen 1958). However, even with ample supplies of drone comb, colonies will restrict the amount of drone brood present (Allen 1965; Free 1977). The laying of drone eggs is regulated by the queen (Koeniger 1969, 1970) but workers also regulate the amount of drone brood by destroying and eating drone eggs, larvae and occasionally pupae (Weiss 1962; Free and Williams 1975; Fukuda and Ohtani 1977; Woyke 1977). Production of drone brood can be affected by the queen, the time of year, by reduced intake of pollen and nectar, and by low temperatures (Allen 1958; Gorbaczaw 1961; Allen 1963; Taber 1964; Louveaux *et al*. 1973; Mesquida 1976; Fukuda and Ohtani 1977).

Worker bees also regulate the numbers of adult drones in a colony by evicting drones in the fall and under periods of nectar dearth (Ribbands 1953; Levenets 1956; Ruttner 1956; Weiss 1962). Certain workers specialize in aggressive acts against drones (Free 1957; Dathe 1975). These workers chew and maul drones and sometimes pull them from the hive (Free 1957; Mindt 1962; Morse *et al*. 1967; Ohtani 1974). Expulsion of drones is a very gradual process that takes several weeks in the fall (Morse *et al*. 1967).

Factors that initiate the rejection of drones from colonies include: low temperatures, the presence of a queen, the age of a queen, the amounts of sealed and unsealed brood, odours of drones, the activity of a colony, the amount of forage collected, the amount and condition of honey stores, and the genetic strain (Alber 1955; Levenets 1956; Free 1957; Orosi Pal 1959; Taber 1964; Morse *et al*. 1967; Holmes and Henniker 1972; Free 1977; Woyke 1977). The amount of forage collected and the type of food stored are probably the most important factors controlling drone eviction (Langstroth and Dadant 1922; Phillips 1928; Wedmore 1932; Free and Williams 1975).

The presence of a queen, the amount of forage collected and stored and the environmental conditions at the time of introduction also appear to influence the acceptance of marked drones that are introduced into colonies (Currie 1982). Acceptance of introduced drones can vary between queenless and queenright colonies, between different colonies on the same date and throughout the season (Currie 1982). Currie (1982) found that 36 percent of the marked drones were lost after one day but only 6 percent are lost from the first to the fifth day after introduction. The initial loss of marked drones may have resulted from paint marks coming off, death from injury during marking, transport or introduction of the drones and from rejection of the drones by the workers of a colony. However, the most important cause of drone loss is probably the rejection of drones by workers of a colony (Currie 1982). Witherell (1972) achieved 87% acceptance of marked drones by throwing evicted drones back into the colony, but this method is impractical for large numbers of drones and/or colonies. The proportion of drones accepted might be increased and variability in acceptance between hives reduced if drones could be confined for the first 24 hours or until environmental conditions are favourable.

## Flight Activity

Drone flight activity may begin when adult drones are four days old (Howell and Usinger 1933; Kurennoi 1953a; Kepena 1963). Drones take their first flights when they are from 5-7 days old (Howell and Usinger 1933; Witherell 1970). Eighty to ninety percent of drones make their first flights before 6-12 days of age (Kurennoi 1953b; Kepena 1963) and all drones have usually made their first flight by 18 days of age (Howell and Usinger 1933; Kurennoi 1953b; Drescher 1969).

Most drone flight takes place in a concentrated period during the afternoon and occurs at the same time of day in different geographical locations (Lavrekhin 1960; Taber 1964). The flight of *A. mellifera* drones is temporally separated from drones of *Apis cerana*, *Apis florea* and *Apis dorsata* (Lavrekhin 1960; Ruttner *et al*. 1972; Koeniger and Wijayagunasekera 1976). Flight of *A. mellifera* drones begins between 11:00 and 14:00 hours and ends between 16:00 and 18:00 hours (Kurennoi

1953b; Oertel 1956; Lavrekhin 1960; Ruttner 1966; Drescher 1969; Garofallo 1972; Strang 1971). Peak flight activity occurs between 14:00 to 16:00 hours. Drones can begin flying as early as 09:00 hours (Tuchashivili 1969) and will join swarms that leave the colony between 09:00 and 13:00 hours (Avitable and Kasinskas 1977).

The period of peak flight activity can vary with the time of the year. Drone flight activity begins earlier in the spring and fall than in the summer (Taber 1964; Lensky et al. 1985; Lensky and Demter 1985). Bol'Shakova (1978) found that the duration of flight activity was reduced from 4 hours in the middle of the flight season to 2.5 hours at the end of the season.

The time of day that drone flight activity begins has been correlated with daily temperature, relative humidity, light intensity and may be regulated by a circadian rhythm or an interval timer. However, the exact mechanism regulating drone flight activity is not known (Howell and Usinger 1933; Tuchashivili 1969; Witherell 1970; Lensky et al. 1985). Queen flight activity begins at a fixed number of hours before sunset (Lensky and Demter 1985). Therefore, the flight activity of queens and drones may be regulated by an interval timer that operates using the time from sunset as a cue.

Drones may begin flying earlier in the day in hives where they were prevented from flying on the previous day and in hives with entrances facing towards the southeast (Taber 1964). When weather conditions prevent drones from flying, drone flight occurs earlier on the following day (Oertel 1956; Taber 1964; Lensky et al. 1985). Taber (1964) observed that drones in southeast-facing hives tended to fly earlier than did drones in hives facing southwest.

Drones will not fly if environmental conditions are unfavourable and take only short flights on cloudy or windy days (Witherell 1971). Drones usually will not fly unless temperatures are above 18-20$^{0}$C. They can fly at temperatures as low as 15$^{0}$C, but flights at this temperature last only 1-2 minutes (Drescher 1969; Bol'Shakova 1978; Lensky and Demter 1985). Drone flight is not affected by winds at speeds of up to 25 km/hr, but mating with queens is hindered at wind speeds greater than 18 km/hr (Bol'Shakova 1978). Lensky and Demter (1985) found that no drones or queens flew when wind speeds were greater than 14 km/hr. Released drones cannot return to colonies when temperatures are low and wind speeds are from 8-16 km/hr (Bol'Shakova 1978). If the sky is totally overcast then few drones fly (Witherell 1971; Bol'Shakova 1978; Lensky and Demter 1985).

Drones fly for the purposes of orientation, defecation and mating (Witherell 1971). The first to fifth flights made by drones are usually for orientation and last from 1-6 minutes (Howell and Usinger 1933; Drescher 1969). The first flight of the day tends to be the longest (33 minutes) while second and third flights average 16 and 30 minutes respectively (Witherell 1971). The duration of flights tends to increase with the age of the drone, with the longest flights being taken by the oldest (31-40 day old) drones (Witherell 1971). Drone mating flights last 20-33 minutes (Howel and Usinger 1933; Butler 1939; Drescher 1969; Witherell 1971). The duration of flights also varies with season. Drones tend to take longer flights in the summer (36 minutes) than in the spring (26 minutes) (Garofalo 1972). Drones average 2-4 flights per day (Howell and Usinger 1933; Kurennoi 1953b;

Drescher 1969). The number of flights per day can be as high as 17 (Howell and Usinger 1933).

## Attraction of Drones to Virgin Queens

Drones locate and mate with virgin queens while in flight (Taber and Wendel 1958). Drones orient upwind to a volatile pheromone produced by the queen (i.e. anemotaxis)(Bossert and Wilson 1963; Butler and Fairey 1964). The queen's pheromone attracts drones up to 60 m down wind of her (Butler and Fairey 1964). If the pheromone concentration falls below a minimum threshold the drone flies in random directions until it locates the pheromone again and then continues upwind. The drone will fly upwind until it either sees the queen or loses the queen's scent entirely. Drones must pass within one metre of a queen to see her (Butler and Fairey 1964). In the presence of sex pheromone drones are attracted to dark colours, compact shapes or moving objects (Strang 1970; Gerig 1971). Drones are not attracted to virgin queens while in the colony (Pain 1973).

The queen's sex attractant is thought to attract drones only when drones and queens are in flight, above heights of 5-10 m. (Gary 1962; Ruttner and Ruttner 1971; Pain 1973). The height at which drones are attracted varies inversely with wind speed (Butler and Fairey 1964). On very windy days drones may be found within 2 m of the ground (Tribe 1982). Gerig and Gerig (1976, 1982, 1983) have sampled drones in flight using radio controlled aircraft and found drones within 1-4 m of the ground. A higher proportion of sexually mature drones were found at heights below 4 m (48 %) than higher up (19 %).

Virgin queen's sex pheromones are produced and released primarily from the queen's mandibular glands (Gary 1962, 1963). Morse et al. (1962) found that virgin queens with extirpated mandibular glands could mate. However, Gary (1962) reported that during the removal of the mandibular glands some leakage of the contents was unavoidable. If the glands were not fully removed or if some sex pheromone remained this may explain why drones were able to locate queens with extirpated mandibular glands.

The queen's mandibular gland secretion consists of up to 32 different compounds, 15 of which have been identified (Callow et al. 1964; Simpson 1979). Two components of the mandibular gland are attractive to drones, 9-oxo-trans-2-decenoic acid and 9-hydroxy-decenoic acid (Butler and Fairey 1964). Butler and Fairey (1964) found that 9-hydroxy-decenoic acid attracted 1/4 as many drones as 9-oxo-trans-2-decenoic acid. However, Blum et al. (1971) and Boch et al. (1975) were unable to attract drones with 9-hydroxy-trans-2-decenoic acid, even at high concentrations. Winston et al. (1982) have shown that different enantiomers of 9-hydroxy-(E)-2-decenoic acid affect swarm clustering of worker bees and suggested that the function of this compound in drone attraction should be re-evaluated.

Whole extracts of queen's mandibular glands are slightly more attractive to drones than 9-oxo-trans-2-decenoic acid alone (Gary 1962; Pain and Ruttner 1963; Boch et al. 1975). However, 9-oxo-trans-2-decenoic acid is the major component of the sex pheromone that attracts drones from a distance (Boch et al. 1975). One hundred μg of 9-oxo-trans-2-decenoic acid is as attractive as the quantity of pheromone released by a virgin queen (Butler and Fairey 1964).

There are two isomers of 9-oxo-2-decenoic acid in queen mandibular gland secretions and each has a different function within the colony (Doolittle et al. 1970; Pain 1973). The trans isomer is 200-400 times more attractive to drones than is the cis isomer (Adler et al. 1973). The cis isomer can be photoisomerized to the trans isomer after prolonged exposure to sunlight (Doolittle et al. 1970). However, the cis isomer has no activity in masking the activity of the trans isomer (Doolittle et al. 1970; Blum et al. 1971). No other compounds have been identified that either mask or act synergistically with 9-oxo-trans-2-decenoic acid (Blum et al. 1971). However, fatty acids produced somewhere in the queen's head may act as "keeper substances" to ensure gradual release of the pheromone (Butler 1969; Boch et al. 1975), while other substances produced in the queen's head cause drones to hover near a lure (Boch et al. 1975).

Drones detect the pheromone through highly specific pore plate receptor sites on the antennae that complement the rigid spatial conformation of the molecule (Lacher and Schneider 1963; Lacher 1964; Kaissling and Renner 1968; Vareschi 1971; Blum et al. 1971).

Pheromones that are produced by queens change quantitatively with the age of the queen and following mating (Gary 1961; Butler and Paton 1962; Butler and Fairey 1963, 1964; Butler 1967; Boch et al. 1975; Simpson 1979). Drones are attracted by both virgin and mated queens used as lures (Butler and Fairey 1964; Boch et al. 1975). Mated queens can be more attractive to drones than are virgin queens (Boch et al. 1975). The relative attractiveness of different queens is proportional to the quantity of 9-oxo-trans-2-decenoic acid in their mandibular glands (Boch et al. 1975). Newly emerged virgin queens have very little pheromone, but develop about the same quantity as that found in mated queens by the time they are 5-10 days old (Butler 1961; Butler and Fairey 1964). Boch *et al*. (1975) found that mated queens have more 9-oxo-*trans*-2-decenoic acid than do virgin queens that are 4 to 18 days old.

There are also qualitative differences between the pheromones produced by virgin and mated queens. Mated queens produce pheromones not found in virgin queens such as those that can prevent oogenesis in workers (Butler and Fairey 1963, 1964). Virgin queens have a clearly perceptible odour that may function in the mating process (Renner and Baumann 1964; Boch *et al*. 1975). This odour may be produced by glands located in the abdominal tergites or abdominal sternites; it is not found in very young virgin queens or very young mated laying queens (Renner and Baumann 1964; Boch *et al*. 1975). However, this compound has not yet been identified and its function has not been determined (Boch *et al*. 1975). The Koschewnikow's gland also releases a pheromone when the setaceous membrane of the queen is extruded during the mating flight (Butler 1967; Grandperrin and Cassier 1983). This gland degenerates in older queens (Grandperrin and Cassier 1983).

The honey bee queen's sex attractant pheromone is produced by different species and castes and possibly by drones. 9-oxo-*trans*-2-decenoic acid is produced by queens of *A. cerana*, *A. dorsata* and *A. florea* (Butler *et al*. 1967; Ruttner and Kaissling 1968; Shearer *et al*. 1970). However, the flight times of these species are different (Ruttner and Kaissling 1968). 9-oxo-*trans*-2-decenoic acid is also produced in the mandibular glands of workers in some queenless colonies (Crewe and Velthuis 1980). Drones also produce secretions from their mandibular glands that can attract other drones (Gerig 1972; Lensky et al. 1985). However, these glands degenerate in drones older than 9 days (Lensky et al. 1985).

## Mating

Where drones and queens go on mating flights and the processes that they use in orienting to their colonies are not fully understood. Drones can mate with queens from colonies up to 16.2 km away (Peer and Farrar 1956; Peer 1957). Mating may occur in areas termed "drone congregation areas" or "drone assemblies" (Ruh 1960; Ruttner and Ruttner 1963; Zmarlicki and Morse 1963). Congregation areas are defined as areas where drones gather regularly, irrespective of the presence of a queen, in a location that remains constant over time (Ruttner and Ruttner 1971). There is some evidence to support the theory of drone congregation areas. Drones regularly visit the same area (Ruttner and Ruttner 1963, 1966, 1968), and these areas remain constant over time (Ruttner and Ruttner 1965a, 1968; Strang 1970; Ruttner and Ruttner 1972). Drones produce a pheromone that may be used to attract other drones to congregation areas (Gerig 1972; Lensky et al. 1985). Drones sometimes follow virgin queens vigorously within congregation areas but only short distances beyond them ( Ruttner and Ruttner 1963; Zmarlicki and Morse 1963; Ruttner and Ruttner 1965a). However, Tribe (1982) reported that drones of *A. mellifera adansonii* Latreille followed mating lures for up to 2 km outside of congregation areas. During the honey flow congregation areas of *A. m. adansonii* are virtually indistinguishable from other sites (Tribe 1982).

Drones may rely primarily on visual cues to locate congregation areas (Ruttner and Ruttner 1972; Praagh and Ruttner 1975) but Tribe (1982) believes that wind may be an important cue. The boundaries of congregation areas appear to be marked by some form of vertical relief in the landscape (Strang 1970; Ruttner and Ruttner 1971).

Congregation areas may be an artifact of the experimental techniques used to locate them. Butler (1967) and Strang (1970) created congregation areas artificially by training drones to visit a site where they regularly exposed large amounts of synthetic sex attractant pheromone. Tribe (1982) found that congregation areas were in areas where the topography caused air turbulence that circulated the pheromone more effectively and caused it to be present for a longer period of time. In flat country congregation areas are not well defined (Ruttner and Ruttner 1965b). Quantitative data showing that congregation areas form irrespective of the presence of a queen's pheromones and that queens fly to these areas to mate with drones are required before the theory of congregation areas can be fully accepted.

Alternatively, congregation areas may not be required for mating. Butler and Fairey (1964) suggested that because drones find queens rapidly, that drones are abundant and widely dispersed. They found no areas in which drones congregated. Because queens have an efficient sex pheromone system for attracting drones Butler and Fairey concluded that drones leaving a hive in search of virgin queens probably cruise at random, seldom venturing more than 3 km. from there hives. Little is known about the flight paths of virgin queens.

## Drift and Orientation to the Hive

Little is known about the orientation cues drones use in locating their colonies. Drones fly up to 7 km from their colony (Ruttner and Ruttner 1966). The number of drones that return to their hive appears to decrease as the distance from the hive increases (Levenets 1954; Konopacka 1968). Less than 1 % of the drones returned from 3.2 km while 21 % and 47 % returned from distances of 1600 and 800 m respectively. The direction from which drones are released does not affect the rate of return and drones are capable of returning even with their antennae removed (Oertel 1956). Drones may rely on the use of landmarks, the sun, or on a magnetic compass (or any of the above) to aid in orientation to their colonies (Oertel 1956; Gould et al. 1978; Gould 1980; Currie 1982).

When returning to their colonies drones often make orientation errors and enter the wrong hive. The movement of drones to colonies other than their hive of origin is termed "drifting" (Butler 1939). Some authors reported that levels of drone drift can be quite low (0-12 percent) (Butler 1939; Levenets 1951; Witherell 1965b) while others have found that higher proportions (50-80%) of drones drift (Goetze 1954; Free 1958; Currie 1982). The estimated amount of drone drift varies with the sampling technique used, the method of calculating drift, the age of the drones at the time of sampling, the apiary layout used, the environmental conditions and the topography of the study area (Currie 1982). Drones begin drifting when 5-7 days old and drift at all ages (Currie 1982). Very few workers drift between pairs of hives spaced 1 m apart (Jay 1966) however, the level of drone drift is high even in a paired colony layout (Currie 1982). Drones drift quite frequently (up to 3 or more times) therefore, the amount of drone drift may be underestimated (Currie 1982). Currie (1982) showed that mass marking of drones gives accurate estimates of the proportion of drones drifting when compared to individual marking techniques. However, because drones drift more than once, mass marking does not allow an accurate determination of the amount of drift that occurs on a daily basis, how the number of drones drifting might change over time or how the number of drones drifting might change with the age of drones. Individual marking is required to accurately determine the amount of drone drift and the frequency with which drones drift under different conditions.

## Sun Position

Oertel (1956) believed that drones do not use the sun as an orientation cue. However, Tribe (1982) suggested that drone flight to congregation areas may be influenced by the position of the sun. Currie (1982) found that drones placed in the centre hives of rows that faced N, S, E and W at Glenlea, Manitoba ($49^{0}$ 38' N, $97^{0}$ 09' W), tended to drift southward along east and west facing rows, and westward along north and south facing rows. Drones in pairs of hives that faced south also tended to drift in a westwardly direction. These effects have been observed in worker bees (Jay 1966, 1968, 1971) although there was only a weak tendency for westward movement in north and south-facing hives. Differences in the direction that drones drift appear to be correlated with the sun's position in the sky and its apparent movement throughout the day (Jay 1971; Currie 1982; Jay and Warr 1984), or possibly with a sun-related phenomenon such as a hive-shadow effect (Jay and Warr 1984). At latitudes where the sun passes directly overhead (e.g. Kingston, Jamacia, $18^{0}$ 00' N, $76^{0}$ 45' W; Jay 1971) and at latitudes where the sun passes towards the north of the hive (e. g. Tauranga, New Zealand, 37° 40' S, 176° 12' E, Jay and Warr 1984) the direction in which workers drift remains correlated with the sun's position and its apparent movement across the sky. Vollbehr (1975) found that 5 day old worker bees on orientation flights tend to leave the colony in the direction of the sun and probably approach the home site from the direction of the sun. He hypothesized that a bee would tend to make more orientation errors to hives in the direction of the sun. More research is required to test Vollbehr's hypothesis to determine if this mechanism operates on bees of different ages (Jay and Warr 1984) and if the same mechanisms operate in hives that face different directions.

## The Effect of a Colony's Queen Type on Drift

The presence or absence of a queen as well as the colony's queen type may influence the drifting of drones. The presence of virgin queens within a colony is not thought to affect the number of drones attracted to the colony or retained by the colony (Butler 1939; Levenets 1951). However, Currie (1982) noticed that large numbers of drones drift to colonies which become queenless and in which virgin queens then emerged. Free and Spencer-Booth (1961) found that drift of both workers and drones was higher to colonies that were queenless than to queenright colonies. The differences in the rate of drift may have been due to differences between the number of intruders repelled by queenless and queenright colonies. More research is required to determine if the presence of a virgin queen in a colony can attract drones from other colonies and if colonies with virgin queens retain their own drones. If drift to colonies with virgin queens is greater it should be determined whether the drones are attracted by the virgin queen's pheromones, or if this is caused by differences in the rate of rejection of drones in colonies with different queen types.

## Marking Techniques

Honey bees can be individually marked using several paint marks of more than one colour (Harris 1979), by marking bees with individually numbered coloured plastic or ferrous tags (Gary 1971a), or by marking them with individually numbered tags from photographic prints (Verron and Barreau 1974; Fresneau and Charpin 1977). These techniques have disadvantages when it is necessary to mark large numbers of bees. Plastic tags can be difficult to obtain from the supplier and are available in a limited number of colours (5) and numbers (1-99). Photographic tags are available and can be labeled with any code needed but they are difficult to make. A technique is needed to rapidly mark large numbers of drones with individually numbered tags of up to 8 or more colours.

# CHAPTER III

# MATERIALS AND METHODS

## General Methods

### Drone Rearing

Drones were reared from seven colonies each of which contained a dark strain of queens. Queens were placed on drone comb and enclosed in a single frame queen excluder within each colony. Young worker bees, worker brood and pollen supplements were supplied continuously to the colonies. After a period of two days the drone combs containing eggs were removed from beneath the queen excluder and placed between frames of worker brood within the colony. Thus the queens were allowed to lay eggs only in the drone comb. When the brood was capped it was transferred to other colonies to complete its development. When the drone brood was ready to emerge (after 24 days), all of the adult bees that were on the frames were brushed off and the frames were transferred to an incubator at $30^{0}C$. Drones that emerged in the incubator overnight were marked the following day.

### Marking and Introduction Techniques

Newly emerged drones that showed no signs of external morphological deformities were selected, marked, and randomly assigned to each treatment. Drones were individually marked with uniquely numbered and coloured tags (8 different available colours). Tags were glued to the drone's thorax with a mixture of Cutex[1] ® nail polish and white Aero Gloss[2] ® model airplane dope. Each group of 50 marked drones was stored in a 7x10x5 cm plexiglass cage with screened sides. The drones were fed a solution of sugar syrup (1:1 sugar to water) and water and stored in an incubator at 30°C until introduction into hives.

Drones were introduced into colonies after drone flight activity had ended for the day. A queen excluder was placed between the bottom board of the colony and its bottom box to prevent drones from leaving the hive. The drones were released onto the top bars of the hive while heavily smoking the colonies. The queen excluder was removed the following day to permit drone flight.

## Description of Colonies

All experimental colonies consisted of single chamber Langstroth hives. All hives were painted white, had similar lids and bottom boards and were placed on 9 cm high hive stands. At the beginning of each experiment, colony populations were equalized so that each colony contained six frames of worker bees and 3 frames of brood and each colony was given equal amounts of stored honey and pollen.

## Sampling Method

All frames, lids, sidewalls and bottom boards of each hive were carefully searched early in the morning, before drone flight began, and the numbers and colours of the marked drones present in each hive were recorded.

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[1] Chesebrough Pond's (Canada) Inc. Markham, Ontario. L3P 1W3.
[2] Pactra Inc. Los Angeles, CA. U.S.A. 90028.

Statistical Analyses

The assumptions of the analysis of variance (ANOVA) were tested by using "Bartlett's test for homogeneity of variance" and by plotting the log variance against the log mean (Little and Hills 1978; Southwood 1978). Appropriate transformations were determined by using Taylor's power law (Southwood 1978). However, all data are presented as untransformed means.

## Experiments

The Effects of Marking Technique on the Longevity of Caged Worker Bees

Seven hundred and fifty worker bees of unknown ages were selected from the centre of a cluster of bees of an over-wintered colony during February, 1983. The bees were divided into 30 groups of 25 bees each which were then randomly assigned to five treatments: (1) bees with no anaesthetic, (2) bees anaesthetized and not marked, (3) bees anaesthetized and marked with Aero Gloss ® Dope paint, (4) bees anaesthetized and marked with a plastic tag using Testors[1] ® model glue, and (5) bees anaesthetized and marked with a plastic tag using Cutex ® nail polish (as a glue).

Bees were anaesthetized by placing them in a freezer at $0^{o}C$ for 10 to 15 minutes. Bees (100) of each replicate were anaesthetized at the same time, so that all of the bees in each treatment received the same exposure to the anaesthetic.

Plastic tags were made from Letrafilm Matt[2] ®. Round discs, 2.8 mm in diameter that weighed 2.79 μg were made using a single hole punch.

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[1] Testors Corporation of Canada Ltd. Weston Ontario. M9L 1Z9.
[2] Letrafilm, Letraset Canada Ltd. Markham Ontario. L3R 3L5.

The tags were numbered, using India ink, with a .01-mm technical pen. Two types of glue were used to secure the tags; Testors ® plastic model cement and Cutex ® clear nail polish. The Testors ® plastic model cement was mixed with Liquid paper[1] ® (White) and the Cutex ® clear nail polish was mixed with white Aero Gloss ® model airplane dope, in a ratio of six parts glue to one part colouring, to obtain opaque glues. The tags were then glued onto the bees' thoraces.

Bees were marked with Aero Gloss ® model dope using the technique of Harris (1979). A single mark was applied to the thorax of each bee in each treatment.

The marked bees were placed into 5x7x10 cm plexiglass cages with screened sides. Six cages with 25 bees per cage were assigned to each treatment (*i. e.* 30 cages in all). The bees were fed sugar syrup (1.5:1 sugar to water) and supplied with water *ad libitum*. The cages were randomly distributed throughout an incubator which was maintained at 30°C. Cages were checked daily and the mortality of workers and tag losses recorded.

The data were analyzed using ANOVA on a randomized complete block design. The five treatments were blocked by replicate. Differences between treatment means were compared using orthogonal contrasts (Snedecor and Cochran 1980).

## The Effects of Glue Type on Tag Retention by Marked Drones

Newly emerged drones that had no external morphological deformities were randomly assigned to either treatment. Drones in each treatment were marked with coloured numbered discs available from Chr.

[1] Gillette Canada Inc. Montreal, Quebec, H4P 1A4

Graze Institute[1]. In the first treatment tags were glued onto the drones using the glue supplied with the tags (German Glue) and in the second treatment, the tags were attached with Cutex ® clear nail polish. One hundred marked drones (50 from each treatment) were introduced into each of four colonies on 4 July, 1983 and on 12 August, 1984 and the number of marked drones remaining in each treatment was counted daily, for a period of 5 days.

The data were analyzed using ANOVA in a randomized block design with repeated measurements over time. Introduction dates were treated as blocks and the daily counts were treated as repeated measurements. The data were transformed to log +1. Differences between treatment means were compared by using Tukey's multiple range test (Snedecor and Cochran 1980).

## Factors Affecting Drone Acceptance

Drones were introduced into colonies using two different introduction techniques, each tested at three different times throughout the day. Equal numbers of drones were introduced into each of six colonies that made up the different treatment combinations of a single replicate. The standard introduction technique (no excluder) used by Currie (1982) was compared to a modified technique (excluder) in which queen excluders were used to prevent drones from leaving the hive.

### Technique Used and Time of Day

In the "no-excluder" technique, hardware cloth with 8 mm squares (*i. e*. three squares to the inch) was placed between the brood chamber

[1] Strumpfelbacher Strabezl, Postfach 2107, 7056 Weinstadt-Endersbach, Germany

and an empty hive box. Marked drones were then released onto the hardware cloth, the bees were gently smoked and the lids replaced on top of the empty boxes. When all of the drones had moved down through the hardware cloth and into the hive the hardware cloth and the empty box was removed.

The excluder technique differed from the no-excluder technique in that a queen excluder was placed between the bottom box and the bottom board of each hive. Excluders were put in place at the time of each introduction. The queen excluders were removed before the next period of drone flight began.

The two techniques were compared when drones were introduced at three different times of the day; i. e. in the afternoon (at 14:00 h, C.S.T.), in the evening (at 21:00 h, C.S.T.) and on the following morning (at 07:00 h, C.S.T.). Newly emerged drones were marked and introduced into each of 6 colonies. The experiment was replicated 10 times on six different dates.

### Number of Drones Introduced

Equal numbers of drones were introduced into each hive within a replicate but the number that was introduced varied between replicates. The dates of introduction and the numbers introduced per hive (in brackets) in each replicate were 9 June, 1983 (18), 13 June, 1983 (63), 17 June, 1983 (37), 4 July, 1983 (100), 10 July, 1983 (25, 50, and 95) and 12 Aug, 1984 (25, 50 and 95). Experiments on 10 July, 1983 and on 12 August, 1984 were analyzed separately to determine if variation in the number of drones introduced had an effect on the level of acceptance over time.

All of the hives were examined and the number of drones accepted in each hive was recorded on each of five consecutive days after drones were introduced. The data were analysed using a factorial ANOVA with time as a repeated measurement. Replicates were used as blocks to control for the variation in acceptance between introduction dates. Drones introduced on 10 July, 1983 and on 12 August, 1984 were analysed to determine if variation in the number of drones introduced had an effect on the level of acceptance over time. The data were transformed to square roots + 1/2. Differences between treatment means were compared by using Tukey's multiple range test (Snedecor and Cochran 1980).

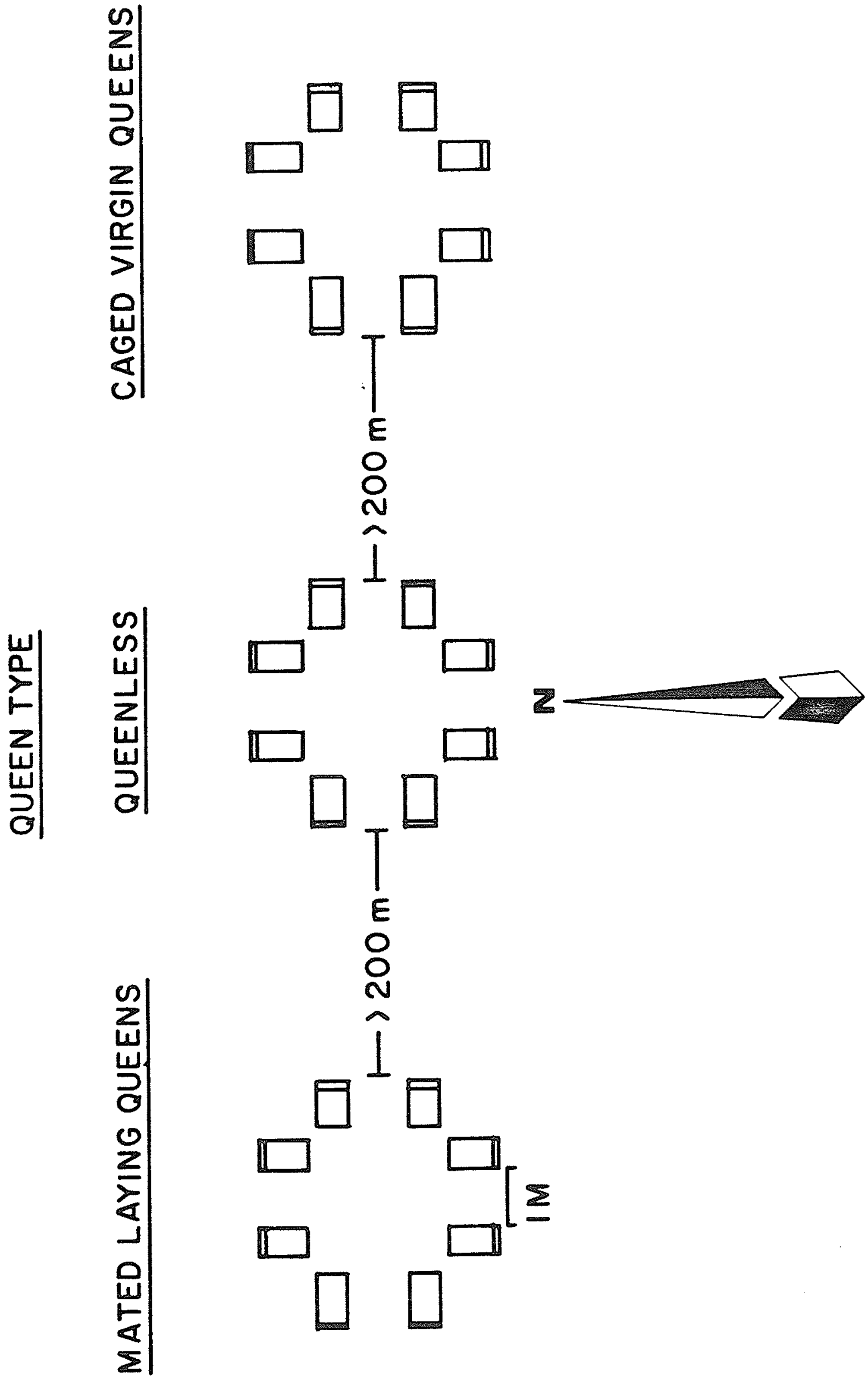
Factors Affecting the Amount and Direction of Drone Drift

The effects that the direction hive entrances face has on the direction of drone drift were studied in paired hives. Four different age groups of drones were tested in paired hives that had three different queen types. The proportion of drones drifting from each hive of a pair was examined over five days.

Twenty-four single-chamber Langstroth hives were placed in 12 pairs. Hives of each pair were spaced 1 m apart, and pairs of hives were separated by a minimum distance of 200 m. The hives were positioned so that the hive entrances of three pairs each faced one of the four cardinal points of the compass. The three pairs that faced each direction each had three different "queen states" (Figure 1). In both hives of a pair the colonies were either queenright, queenless or contained caged virgin queens.

Fifty newly-emerged, individually marked drones were introduced into each hive on each of four different dates, at five-day intervals.

Figure 1. The arrangement of hives to determine effects of the drones age and queen type on the amount and direction of drone drift. Each pair was separated by a minimum distance of 200 m.


QUEEN TYPE
MATED LAYING QUEENS
QUEENLESS
CAGED VIRGIN QUEENS
>200 m
>200 m
1M
N

Five colonies were inspected for the next five consecutive days on which drones flew to determine the number of drones drifting on each day. Thus four age groups, of 5-10 day-old, 10-15 day-old, 15-20 day-old and 20-25 day-old drones were present in each hive and the proportion of drones that drifted was measured on four consecutive days. The proportion of drones that drifted was calculated by dividing the number of drones that drifted from a hive each day by the total number of drones that were found in that hive on the previous day. The entire experiment was replicated on 11 and 17 August 1983.

The colonies were dequeened before the first age-group of drones was introduced in the queenless and the virgin queen treatments. The virgin queens were placed in the colonies on the day before the colonies were first examined for marked drones. Virgin queens were reared using the Doolittle method (Laidlaw and Eckert 1974) and/or by placing frames of worker eggs into queenless colonies. Queen rearing was timed so that 7 day-old virgin queens would be available for each experiment. The virgin queens were caged in 3.5x3.5x2.5-cm queen cages with screens on two sides. The cages were then placed between the centre frames, at the top of each hive, approximately 5 cm from the back of the hive.

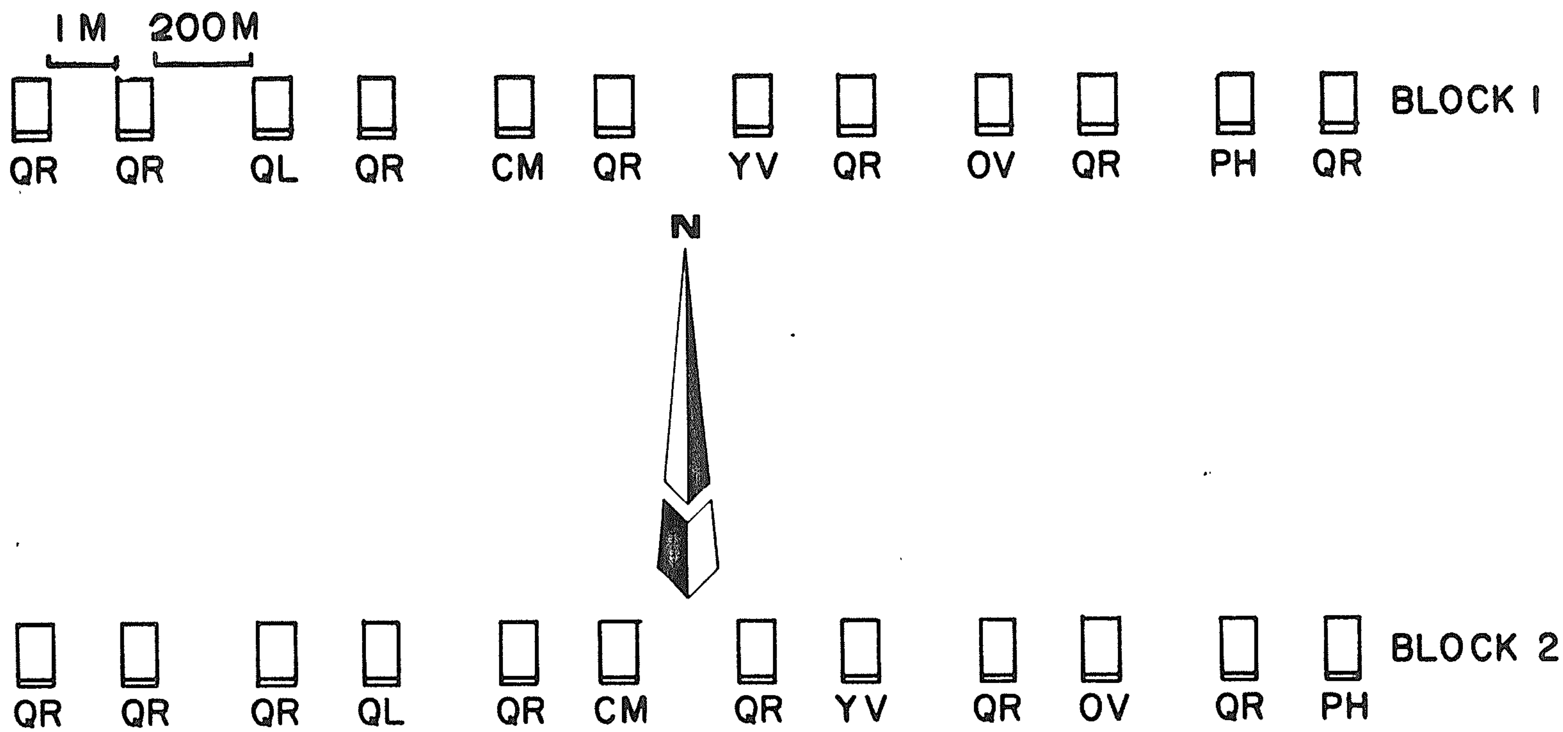
The experiment was arranged in a split-plot design with repeated measurements over time. The queen state and direction that pairs faced were treated as main-plot factors. Daily counts were treated as repeated measurements. Dates on which the experiment was replicated were treated as blocks. A weighted analysis of variance in the logit scale was used to analyse the data (Snedecor and Cochran 1980). A value of 1/2 was added to each cell prior to analysis. Contrasts were used for comparisons between treatments.

Factors Affecting the Relative Number of Drones Attracted to, and Retained by Colonies of Different Queen Types

The colony's queen state was varied and the effects on the proportion of drones drifting between neighboring hives were studied. Four different age groups of drones were tested in paired hives that had 6 different "queen types". Twenty four single-chamber Langstroth hives were randomly assigned to make up 12 pairs of hives that were spaced 1 m apart. Each pair of hives was separated by a minimum distance of 200 m. Colonies were positioned so that all hive entrances faced south. In six of the pairs, the queen types were varied in the west hives of each pair (Block 1) and in the other six pairs the queen types were varied in the east hives of each of the pair (Block 2) (Figure 2). All other hives had mated laying queens. The six different "queen states" tested were mated laying queens, queenless, caged mated queens, 1-7 day old virgin-queens, >7 day-old virgin queens and trans-9-oxodec-2-enoic acid (a component of a queen's pheromones).

Fifty newly emerged, individually marked drones were introduced into each hive on four different dates, at five day intervals. Five days after the last group of drones were introduced, all of the colonies were inspected for the next five consecutive days on which drones flew to determine the number of drones drifting on each day. Thus four age groups, 5-10 day-old, 10-15 day-old, 15-20 day-old and 20-25 day-old drones were present in each hive and the proportion of drones that drifted was measured on each of four consecutive days. The proportion of drones that drifted was calculated by dividing the number of drones

Figure 2. The arrangement of hives to determine effects of queen type on the proportion of drones that were attracted to and retained by colonies. Each pair was separated by a minimum distance of 200 m.




QR = MATED LAYING QUEEN

QL = QUEENLESS

CM = CAGED MATED QUEEN

YV = CAGED YOUNG VIRGIN QUEEN (< 7 DAYS OLD)

OV = CAGED OLD VIRGIN QUEEN (> 7 DAYS OLD)

PH = PHEROMONE (100 μg TRANS-9-OXODEC-2-ENOIC ACID) that drifted from a hive each day by the total number of drones that were found in that hive on the previous day. The entire experiment was replicated on 9 September, 1983, 23 July 6 and 13 August, 1984.

Mated laying queens had unrestricted movement in the colony throughout the experimental period. In each of the five other treatments the colony's queen was removed before any drones were introduced into the hive. Treatments (i.e. queen states) were altered in these colonies on the day before colonies were examined for marked drones. Virgin queens were reared using the Doolittle method (Laidlaw and Eckert 1974) and/or by placing frames of worker eggs into queenless colonies. Queen rearing was timed so that newly emerged and 7 day-old virgin queens were present at the start of each experiment.

Mated and virgin queens were both caged in 3.5x3.5x2.5 cm cages, screened on two sides. The cages were then placed between the centre frames at the top of each hive approximately 5 cm from the back of the hive.

The pheromone, *trans*-9-oxodec-2-enoic acid (Batch number HC 3861-2) was obtained from Galaxo Group Limited[1]. The pheromone was dissolved in methanol and 100 μg/25 μl of the pheromone was applied to 5x5x2 mm square blocks of plastic foam after Boch *et al*. (1975). The pheromone blocks were suspended in the hives from a wire, in the same relative position as were caged queens. Blocks with pheromone were replaced daily.

The experiment was arranged as a factorial ANOVA with repeated measurements over time. Separate analyses were performed on the drones

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[1] Galaxo Group Ltd., Clarges house 6-12 Clarges street, London W1Y 8DH.

drifting to the treated colonies ("attractance") and the drones drifting from the treated colonies ("retention"). The position of the treated colonies were treated as blocks (Figure 2). A weighted analysis of variance in the logit scale was performed on the data (Snedecor and Cochran 1980). A value of 1/2 was added to each cell before the data were analysed. Contrasts were used for comparisons between treatments.

Observations of the Flight Activity and Behaviour of Drifting Drones

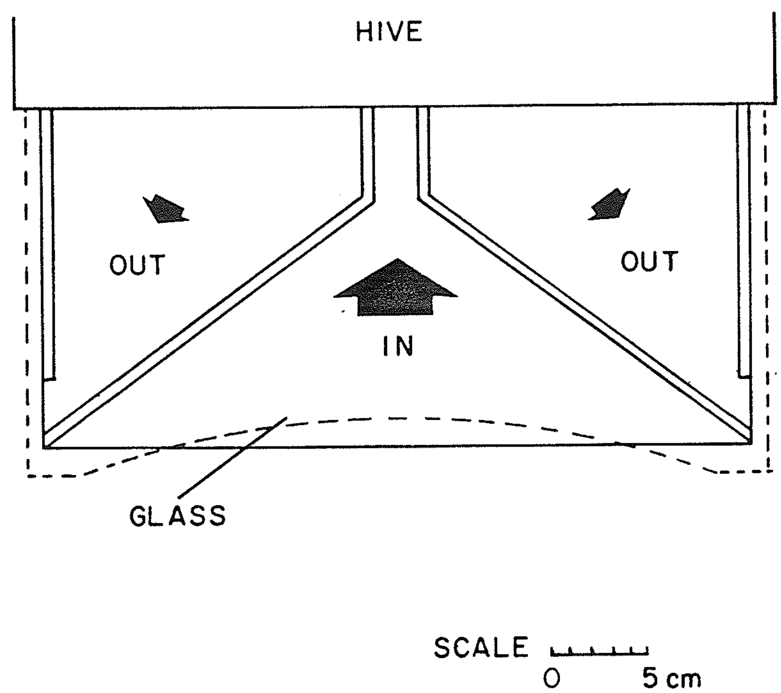
Four different age groups of drones were observed in paired hives with three different queen types. Single-chamber Langstroth hives were spaced 1 m apart and arranged in pairs with entrances facing south. An observation board (Figure 3) was placed on the entrance of each hive to allow an observer to read the tag numbers of drones flying from or returning to, the colony. Two observers were positioned on either side of the pair of hives. The position of each observer remained constant throughout the experiment. Colonies were observed throughout the entire flight period of the drones, and the time for each drone flying from, and returning to, each colony was recorded.

In all other aspects the experiments were as described in the previous section for Block 2 (see Figure 2). Colonies were observed for up to five days and four age groups of drones, 5-10 days old, 10-15 days old, 15-20 days old and 20-25 days old were observed in each hive.

The flight activity of drifting drones was observed in queenright pairs, in a pair with one queenless hive and in a pair where one hive had a virgin queen. The queenless hives and hives with virgin queens were in the east hive of the pair (as in Block 2, Figure 2).

Queenright pairs of hives were observed on 14, 19, 20, 21, and 22

Figure 3. Top view of the modified entrance board that was placed on each hive of a pair to allow observation of the flight activity of drones. The arrows indicate the direction of flow of bees as they returned from or left on flights.


HIVE
OUT
OUT
IN
GLASS
SCALE
0
5 cm

July, 1983; 23, 28, 29, 31 July, 1984 ; on 01, and 03 August, 1984. Pairs with a virgin queen were observed on 06, 08, 10, 11, and 12 August, 1984; pairs with a queenless colony were observed on 13, 14, 15, 16, 17, 18, and 19 August, 1984.

The data on the duration of flights, the time of day when drones flew, the number of flights taken per day, and the flight after which drifting occurred were analysed for individual drones using ANOVA with a randomized block design. The data on the number of flights taken and the flight after which drifting occurred, were transformed to reciprocal square roots and the data on time of day when drones flew and the duration of their flights were transformed to reciprocals. Differences between treatment means were compared by using Tukey's multiple range test ($p<.05$). The frequency distributions were analyzed using a Chi Square based on the number of times that individual drones drifted in the different treatments.

# CHAPTER IV

# RESULTS

## The Effects of Marking Technique on the Longevity of Caged Worker Bees

Differences in the mean longevity of workers were found in the different treatments ($p<.03$, Table 1). Worker bees that were marked using the plastic model cement or nail polish treatments lived significantly longer ($p<.007$) than did the workers marked with Aero Gloss ® dope. The marked bees (from all marking techniques) did not have significantly shorter ($p>.05$) life spans than did the anaesthetized unmarked bees. The life spans of anaesthetized bees did not differ significantly from those of the treated bees ($p>.05$).

Tag-retention times of the marked bees were calculated and compared to the life spans of the workers in the other treatments (Table 1). When the tag retention time was taken into consideration, no significant differences ($p>.05$) between treatments were found.

## The Effects of Glue Type on Tag Retention by Marked Drones

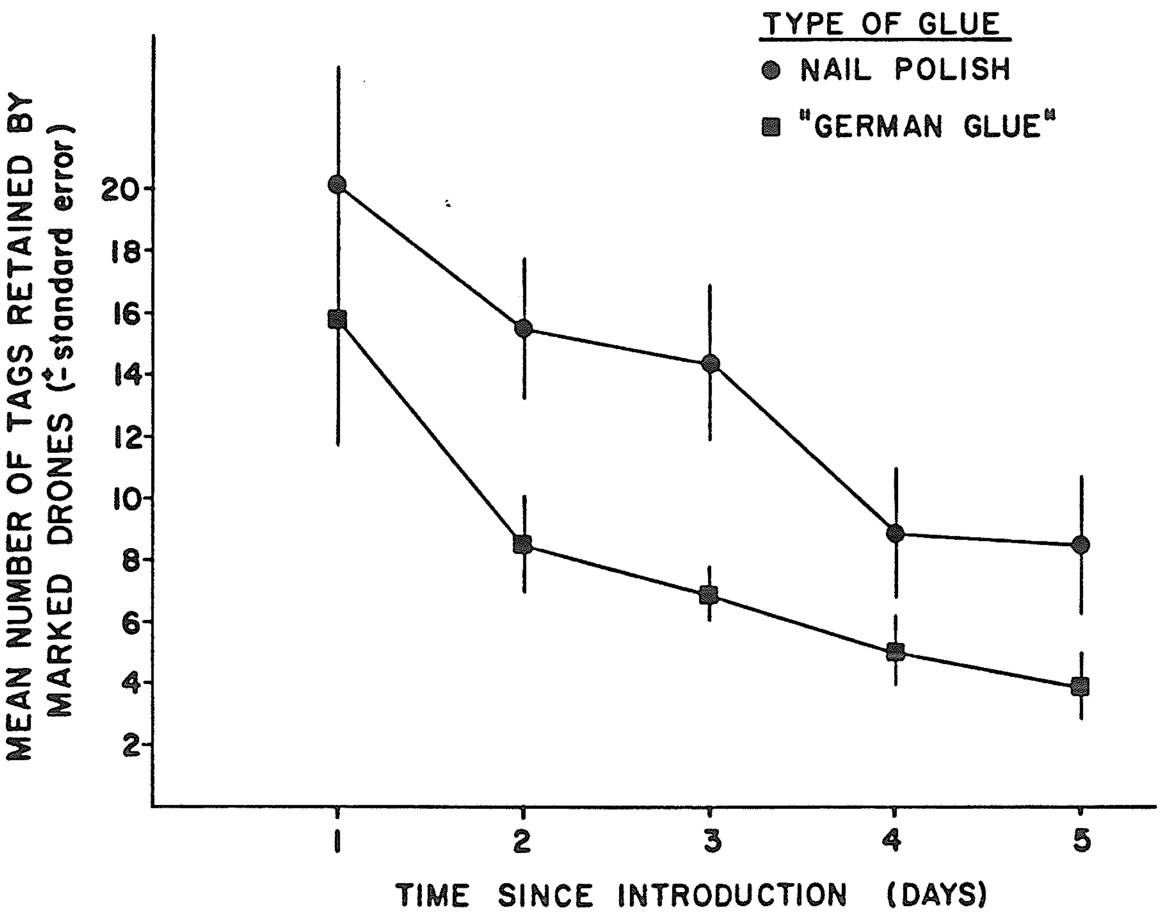
Significantly ($p<.02$) more tags were retained by the drones when the tags were attached with nail polish than with the German glue (figure 4). The relative number of marked drones that retained their tags in either treatment did not decrease significantly ($p>.05$) during the five day period.

TABLE 1. The effect of various marking techniques on the mean longevity and tag retention time from caged worker honey bees (Apis mellifera) taken from overwintered colonies of bees.

| Treatment* | Mean† longevity of worker bees (days)** | Mean tag retention time |
|---|---|---|
| Untreated workers | 10.5±.5 | 10.5±.5 |
| Unmarked workers anaesthetized using cold exposure at $0^0C$ | 9.6±.4 | 9.6±.4 |
| Workers marked with plastic tags and Testors ® glue | 10.4±.5 | 9.8±.5 |
| Workers marked with plastic tags and Cutex ® nail polish | 11.2±.5 | 9.8±.5 |
| Workers marked with Aero Gloss ® dope | 9.1±.6 | 9.1±.6 |

* 150 worker bees/treatment
** Significant differences between means ($p<.05$)
† ± Standard error

Figure 4. Comparison of two different glue types on the retention of tags by newly emerged drones that were marked and then introduced into hives.


TYPE OF GLUE
NAIL POLISH
"GERMAN GLUE"
MEAN NUMBER OF TAGS RETAINED BY MARKED DRONES (±standard error)
20
18
16
14
12
10
8
6
4
2
1
2
3
4
5
TIME SINCE INTRODUCTION (DAYS)

## Factors Affecting Drone Acceptance

### Technique Used and Time of Day

Significantly ($p<.003$) more drones were accepted by colonies when a queen excluder was used than when no excluder was used (Figure 5). The number of drones accepted by colonies also varied with the time of day when drones were introduced ($p<.02$). Acceptance of marked drones was significantly higher ($p<.05$) in the afternoon and evening than in the morning.

There was a significant interaction ($p<.001$) between the introduction technique used and the time of day when drones were introduced (Figure 5). When the excluder technique was used acceptance was highest in the afternoon, but when no excluder was used, the acceptance of introduced drones was highest in the evening. Acceptance of drones was the lowest techniques when drones were introduced in the morning.

The relative number of drones accepted under each introduction technique did not decrease significantly over time (Figure 6). However, there was a significant ($p<.05$) reduction in the number of drones that were accepted over the five day period.

### Number of Drones Introduced

There were significant differences ($p<.005$) in the mean number of drones accepted by colonies when different numbers of drones were introduced (Figure 7). The number of drones accepted after five days was significantly higher ($p<.05$) when 50 or 95 drones were introduced than when 25 drones were introduced. However, the number of drones accepted after five days was not significantly different ($p>.05$) when either 50 or 95 drones were introduced.

Figure 5. The relative effectiveness of two different techniques for introducing drone honey bees into colonies at three different times of the day.

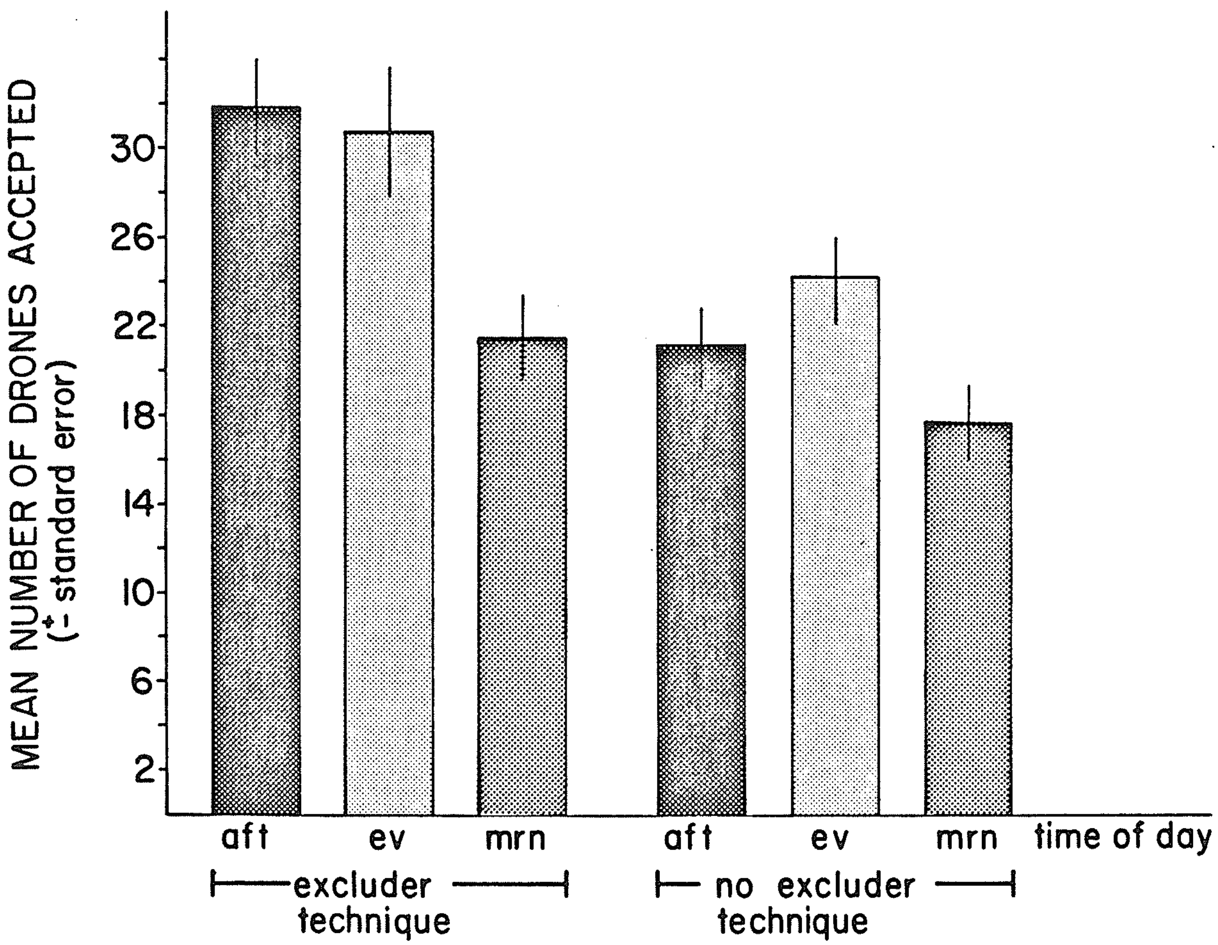
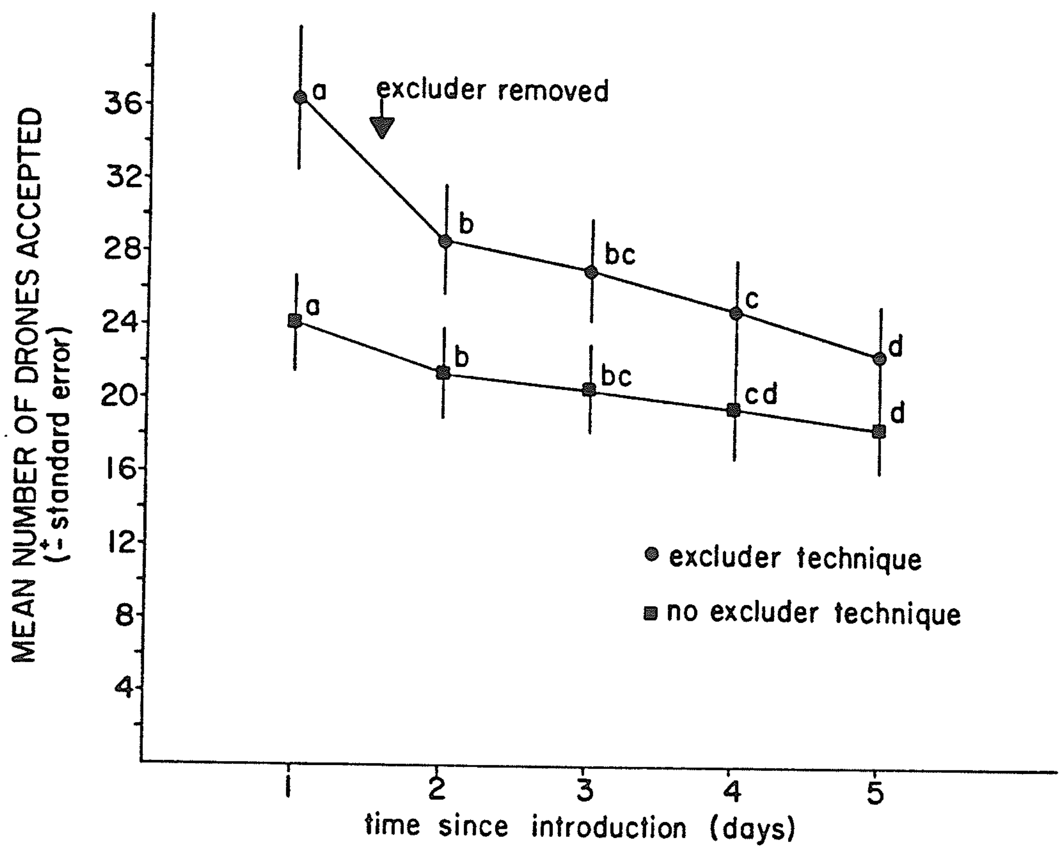
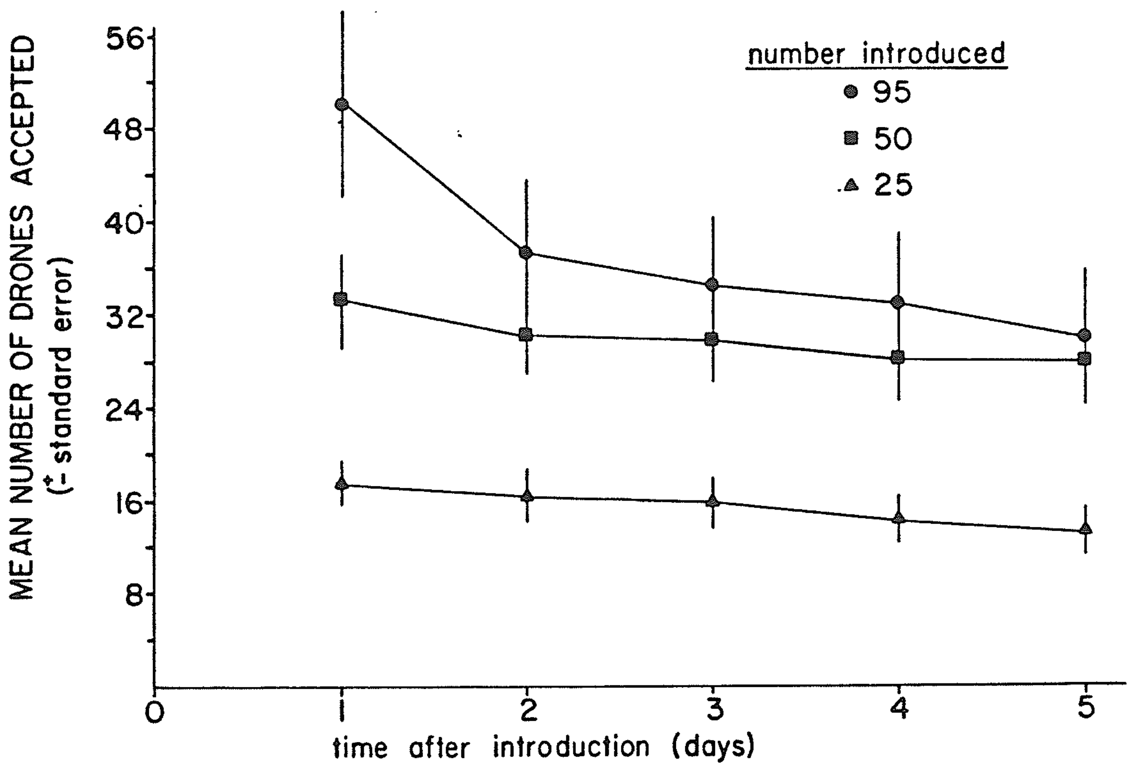

MEAN NUMBER OF DRONES ACCEPTED
(± standard error)
30
26
22
18
14
10
6
2
aft
ev
mrn
aft
ev
mrn
time of day
excluder technique
no excluder technique

Figure 6. The relative effectiveness of two different techniques for introducing drone honey bees into colonies during the first five days after introduction. Points on a line followed by the same letter are not significantly different.



excluder removed
MEAN NUMBER OF DRONES ACCEPTED
(± standard error)
36
32
28
24
20
16
12
8
4
a
b
bc
c
d
a
b
bc
cd
d
excluder technique
no excluder technique
1
2
3
4
5
time since introduction (days)

Figure 7. The effect of the number of drones that were introduced had on the numbers that were accepted on the first five days after introduction.



MEAN NUMBER OF DRONES ACCEPTED
(± standard error)
56
48
40
32
24
16
8
0
1
2
3
4
5
time after introduction (days)
number introduced
95
50
25

There was no significant interaction between the number of drones introduced into colonies and the number accepted by colonies over five days (Figure 7). Acceptance of drones was significantly higher on the first day after introduction (before removal of the queen excluders) than on the following four days.

Factors Affecting the Amount and Direction of Drone Drift

Effect of Introduction Date on the Amount of Drift

The proportion of drones that drifted from hives was significantly ($p<.02$) higher on 17 August than on 11 August (Figure 8). However, the direction that drones drifted in paired hives, did not differ significantly between the two different dates regardless of hive orientation.

Effects of the Direction that Pairs of Hives Faced and the Colony's Queen State, on the Amount of Drift

There was a significant interaction ($p<.0001$) between the colony's queen State and the direction that pairs of hives faced (Figure 9). There were no significant differences between the proportion of drones that drifted from paired hives facing different directions when the colonies had mated laying queens (Figure 9A). However, the proportion of drones that drifted was significantly greater ($p<.05$) in pairs that faced east or west than in pairs that faced north or south when colonies were queenless (Figure 9B) or had virgin queens (Figure 9C).

The proportion of drones that drifted in colonies with different queen types were not significantly different ($p>.05$) in pairs of hives that faced north or south (Figure 9). However, drift from queenless

Figure 8. The effect of date when experiments were replicated on the proportion of drones that drifted between pairs of hives spaced 1 m apart. n= the total number of drones observed (on which proportions were based).

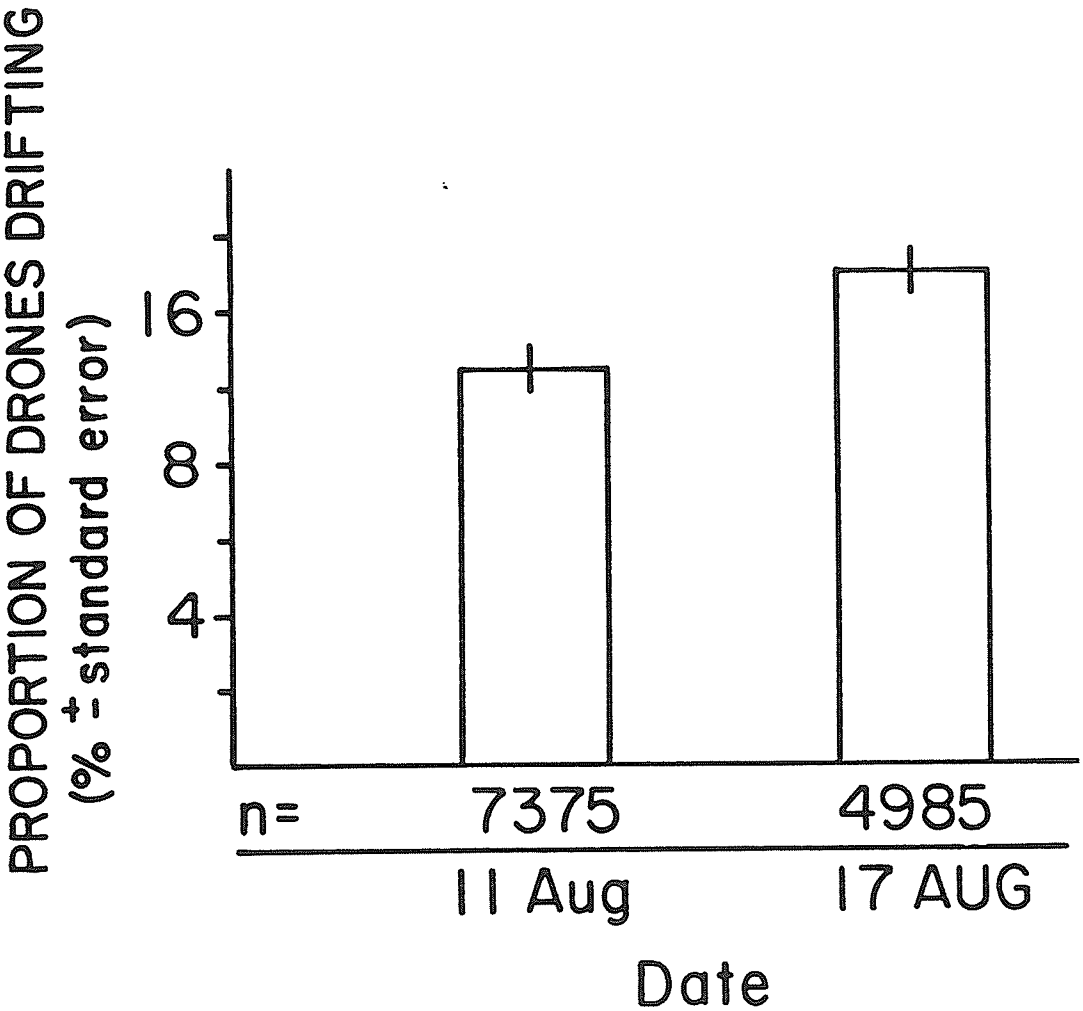
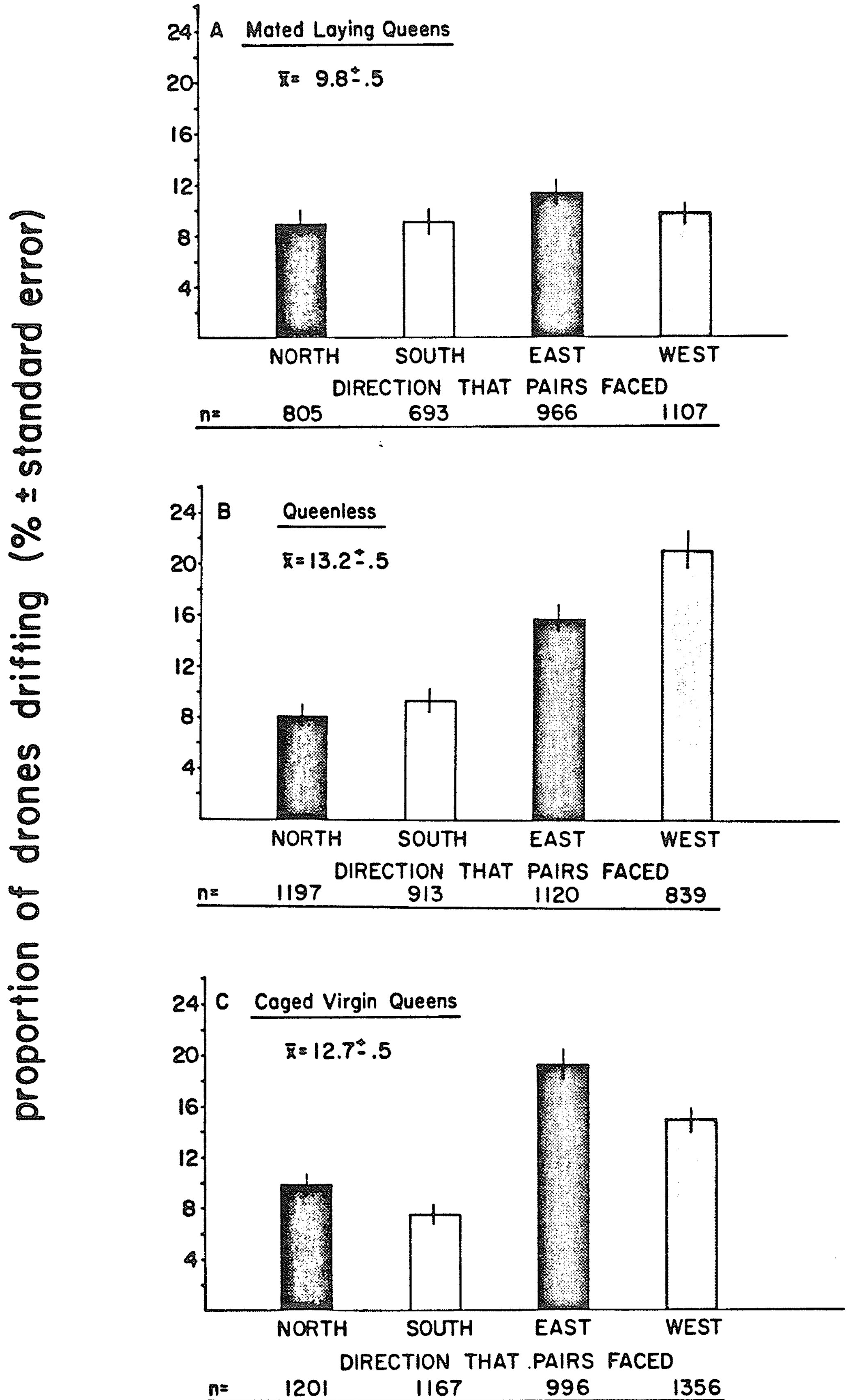

PROPORTION OF DRONES DRIFTING
(% ± standard error)
16
8
4
n=
7375
4985
11 Aug
17 AUG
Date

Figure 9. The effects that queen type and the direction that pairs of hives faced had on the proportion of drones that drifted. n=the total number of drones observed (on which proportions were based). x represents the overall means.


proportion of drones drifting (% ± standard error)
A Mated Laying Queens
x̄ = 9.8 ± .5
NORTH SOUTH EAST WEST
DIRECTION THAT PAIRS FACED
n= 805 693 966 1107
B Queenless
x̄ = 13.2 ± .5
NORTH SOUTH EAST WEST
DIRECTION THAT PAIRS FACED
n= 1197 913 1120 839
C Caged Virgin Queens
x̄ = 12.7 ± .5
NORTH SOUTH EAST WEST
DIRECTION THAT PAIRS FACED
n= 1201 1167 996 1356

colonies (Figure 9B) and from colonies with virgin queens (Figure 9C) was significantly greater ($p<.004$) than drift from colonies that had mated laying queens (Figure 9A) in pairs of hives facing east or west. The proportion of drones that drifted from queenless colonies (Figure 9B) was not significantly different from that of virgin queen colonies (Figure 9C) when pairs faced east or west.

Effect of Drone Age on the Amount of Drift

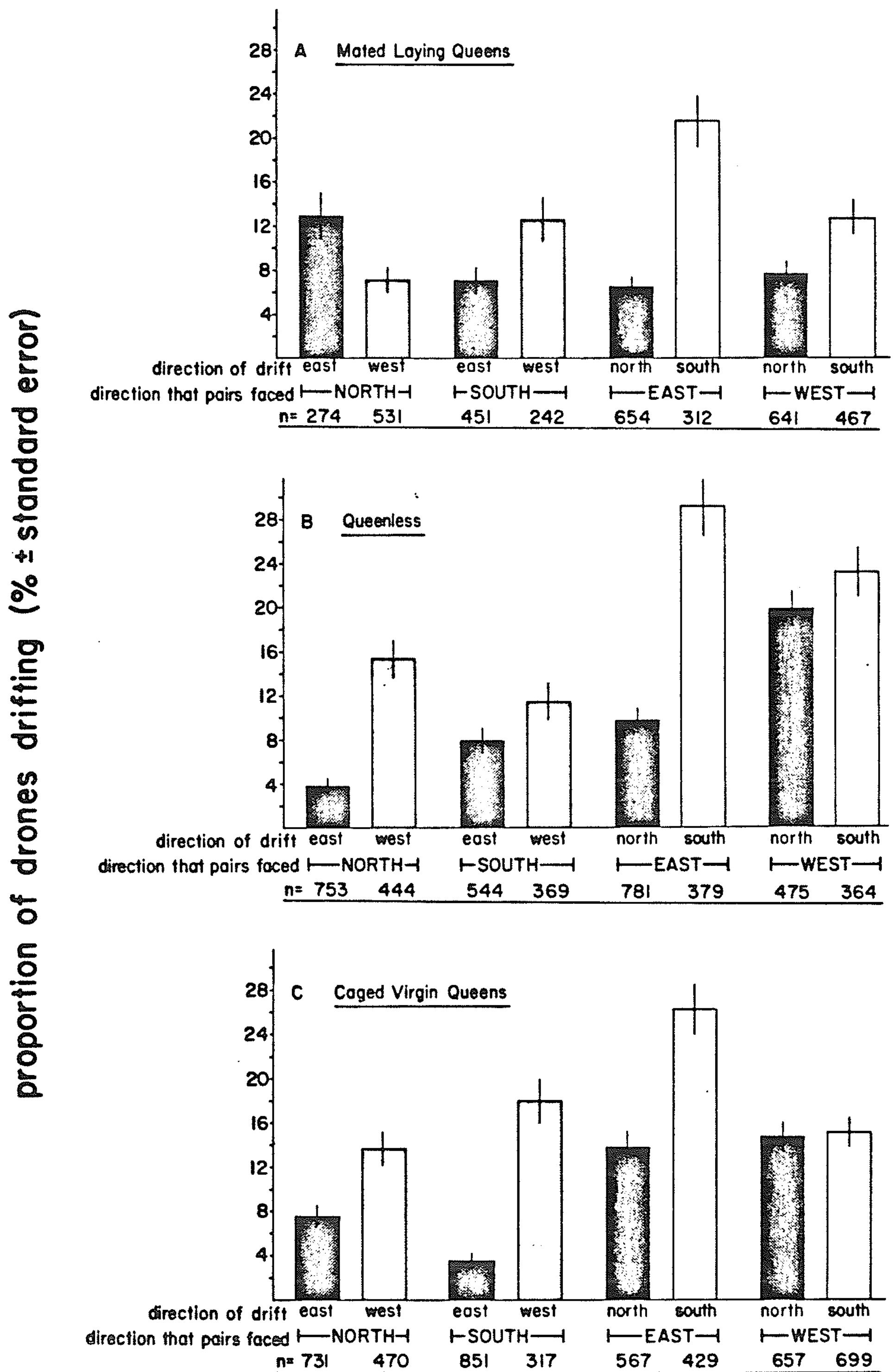
There was no significant ($p>.05$) effect of age group on the proportion of drones that drifted, but the proportion tended to decrease among the older drones (Figures 11A to 11D).

Effect of Queen Type on the Direction that Drones Drift

The direction in which drift was higher varied with the queen type in pairs that faced north ($p<.046$) (Figure 10). The proportion of drones that drifted towards the east tended to be higher than the proportion that drifted towards the west when colonies contained mated laying queens. However, in colonies that were queenless, or had caged virgin queens, the proportion of drones that drifted towards the west tended to be greater than towards the east.

In pairs of hives that faced south (Figure 10), significantly ($p<.005$) more drones drifted towards the west than towards the east. The direction of drift was consistent between colonies with different queen types but the relative numbers of drones drifting in each direction varied ($p<.005$). Westward drift was relatively higher in colonies with virgin queens (Figure 10C) than in colonies that were queenless (Figure 10B).

Figure 10. The effects that queen type and the direction that pairs of hives faced had on the direction of drift. n=the total number of drones observed (on which proportions were based).


proportion of drones drifting (% ± standard error)
A Mated Laying Queens
28
24
20
16
12
8
4
direction of drift east west east west north south north south
direction that pairs faced NORTH SOUTH EAST WEST
n= 274 531 451 242 654 312 641 467
B Queenless
28
24
20
16
12
8
4
direction of drift east west east west north south north south
direction that pairs faced NORTH SOUTH EAST WEST
n= 753 444 544 369 781 379 475 364
C Caged Virgin Queens
28
24
20
16
12
8
4
direction of drift east west east west north south north south
direction that pairs faced NORTH SOUTH EAST WEST
n= 731 470 851 317 567 429 657 699

In pairs of hives that faced east (Figure 10) significantly more ($p<.0001$) drones drifted towards the south than towards the north. This trend did not vary significantly with queen type.

In pairs of hives that faced west (Figure 10) more drones drifted towards the south than towards the north but this trend was not significant and did not vary with queen type.

### Effect of Drone Age on the Direction that Drones Drift

The proportion of drones that drifted towards the west tended to be greater than towards the east in pairs of hives that faced north or south, irrespective of drone age (Figure 11). Higher proportions of drones tended to drift towards the south than towards the north in pairs of hives that faced either east or west (Figure 11). In pairs of hives that faced west a higher proportion of the 20-25 day-old drones drifted towards the north (Figure 11D), but the interaction between drone age and the direction of drift was not significant ($p>.05$).

Drone age had a significant effect ($p<.0001$) on drift in east facing hives. Relatively more 5-10 and 10-15 day-old drones drifted towards the south than did 15-20 day-old drones.

## Factors Affecting the Relative Proportion of Drones Attracted to, or Retained by Colonies With Different Queen Types

### Variation Among Introduction Dates

The proportion of drones that drifted to colonies treated with different "queen types" was significantly different on different introduction dates (Figure 12). Drift to treated colonies was significantly higher on 9 September, 1983 and 23 July, 1984, than on either 6 August,

Figure 11. The effect that the age of drones and the direction that pairs of hives faced had on the direction of drift. n=the total number of drones observed (on which proportions were based).

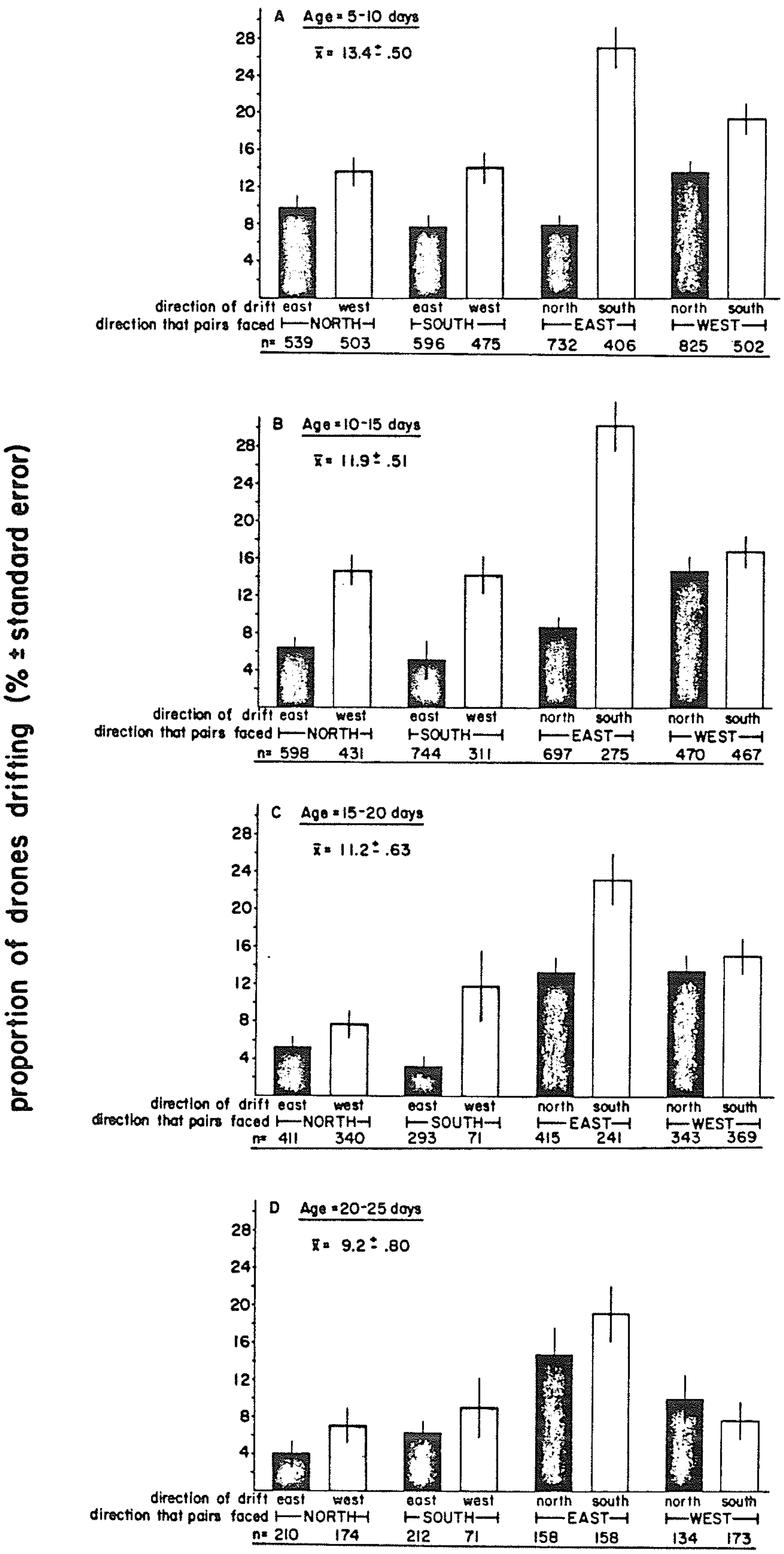
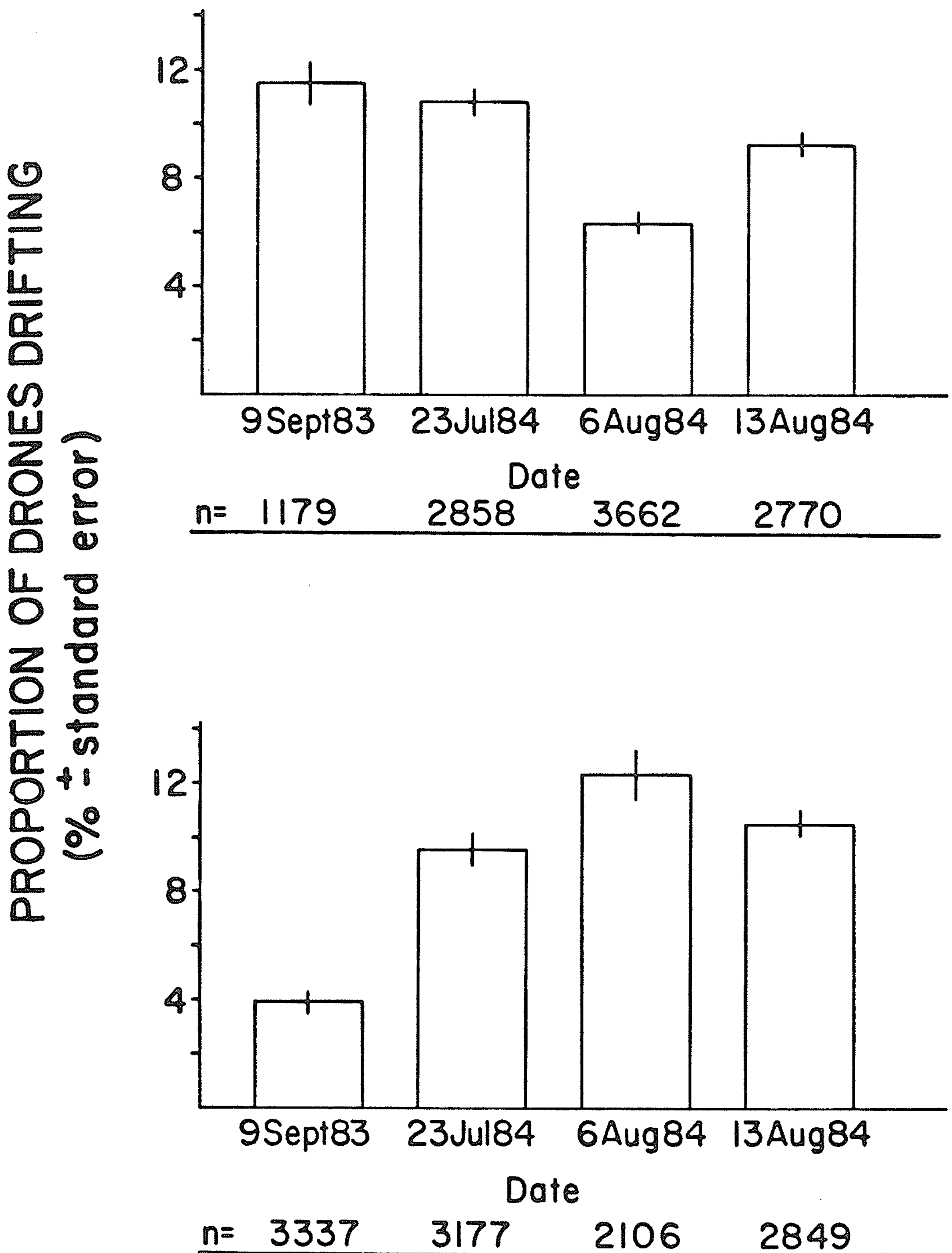

proportion of drones drifting (% ± standard error)
A Age = 5-10 days
x̄ = 13.4 ± .50
direction of drift east west east west north south north south
direction that pairs faced NORTH SOUTH EAST WEST
n= 539 503 596 475 732 406 825 502
B Age = 10-15 days
x̄ = 11.9 ± .51
n= 598 431 744 311 697 275 470 467
C Age = 15-20 days
x̄ = 11.2 ± .63
n= 411 340 293 71 415 241 343 369
D Age = 20-25 days
x̄ = 9.2 ± .80
n= 210 174 212 71 158 158 134 173

Figure 12. The proportion of drones that drifted to all of the colonies treated with different queen types on the four dates when the experiments were replicated. n=the total number of drones observed (on which proportions were based).

Figure 13. The proportion of drones that drifted from all of the colonies treated with different queen types on the four dates when the experiments were replicated. n=the total number of drones observed (on which proportions were based).


PROPORTION OF DRONES DRIFTING
(% ± standard error)
12
8
4
9Sept83
23Jul84
6Aug84
13Aug84
Date
n= 1179
2858
3662
2770
12
8
4
9Sept83
23Jul84
6Aug84
13Aug84
Date
n= 3337
3177
2106
2849

1984 or 13 August, 1984. The proportion of drones drifting to treated colonies on 6 August, 1984 was significantly lower ($p<.05$) than on 13 August, 1984.

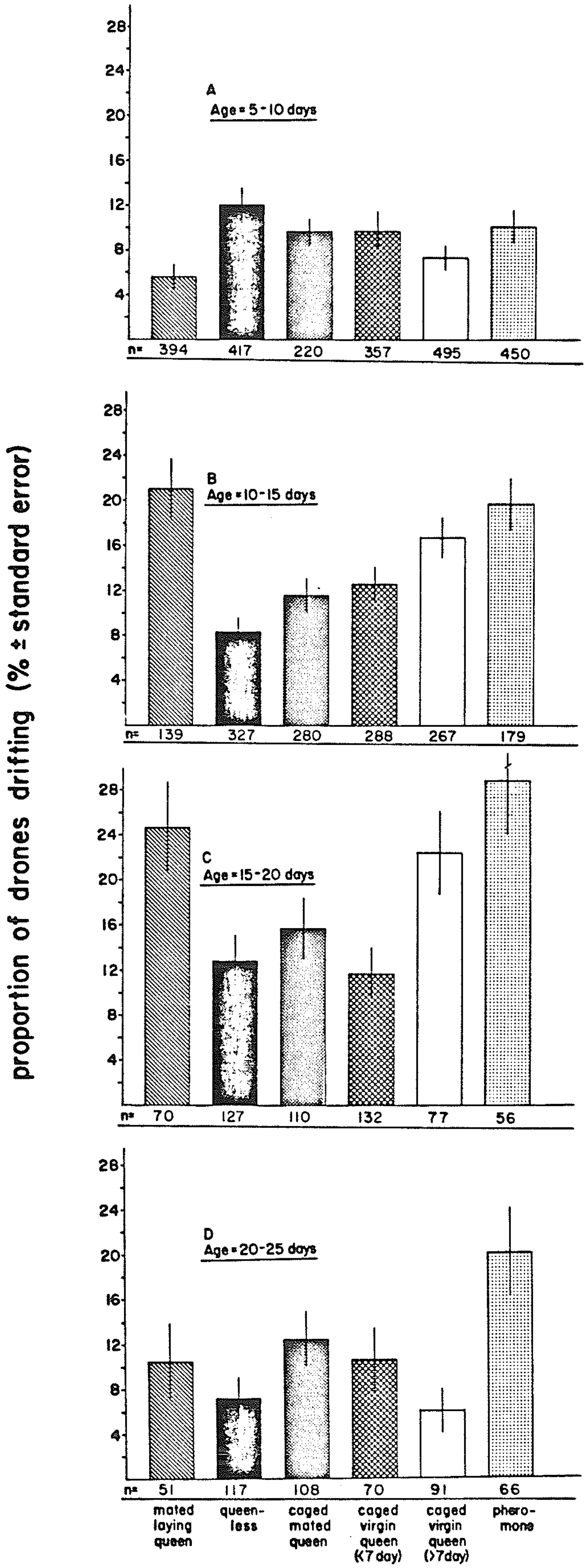
Fewer drones drifted from treated colonies on 9 September, 1983, than on the other dates ($p<.0001$) (Figure 13). Retention of drones by treated colonies was lower on 23 July, 1984, and 13 August, 1984, than on 6 August, 1984 ($p<.0001$).

The Relative Attractiveness of Different Queen Types to Drones that Drifted West

The relative attractiveness of different queen types to drifting drones varied significantly ($p<.0001$) with the position of the treated hive (i. e. the block, in Figure 2) and with the age group of drones sampled.

Both queen type ($p<.0001$) and drone age ($p<.05$) had significant effects on the proportion of drones drifting (Figure 14). More drones drifted to colonies with mated laying queens (queenright colonies) than to queenless colonies ($p<.002$) but drift to queenright colonies did not differ significantly from drift to colonies with mated caged queens ($p>.05$) (Figure 14). Significantly more drones drifted to colonies with virgin queens (to colonies with young virgin queens < 7 days old and to colonies with old virgin queens > 7 days old), than to queenless colonies ($p<.009$). The proportion of drones drifting to colonies with old virgin queens did not differ from colonies with young virgin queens ($p>.05$). Drift of drones to colonies with virgin queens was not significantly different from drift to colonies with caged mated queens, but was lower ($p<.05$) than drift to colonies with the synthetic pheromone.

Figure 14. The proportion of four age groups of drones that drifted west to colonies 1 m away that contained different queen types. n=the total number of drones observed (on which proportions were based).


proportion of drones drifting (% ± standard error)
A
Age = 5-10 days
n= 394 417 220 357 495 450
B
Age = 10-15 days
n= 139 327 280 288 267 179
C
Age = 15-20 days
n= 70 127 110 132 77 56
D
Age = 20-25 days
n= 51 117 108 70 91 66
mated laying queen
queen-less
caged mated queen
caged virgin queen (<7 day)
caged virgin queen (>7day)
phero-mone

The relative number of drones attracted to each queen type varied between different age groups of drones ($p<.0001$) (Figure 14). The proportion of drones that drifted to queenright colonies was higher than the proportion that drifted to colonies with virgin queens when the drones were between 10-20 days old (Figure 14). However, the proportion of drones that drifted to colonies with virgin queens increased ($p<.02$) relative to queenright colonies in the 5-10 and 20-25 day-old age groups of drones.

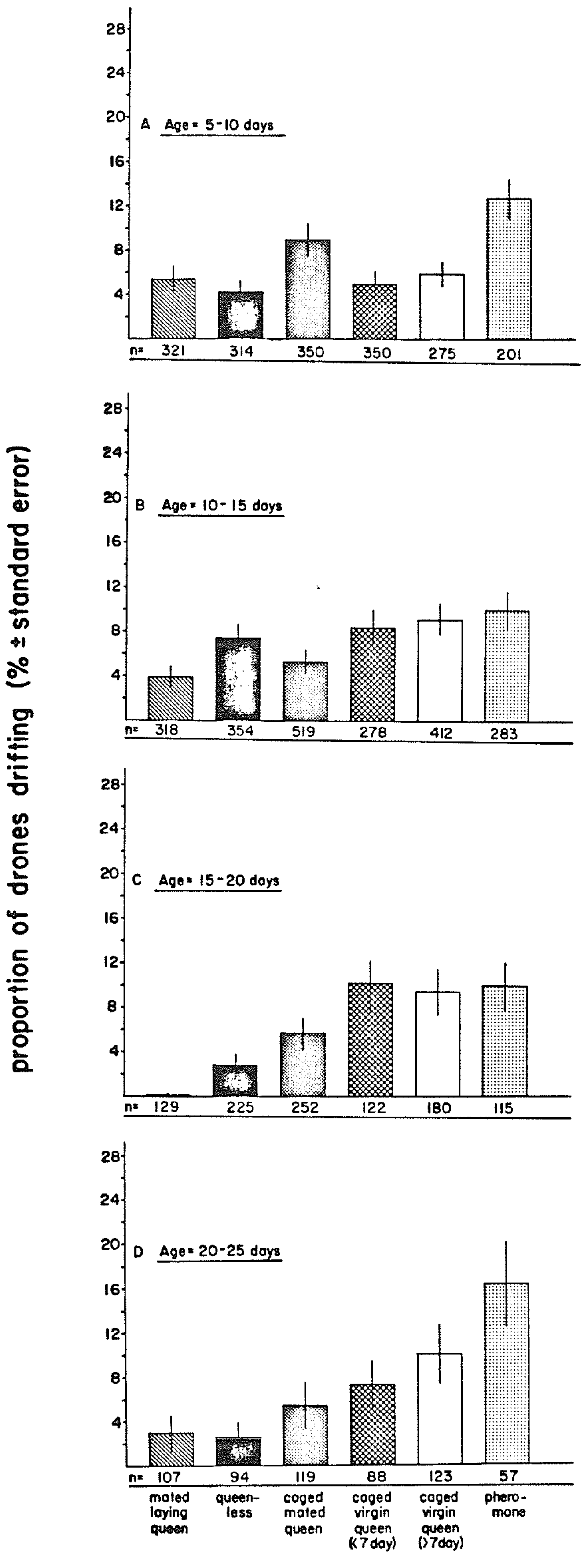
With an in-crease in the age of the drones, the proportion of drones drifting to colonies with caged mated queens or to colonies with pheromone, increased ($p<.02$ and $p<.0001$ respectively) relative to colonies that had virgin queens (Figure 14).

The proportion of drones drifting to colonies with virgin queens increased relative to queenless colonies with an increase in the age of the drones from 5-10 to 20-25 days old ($p<.05$)(Figure 14). The proportions of drones drifting to colonies with young virgin queens did not change relative to old virgin queens in different age groups of drones.

## The Relative Attractiveness of Different Queen Types to Drones that Drifted East

Drone age ($p<.01$) and queen type ($p<.0001$) also had significant effects on the proportion of drones drifting towards the east (Figure 15). Drift to queenright colonies did not differ significantly ($P>.05$) from drift to colonies with caged mated queens or queenless colonies (Figure 15). Significantly more drones drifted to colonies with virgin queens than to either colonies with caged mated queens ($p<.004$) or queenless colonies ($p<.007$). The proportion of drones that drifted to

Figure 15. The proportion of four age groups of drones that drifted east, to colonies 1 m away, that contained different queen types. n=the total number of drones observed (on which proportions were based).


proportion of drones drifting (% ± standard error)
A Age = 5-10 days
n= 321 314 350 350 275 201
B Age = 10-15 days
n= 318 354 519 278 412 283
C Age = 15-20 days
n= 129 225 252 122 180 115
D Age = 20-25 days
n= 107 94 119 88 123 57
mated laying queen
queen-less
caged mated queen
caged virgin queen (<7 day)
caged virgin queen (>7 day)
phero-mone

colonies with old virgin queens was significantly lower ($p<.02$) than the proportion that drifted to colonies with pheromone (Figure 15).

The relative number of drones attracted to each queen type varied between different age groups of drones ($p<.005$, Figure 15). The proportion of drones that drifted to colonies with young virgin queens increased relative to both colonies with caged mated queens ($p<.02$) and queenless colonies ($p<.0015$), as the age group of the drones increased from 5-10 days old to 20-25 days old.

The proportion of drones that drifted towards colonies with old virgin queens increased relative to the proportion drifting to young virgin queens with an increase in the age of drones from 5-10 to 20-25 days old ($p<.05$) (Figure 15). Drift towards colonies with pheromone increased relative to colonies with young virgin queens with an increase in the age of drones from 10-15 to 20-25 days old ($p<.002$).

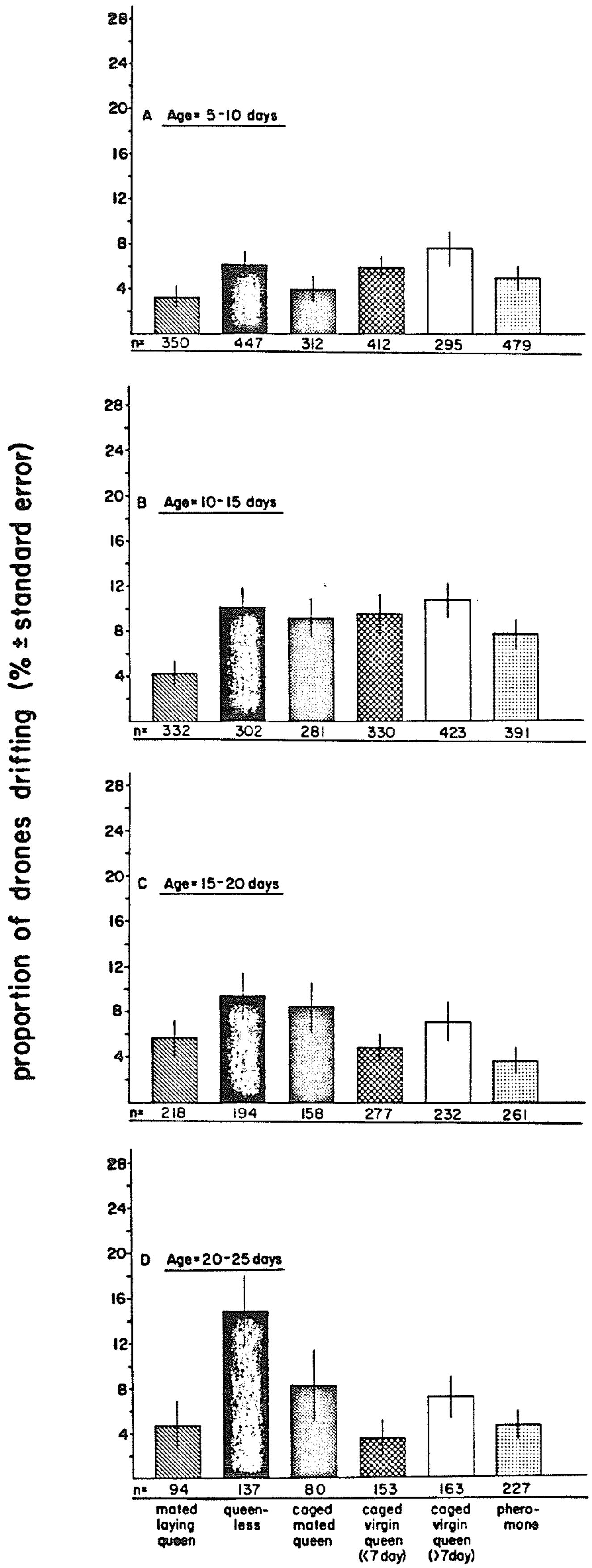
## The Relative Proportion of Drones that Drifted East That Were Retained by Colonies of Different Queen Types

Queen type had no apparent effects on the proportion of drones that were retained by colonies when drones drifted towards the east (Figure 16). The relative number of drones retained by colonies with different queen types did not vary significantly with the age group of the drones. However, the proportion of 10-15 day old drones that drifted was higher ($p<.002$) than 20-25 day old drones.

## The Relative Proportion of Drones that Drifted West that were Retained by Colonies of Different Queen Types

Both queen type ($p<.004$) and drone age ($p<.0001$) had significant effects on the proportion of drones retained by colonies of different

Figure 16. The proportion of four age groups of drones that drifted east, away from colonies that contained different queen types to queenright colonies that were 1 m away. n=the total number of drones observed (on which proportions were based).


proportion of drones drifting (% ± standard error)
A Age= 5-10 days
n= 350 447 312 412 295 479
B Age= 10-15 days
n= 332 302 281 330 423 391
C Age= 15-20 days
n= 218 194 158 277 232 261
D Age= 20-25 days
n= 94 137 80 153 163 227
mated laying queen
queen-less
caged mated queen
caged virgin queen (<7 day)
caged virgin queen (>7 day)
phero-mone

queen types when drones drifted towards the west (Figure 17). Drift from queenright colonies was significantly lower ($p<.025$) than from colonies with caged mated queens. The proportion of drones that drifted from colonies treated with pheromone was significantly lower than drift from colonies with virgin queens ($p<.0006$).

The relative number of drones retained by colonies with different queen types varied with the age groups of the drones ($p<.001$) (Figure 17). The proportion of drones that drifted from colonies with virgin queens increased relative to queenright colonies as the age of the drones increased ($p<.04$) from 5-15 days to 20-25 days. Drift from colonies with young virgin queens increased ($p<.05$) relative to colonies with caged mated queens as the age of the drones increased from 15-20 days to 20-25 days.

## Observations on the Flight Activity of Drifting Drones

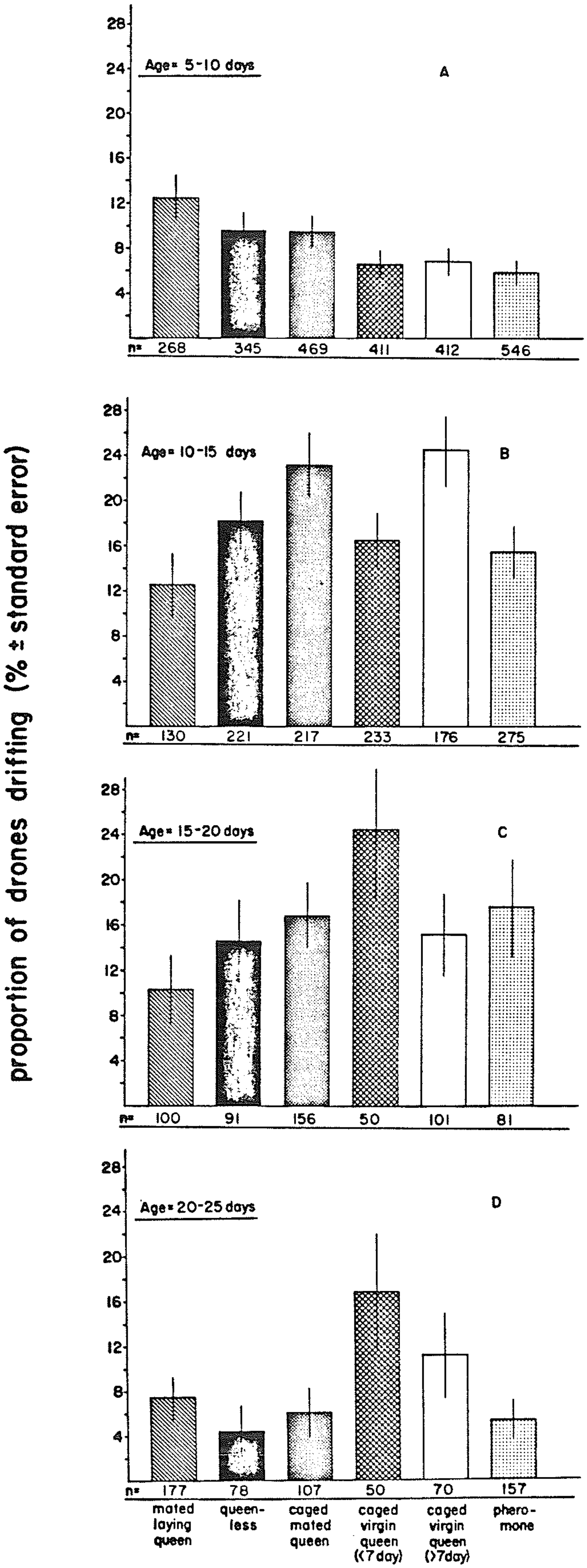
### Daily Pattern of Flight Activity

Drone flight activity occurred between 12:00 and 17:45 (Central Standard Time)(Figures 18-21). The peak period of flight activity occurred at the same time of the day during each time period when the observations of the different treatments took place (between 14 July and 19 August).

### Number of Flights Taken Per Day

The number of flights taken by drifting drones did not vary significantly between the three different queen types or among drones that drifted in different directions (Table 2). The number of flights taken per day varied significantly among different age groups of drones in the

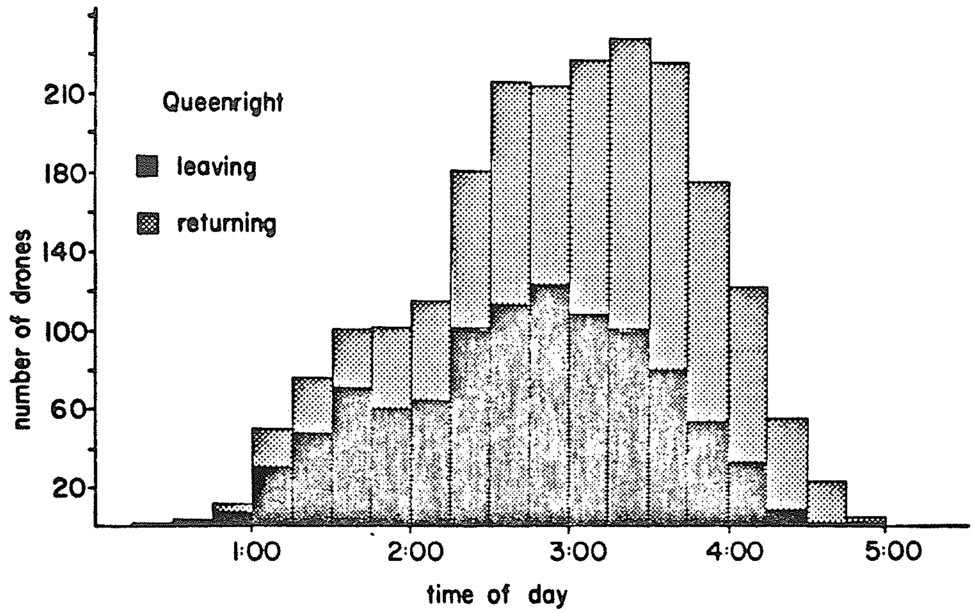
Figure 17. The proportion of four age groups of drones that drifted west, away from colonies that contained different queen types to queenright colonies that were 1 m away. n=the total number of drones observed (on which proportions were based).



proportion of drones drifting (% ± standard error)
Age= 5-10 days
A
n= 268 345 469 411 412 546
Age= 10-15 days
B
n= 130 221 217 233 176 275
Age= 15-20 days
C
n= 100 91 156 50 101 81
Age= 20-25 days
D
n= 177 78 107 50 70 157
mated laying queen
queen-less
caged mated queen
caged virgin queen (<7 day)
caged virgin queen (>7day)
phero-mone

Figure 18. The frequency distribution denoting the time of day when all marked drones were observed leaving on, and returning from, flights from pairs of queenright hives. Hives were observed from 14-22 July, 1983.

Figure 19. The frequency distribution denoting the time of day when all marked drones were observed leaving on, and returning from, flights from pairs of queenright hives. Hives were observed from 23 July to 03 August, 1984.


Queenright
leaving
returning
number of drones
210
180
140
100
60
20
1:00
2:00
3:00
4:00
5:00
time of day

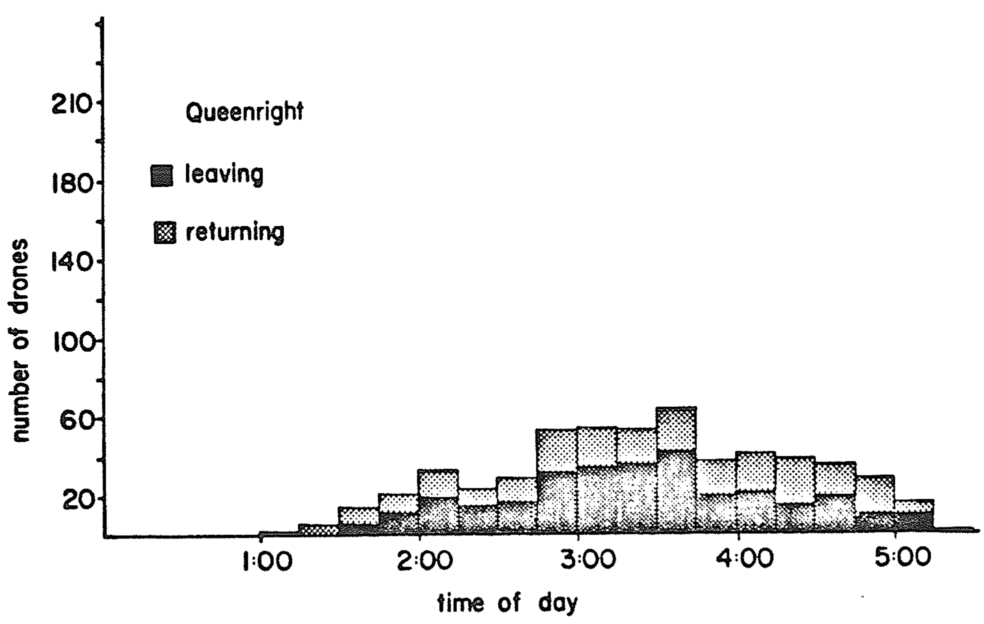
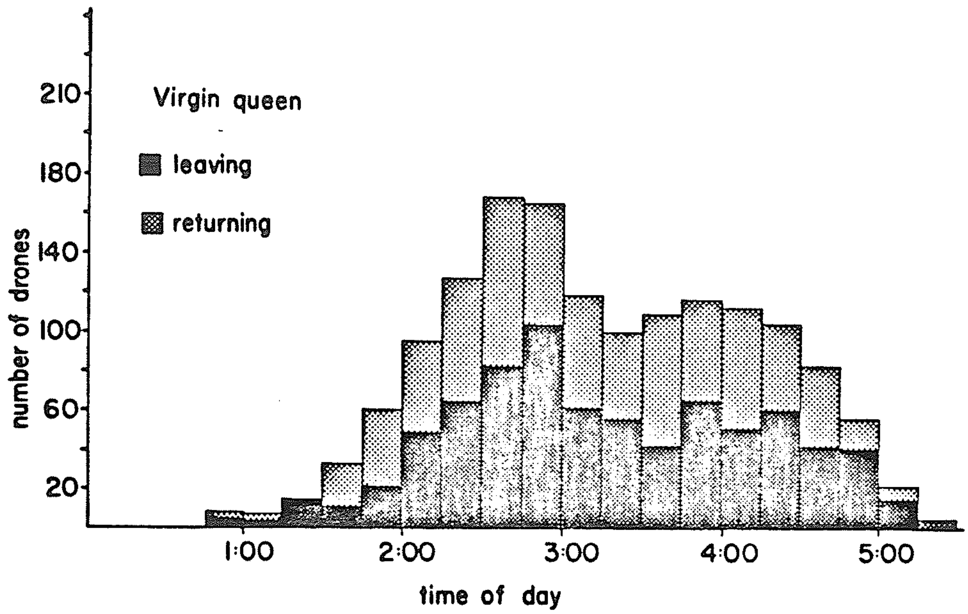

Queenright
leaving
returning
number of drones
210
180
140
100
60
20
1:00
2:00
3:00
4:00
5:00
time of day

Figure 20. The frequency distribution denoting the time of day when all marked drones were observed leaving on, and returning from, flights from pairs with one queenright colony and one colony with a virgin queen. Hives were observed from 06 to 12 August, 1984.

Figure 21. The frequency distribution denoting the time of day when all marked drones were observed leaving on, and returning from, flights from pairs with one queenright colony and one queenless colony. Hives were observed from 13 to 19 August, 1984.


Virgin queen
leaving
returning
210
180
140
100
60
20
number of drones
1:00
2:00
3:00
4:00
5:00
time of day

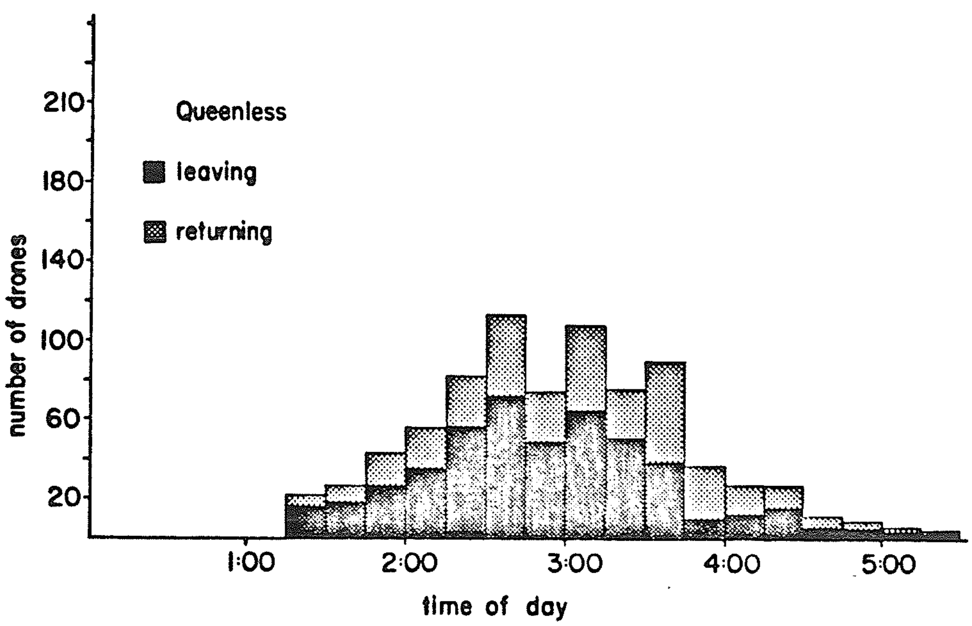

Queenless
leaving
returning
210
180
140
100
60
20
number of drones
1:00
2:00
3:00
4:00
5:00
time of day

TABLE 2. The mean number of flights per day (± standard error)[1] taken by drones that drifted to colonies with different queen types, in pairs of hives that faced south.[2]*

| Type of queen in colonies from which drones drifted | Type of queen in colonies to which drones drifted | Direction of drift | Age group of drones 5-10 | 10-15 | 15-20 | 20-25 | Overall Average |
|---|---|---|---|---|---|---|---|
| Queenright | QR | east | 2.5±.65 (10) | 3.8±.49 (18) | 2.7±.73 (8) | 4.2±0.92 (5) | 3.3±.30 |
| Queenright | QR | west | 3.0±.55 (14) | 3.7±.43 (23) | 2.1±.60 (12) | 5.0± (1) | 3.4±.47 |
| | | subtotal | 2.7±.43a | 3.8±.32a | 2.4±.47a | 4.6±1.1b | |
| Queenright | VQ | east | – (–) | 4.0±.29 (52) | 2.3±.52 (16) | 3.2±.53 (15) | 3.1±.27 |
| Virgin queen | QR | west | – (–) | 4.0±.29 (51) | 3.1±.65 (10) | 3.0±.52 (16) | 3.4±.30 |
| | | subtotal | – | 4.0±.20b | 2.7±.42a | 3.1±.37ab | |
| Queenright | QL | east | 4.4±.41 (17) | 3.3±.84 (6) | – (–) | – (–) | 3.9±.56 |
| Queenless | QR | west | 4.0±.42 (24) | 3.2±.57 (13) | – (–) | – (–) | 3.61±.41 |
| | | subtotal | 4.2±.33a | 3.2±.51a | – | – | |

[1] Number of drones observed in brackets.
[2] Means followed by the same letter within subtotal rows are not significantly different.
* Dashes represent missing observations.

queenright ($p<.05$) and virgin queen ($p<.01$) treatments.

## Flight After Which Drifting Occurred

The flight on which drones drifted did not vary with the type of queen or with the direction in which drones drifted (Table 3). The flight on which drones drifted varied significantly ($p<.03$) with the drones' ages in the queenright colonies.

## Time of Day When Drifting Occurred

The time of day when drifting occurred did not vary with the queen type or with the direction in which drones drifted (Table 4). The time of day when drift occurred, varied significantly ($p<.05$) with the age of the drone in the queenright and queenless treatments.

## Duration of Flights of Drifting Drones

The lengths of flights did not vary significantly among queen states or between different age groups of drones (Table 5). However, the length of flights of drones drifting in different directions did vary significantly ($p<.02$) with the age of the drone in queenright pairs. The length of the flights of drones that drifted west was significantly longer in the 10-15 ($p<.05$) and 15-20 ($p<.02$) day old drone group than between the same age groups drifting east.

## The Frequency of Drift

The frequencies of drones drifting towards the west varied significantly between the pairs that were treated with different queen types ($p<.02$) (Table 6). Nineteen percent of the drones from the queenless colonies and 10 % of the drones from the queenright colonies drifted

TABLE 3. The mean flight of the day after which drones drifted (± standard error)[1] to colonies with different queen types, in pairs of hives that faced south.[2]*

| Type of queen in colonies from which drones drifted | Type of queen in colonies to which drones drifted | Direction of drift | Age group of drones 5-10 | 10-15 | 15-20 | 20-25 | Overall Average |
|---|---|---|---|---|---|---|---|
| Queenright | QR | east | 1.7±.46 (10) | 2.3±.34 (18) | 1.8±.51 (8) | 3.6±.64 (5) | 2.3±.19 |
| Queenright | QR | west | 1.7±.39 (14) | 2.0±.30 (23) | 1.5±.42 (12) | 4.0 (1) | 2.3±.32 |
| | | subtotal | 1.7±.30a | 2.1±.23a | 1.6±.33a | 3.8±.79b | |
| Queenright | VQ | east | - (-) | 2.5±.20 (52) | 1.6±.36 (16) | 2.3±.37 (15) | 2.2±.20 |
| Virgin queen | QR | west | - (-) | 2.5±.20 (51) | 2.4±.46 (10) | 2.2±.36 (16) | 2.4±.22 |
| | | subtotal | | 2.5±.14a | 2.0±.29a | 2.2±.26a | |
| Queenright | QL | east | 2.2±.35 (17) | 2.8±.59 (6) | - (-) | - (-) | 2.5±.35 |
| Queenless | QR | west | 2.4±.29 (24) | 2.0±.40 (13) | - (-) | - (-) | 2.2±.25 |
| | | subtotal | 2.3±.23a | 2.4±.36a | | | |

[1] Number of drones observed in brackets.
[2] Means followed by the same letter within subtotal rows are not significantly different.
* Dashes represent missing values.

TABLE 4. The time of the day (hours) when drones drifted (± standard error)[1] to colonies with different queen types, in pairs of hives that faced south.[2]*

| Type of queen in colonies from which drones drifted | Type of queen in colonies to which drones drifted | Direction of drift | Age group of drones 5–10 | 10–15 | 15–20 | 20–25 | Overall Average |
|---|---|---|---|---|---|---|---|
| Queenright | QR | east | 15.43±.82 (10) | 15.22±.61 (18) | 15.63±.92 (8) | 16.71±1.2 (5) | 15.75±.17 |
| Queenright | QR | west | 15.25±.69 (14) | 15.67±.54 (23) | 15.94±.75 (12) | 16.24 (1) | 15.77±.27 |
| | | subtotal | 15.34±.54a | 15.45±.41a | 15.78±.59ab | 16.47±1.4b | |
| Queenright | VQ | east | – (–) | 15.26±.36 (52) | 15.43±.65 (16) | 15.54±.67 (15) | 15.41±.45 |
| Virgin queen | QR | west | – (–) | 16.28±.36 (51) | 15.61±.82 (10) | 15.69±.65 (16) | 15.85±.49 |
| | | subtotal | – | 15.77±.26a | 15.52±.52a | 15.62±.47a | |
| Queenright | QL | east | 14.82±.63 (17) | 15.64±1.1 (6) | – (–) | – (–) | 15.23±.18 |
| Queenless | QR | west | 15.16±.53 (24) | 15.47±.72 (13) | – (–) | – (–) | 15.31±.13 |
| | | subtotal | 14.99±.41a | 15.55±.64b | – | – | |

[1] Number of drones observed in brackets.
[2] Means followed by the same letter within subtotal rows are not significantly different.
* Dashes represent missing values.

TABLE 5. The mean duration of flights (hours) of drifting drones (± standard error)[1] to colonies with different queen types, in pairs of hives that faced south.[2]*

| Type of queen in colonies from which drones drifted | Type of queen in colonies to which drones drifted | Direction of drift | Age group of drones | | | | Overall Average |
|---|---|---|---|---|---|---|---|
| | | | 5-10 | 10-15 | 15-20 | 20-25 | |
| Queenright | QR | east | .546±.14 (10) | .443±.11 (18) | .302±.16 (8) | 1.031±.20 (5) | .579±.10 |
| Queenright | QR | west | .687±.12 (14) | .781±.09 (23) | .891±.13 (12) | .633 (1) | .747±.15 |
| | | subtotal | .616±.09a | .612±.07a | .596±.11a | .830±.25a | |
| Queenright | VQ | east | - (-) | .364±.06 (52) | .633±.11 (16) | .596±.12 (15) | .531±.06 |
| Virgin queen | QR | west | - (-) | .562±.06 (51) | .471±.14 (10) | .440±.11 (16) | .491±.06 |
| | | subtotal | | .463±.04a | .552±.09a | .518±.08a | |
| Queenright | QL | east | .311±.11 (17) | .324±.18 (6) | - (-) | - (-) | .317±.07 |
| Queenless | QR | west | .249±.09 (24) | .486±.12 (13) | - (-) | - (-) | .367±.05 |
| | | subtotal | .280±.07a | .405±.11a | | | |

[1] Number of drones observed in brackets.
[2] Means followed by the same letter within subtotal rows are not significantly different.
* Dashes represent missing values.

TABLE 6. The number of times per day that drones drifted to colonies with different queen types, in pairs of hives that faced south.

| Type of queen in colonies from which drones drifted | Type of queen in colonies to which drones drifted | Direction of drift | Number of times that drones drifted * | | | | Number of drones |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | ≥4 | |
| Queenright | QR | east | 69 | 22 | 9 | 0 | 32 |
| Queenright | QR | west | 83 | 10 | 3 | 3 | 29 |
| Queenright | VQ | east | 68 | 23 | 6 | 2 | 47 |
| Virgin queen | QR | west | 48 | 48 | 2 | 2 | 56 |
| Queenright | QL | east | 83 | 0 | 17 | 0 | 6 |
| Queenless | QR | west | 65 | 19 | 7 | 7 | 26 |

* Frequency of drift expressed as a percent.

back to their hive of origin. However, in the virgin queen colonies significantly more drones (48 %) drifted back to their hive of origin (Table 6).

## Behaviour of Drifting Drones

Drones returning to a colony generally entered without hesitation and ran quickly (in less than 15 seconds) into the colony irrespective of the queen type. Flight behaviour of drones that drifted into colonies did not appear to differ from the flight behaviour of non-drifting drones. Very few drifting drones were attacked by guard bees at the hive entrance or after entering the hive. Only 4 out of the 300 (1.3%) drifting drones were mauled by workers. Drones that drifted to a colony during the day were not rejected by that colony before the next sampling period.

# CHAPTER V

# DISCUSSION AND CONCLUSIONS

## The Effects of Marking Technique on the Longevity of Caged Worker Bees

Drones must be individually marked to observe their flight behaviour, to accurately determine the proportion of the different age groups that drift, and to determine the number of times that individual drones drift between hives. Tags for individually marking queens are made by Chr. Graze Inst., are difficult to read, and are available in only five colours in a limited range of numbers (1-99). Other techniques for individually marking bees are available (Gary 1971 a; Smith 1972; Verron and Barreau 1974; Fresneau and Charpin 1977; Harris 1979), but have disadvantages. They can be used to mark only a limited number of bees, are difficult to make or obtain, are time consuming or may require anaesthetizing the bees. A technique for marking drone honey bees in which large numbers of unanaesthetized drones could be rapidly marked with individually numbered tags of up to 8 or more colours was developed. The tags could be numbered from 1-999 (or letter coded if desired) and did not appear to affect the flight activity of the drones.

Cage trials were used so that any loss of tags could be determined. Worker bees were used instead of drones because they are easily maintained in cages and were available at the time of year when the experiments were conducted (i. e. February). Exposure to cold was used to anaesthetize the bees because it does not reduce the longevity of worker bees in the field (Ebadi et al. 1980; Mardan and Rinderer 1980). This mode of anaesthesia did not significantly reduce the longevity of worker bees in cages (Table 1).

The longevity of workers was greater when bees were marked with tags that were glued to the thorax with nail polish or model cement, than in the trials where workers were marked with Aero Gloss dope. However, the longevity of workers marked with Aero Gloss dope did not differ significantly from the controls. Differences in longevity between tagged and painted workers may have resulted from, differences in the solvents used, or from slight scratching of the surface of the bee's cuticle by the needle while marking with paints.

A small number of tags fell off the bees in both of the treatments where tags were used. However, when tag retention was taken into consideration, there were no significant differences between the length of time that tags were retained or the life spans of the bees in the other treatments (Table 1).

Nail polish was chosen as the glue for affixing the tags because the longevity of workers in that treatment was slightly higher (Table 1) and because the tags could be applied quickly and easily when this glue was used. The tags could be applied without restraining the drones if the drones were marked while they were walking on a queen excluder. The drones made no attempt to remove the tags.

## The Effects of Glue Type on Tag Retention by Marked Drones

Nail polish was compared to the glue supplied with the commercially produced tags to determine their relative effectiveness when used for marking drones. The glues were assessed for the first five days after marked drones were introduced into colonies, before the drones began taking flight. The number of commercially produced tags (i. e. German) retained by drones marked with nail polish, was significantly higher than the number of tags that were retained when the glue supplied with the tags was used (Figure 4).

Nail polish was more effective for marking drones than the glue supplied with the tags. The tags have a concave shape that complemets the convex shape of the workers' or queens' thoraces. Drones have a wider and less convex thorax, therefore the tags may not adhere to drone thoraces as well as to queen thoraces. The solvent in the nail polish appears to flatten the German tag somewhat and this makes the tag fit the drone's thorax. This may result in the higher rate of tag retention that occurred when nail polish was used.

## Factors Affecting Drone Acceptance

A honey bee colony regulates its drone population (Free and Williams 1975). The number of drones is limited by temperature, time of year, intake of pollen and nectar, queen age, the amount of sealed and unsealed worker brood, the odours of drones, the activity of the colony, and the amount of forage collected and stored (Alber 1955; Levenets 1956; Orosi Pal 1959; Holmes and Henniker 1972; Free 1977; Woyke 1977). Colonies regulate the number of drones by controlling the amount of drone comb produced, by regulating the amount of drone brood and by evicting adult drones from a colony (Darchen et al. 1957; Chauvin et al. 1961; Morse et al. 1967; Ohtani 1974; Free and Williams 1975).

Large numbers of drones are commonly introduced into colonies during studies of longevity, mating behaviour, orientation behaviour or flight activity of drones. However, very little is known about how colonies respond to foreign drones that are introduced into colonies or about the number of introduced drones that a colony will support.

When large numbers of drones are introduced, their acceptance is highly variable under different environmental conditions, between colonies of different queen types and between different colonies (Currie 1982). Low acceptance of introduced drones is correlated with environmental conditions (e. g. cloud, rain, low temperatures) that prevent the foraging of workers on or around the time of introduction (Currie 1982). Most loss of the introduced drones (36 %), occurs within the first 24 hours after introduction, and only 16 % are lost between the first to fifth day after introduction (Currie 1982). More drones are accepted by queenless colonies than queenright colonies, but the survival rates of the drones that were accepted by queenless colonies are not significantly different from rates for queenright colonies (Currie 1982).

The number of drones accepted by a colony appears to be dependent upon environmental conditions during the first 24 hours (Currie 1982). After that period few drones are rejected or lost even if environmental conditions are poor. Rejection of introduced drones during the first 24 hours may have been caused by worker bees rejecting the foreign drones because of their foriegn odour or because more drones are introduced to the colony than it can support.

A new introduction technique was developed in this study and compared to the technique used by Currie (1982) to determine if the initial rejection of introduced drones was due in part to their recognition by the workers as foreign and to determine how many introduced drones a colony can support. With the new technique introduced drones were prevented from leaving the hive or from being pulled from the hive by workers by placing a queen excluder in between the bottom box and the bottom board.

The acceptance of drones was greater when excluders were used, but varied with the time of day when the two different techniques were used to introduce the drones (Figure 5). Acceptance of drones was highest during the afternoon when excluders were used. However, acceptance was highest in the evening if no excluders were used.

Young drones are highly photopositive (i. e. they are highly attracted to light) (Berthold and Benton 1970). When drones are introduced into colonies they often walk out of the entrance towards the sun when it is low on the horizen (Jay, pers. comm.), although none seem to be attacked or mauled by workers before leaving (pers. obs.). When queen excluders were not used the acceptance was highest in the evening, probably because few drones walked out of the hive as a result of their attraction to light. Acceptance was 34 percent higher in the afternoon than in the evening (when environmental conditions were usually less favorable) when the queen excluders were used to confine the drones within the colony.

The acceptance was significantly lower when drones were held over night and introduced into colonies on the following morning. This may be due, in part, to a weakening of the drones, or death of some of the drones during the period of time that the drones had to be stored. Therefore, keeping marked drones overnight for introduction on the following day has no apparent benefits in terms of increased acceptance of introduced drones.

The queen excluders were used to confine the drones within the hive for about 18 hours. This may have allowed the drones time to acquire hive odours and resulted in fewer drones being evicted by the workers. There was a slight (not significant) reduction in acceptance after the queen excluders were removed (Figure 6). However, the use of excluders did not result in more drones being accepted than the colony would normally support. If this did occur, there should have been a large reduction in the number accepted after the queen excluders were removed. The use of excluders appeared to allow the drones time to be accepted by the colony during the first 24 hours after introduction.

Number of Drones Introduced

The number of drones accepted varied with the number that were introduced (Figure 7). The number of drones accepted after 5 days was approximately equal when either 50 or 95 drones were introduced, but was significantly lower when only 25 drones were introduced. Optimal acceptance was obtained when 50 drones were introduced into the single storey hives (Figure 7).

Currie (1982) found that very high acceptance of drones could be achieved when 100 drones were introduced provided that environmental conditions were favorable. Witherell (1972) achieved high acceptance by throwing drones, that were evicted, back into the hive (although it is not known how many he introduced). The experiments in the present study were conducted under a wide range of environmental conditions (see Appendix 1). Levels of acceptance of introduced drones might have been higher under optimal conditions. The optimal number of drones to introduce into colonies in order to maximize acceptance probably varies with factors that influence worker tolerance of drones e. g., the amount of forage, the queen state, the colony size, and various environmental conditions.

Odours (like vanilla) that mask a bee's acquired odours have been used successfully in increasing the acceptance of introduced queens (Dixon 1979). However, masking of the drone's odours might increase the number of drones accepted above the level that a colony can normally support. Treatment of each hive with the same odour may reduce the ability of guard bees to recognize intruders at the hive entrance (Butler and Free 1952), such effects might might confound the experimental treatment effects and accordingly masking odours were not used in this study.

The excluders were not put on hives until after drone flight was over and were removed before drones began flying the next day. Thus the flights of drones (already present in the hive) would not be interrupted. Drones that were either rejected by workers or died as a result of handling and marking could not be evicted from the hive and thus collected on the queen excluder. The introduction of drones into a colony did not appear to affect populations of drones already present in the hive as no other marked or unmarked drones were found dead on the excluders.

## Factors Affecting the Amount and Direction of Drone Drift

### Effect of Introduction Date on the Amount of Drift

The proportion of drones that drifted between paired hives that faced north, south, east and west was studied to determine if the amount of drone drift varied between hives with different queen types and between hives that faced different directions using drones of different ages. Although the amount of drone drift was higher on 17 August (17 %) than on 11 August (12 %) (Figure 8), the patterns of drift did not vary significantly between dates (Figures 9-11). The differences between the amount of drift were slight, despite the experiment on 17 August taking place after the peak period of honey flow. The worker guards did not eject drifting drones from the colony at the hive entrance, and thus they did not visibly affect the amount of drone drift.

## Effects of the Direction That Pairs of Hives Faced and the Colony's Queen Type on the Amount of Drift

The drift of drones was greater between queenless pairs and between pairs with virgin queens than between queenright pairs in the east- and west-facing hives but not in the north and south facing hives (Figure 9). Drift in queenright colonies in the east and west facing pairs might have been reduced by some factor related to worker rejection of the drifting drones inside the hive. The greater drift of both workers and drones from queenright to queenless colonies than to other queenright colonies reported by Free and Spencer -Booth (1961 was attributed by them to differences between queenless and queenright colonies in numbers of intruders repelled at the hive entrance. However, drift of drones to queenless colonies could appear to be higher to queenless colonies than to queenright colonies if the drones that do drift are evicted more between sampling periods in queenless colonies. Many drones drift more than once (Table 6). Drones that drift to queenless colonies may also be more inclined to remain there than drones that drift to queenright colonies.

Differences in drift between queenright and queenless colonies did not occur in north- or south-facing rows (Figure 9). Therefore, the drift that occurred in these rows must not have been influenced to the same extent by factors related to differences in rejection of drifting drones by the workers of the colony. This observation may be of practical importance to commercial queen breeders. The proportion of drones drifting in queen mating yards that have rows of queenless colonies or rows of colonies with virgin queens could be significantly reduced (by almost 50%), by simply facing colonies towards the north or south rather than towards the east or west. The reduction in drone drift could have benefits in reducing the spread of parasitic mites and diseases known to be vectored by drones within commercial apiaries.

The proportion of drones that drifted between pairs of queenless colonies or between colonies with virgin queens was significantly greater than between queenright colonies when pairs faced either east or west, but not when pairs faced north or south (Figure 9).

The proportion of drones that drifted from paired colonies with virgin queens was not significantly different from queenless pairs, regardless of which direction the colonies faced (Figure 9). Earlier,Currie (1982) had observed that large numbers of drones were attracted to colonies with virgin queens. However, in this study if both colonies were of the same queen state (queenless or virgin queens) the proportion of drones that drifted in colonies with virgin queens was not higher than in queenless colonies; there was no apparent increase in drifting activity between them as a result of the presence of virgin queens in the colony.

### Effect of Drone Age on the Amount of Drift

The proportion of drones drifting between pairs tended to decrease as the age of the drones increased (Figure 11). This decrease in drift was not statistically significant, but older drones may make slightly fewer orientation errors due to their increased flight experience.

### Effect of Queen Type on the Direction that Drones Drift

Honey bee workers and drones often make orientation errors and drift into other hives (Free 1958). The pattern of drift of both workers (Jay 1968, 1971; Volbergh 1975; Jay and Warr 1984) and drones (Currie 1982), in rows that face N, S, E, and W, appears to be influenced by the relative position of the sun and by the apparent movement of the sun across the sky.

When rows of hives are arranged to face east or west in the northern hemisphere where the relative position of the sun is to the south, the drifting of worker bees is greater towards the south than to the north (Jay 1968). When rows of hives that face east or west are placed in the southern hemisphere where the relative position of the sun is towards the north, then more bees tend to drift to the north (Jay and Warr 1984). When rows of hives face east or west located closer to the equator where the sun passes almost directly overhead, then the proportion of bees that drifts towards the north and towards the south are similar (Jay 1971). At all three latitudes there is a tendency for more worker bees to drift towards the west than towards the east (Jay 1968, 1971; Jay and Warr 1984).

The drifting of drones in rows of five hives is also greater towards the south (in east- and west-facing rows) and greater towards the west (in north- and south-facing rows) in the northern hemisphere (Currie 1982). Drone drift is greater towards the west than towards the east in pairs of hives that face south (Currie 1982). During the period of drone flight activity in the present experiments the position of the sun relative to the hives was towards the south west and the sun appeared to move westward across the sky (Appendix 2). The drifting behaviour of drones is influenced by the position, apparent movement of the sun, or both. Although, it was previously thought that drones did not use the sun as a cue in orienting to their hives (Oertel 1956).

The fact that drones appear to be affected in the same way as that of workers are is a further test of Jay's theory that the position and apparent movement of the sun influence drift, because drone flight activity differs from that of workers. Factors that may influence worker drift, like the location of major forage sources (Jay and Warr 1984), do not affect drone drift because drones do not forage. Drifting drones in this study were not examined or attacked by worker guards at the hive entrance whereas guard bees often stop, examine and sometimes evict drifting workers (Butler and Free 1952). Drones fly during a restricted period in the afternoon when the sun's position is in the south to the southwest (Appendix 2), while workers will usually fly throughout the day if environmental conditions are acceptable. Drones also fly under a more limited range of environmental conditions than do workers. Drones will not fly, or will make only short flights, when temperatures are below 15 to 18$^{0}$C, when wind speeds are greater than 8-18 km per hour, or when the sky is totally overcast (Witherell 1971;

Bol'Shakova 1978; Lensky and Dempter 1985).

As large numbers of drones had been observed earlier to drift between members of a pair of hives (Currie 1982), the influence of the position and apparent movement of the sun on the drifting behaviour of drones were studied between pairs of hives. The relative number of drones that drifted in different directions varied with the direction that the pairs faced, the colony's queen state and the age of the drone (Figures 10 and 11). However, more drones tended to drift westward than eastward in pairs of hives that faced towards the north or south, and more drones tended to drift southward than northward in east- and west-facing pairs (Figures 10 and 11). This trend was statistically significant only in south- and east-facing pairs (Figures 10 and 11). These results corroborate those of Currie (1982) and support the hypothesis that drift of drones would be influenced by the position and apparent movement of the sun in paired hives in the same way as when longer rows of hives are used.

The directions in which drones drifted did not vary significantly between colonies with different queen state in either the east- and west-facing pairs, but varied with queen type in the north- and south-facing pairs (Figure 10). In the south-facing pairs drift was consistently greater towards the west and was significantly more pronounced in colonies with virgin queens (Figure 10). In north-facing queenright pairs, more drones drifted towards the east than towards the west but in the queenless pairs, and in the pairs with virgin queens, drift was greater towards the west than towards the east.

In the northern hemisphere the tendency of both workers and drones to drift west, appears to be less consistent in north-facing rows than in south-facing rows (Jay 1968; Currie 1982). Earlier, Currie (1982) attributed the variation in drone drift to differences in worker populations in different hives. In this study, differences in the rates of rejection of drones by workers in different colonies were mimimized by equalizing colony populations and food supplies at the start of each experiment. However, the direction that drones drifted was still more variable in north-facing rows (Figure 10). It is of interest that in the southern hemisphere where the relative position of the sun is towards the north, the westwardly tendency for worker drift was more variable in south-facing rows than in north-facing rows (Jay and Warr 1984).

The influence of the sun's position, its apparent movement, or both, appears to influence drift in north- and south-facing rows to a greater extent when the hive entrances face the sun. Bees that approach hives with their "backs" to the sun may be more susceptible to orientation errors caused by the position or movement of the sun.

The Effect of Drone Age on the Direction that Drones Drift

Vollbehr (1975) observed that young (5 day old) worker bees in west facing rows fly out towards the sun on orientation flights and approach the home site from the direction of the sun. Thus, drifting bees end up further down the row in the direction of the sun with each subsequent flight. More work is needed to test Volberh's hypothesis to determine if this mechanism operates in bees of all ages (Jay and Warr 1984), and if the same mechanism operates in rows that face different directions.

In the present study the relative number of drones that drift towards the west or towards the south tended to decrease slightly with the age of the drone, but this relative decrease was statistically significant only in the east facing pairs (Figure 11). The direction that drones of different age groups drifted remained quite consistent with hive orientation with the exception of west-facing rows (Figure 11), where drones that were 20-25 days old drifted more towards the north than towards the south (Figure 11). Drones that were 20-25 days old (in queenright colonies facing south) drifted significantly later in the day (Table 4), at approximately 15:45 hrs. (Table 4) when the sun's position was to the west (Appendix 2). Younger drones drifted earlier (Table 4) in the day when the sun's position was more towards the south, (Appendix 2) and tended to drift more to the south.

The southward tendency of drift in east-facing pairs was not as great for older drones as for younger drones (Figure 11). This may be because older drones drifted later in the day when the sun's direction was not as far to the south (Appendix 2). In the northern hemisphere, the southward tendency for drift of both workers and drones tends to be less pronounced in west- and east-facing rows (Jay 1968; Currie 1982) and is also apparent in hives in the southern hemisphere (Jay and Warr 1984). This trend was far more pronounced in drones (Figure 10 and 11) than Jay (1968) found it to be for workers, and may be related to differences in the time of day when workers and drones drift. Drones in east-facing rows tend to fly earlier in the afternoon than in west-facing rows (Taber 1964). If drone flight in east facing rows ends earlier in the day, when the sun's positon is still towards the south, this might explain the quantitative differences in the direction that drones drift between east and west facing rows. However, it is not known if similar conditions influence worker bees, as it is not known at what time of day that most workers drift.

A second mechanism by which the sun may influence drift is through its apparent movement across the sky (i. e. the change in azimuth angle) (Jay 1971; Vollbehr 1975; Currie 1982). During the period that a drone is on a mating flight (mean 30 min.) (Witherell 1971), the sun's azimuth angle can change 12 degrees (Appendix 2). If any insect returning to its nest after a 30 minute flight used the sun as an orientation cue and had no mechansim for compensating for the sun's apparent movement it could be displaced by almost 100 m (Gould 1982). This displacement could cause bees to drift in the direction of the apparent movement of the sun (Appendix 3).

However, worker bees can use the sun as a compass for navigating and can compensate for the apparent movement of the sun across the sky when they are both communicating and navigating (Frisch 1967). Workers are thought to extrapolate the most recent rate of change in the azimuth angle of the sun that they have seen, to compensate for the sun's movement during the day (Gould 1982). If honey bees calculate the rate of change of the sun perfectly then the sun's movement should not cause orientation errors. However, extrapolation does not give precise estimates of the sun's position because the azimuth angle does not change at a constant rate throughout the day (Gould 1982) (see also Appendix 2).

Workers require up to 500 flights before they can fully use the sun as a compass (Lindauer 1961), but drones only make up to 94 flights (median 30) during their lifetime (Witherall 1971). Therefore, drones must either learn to make use of the sun as a compass earlier in their life or may not use the sun as a compass at all. The fact that drones get much less flight experience during their life than do workers may be one reason why drones make more orientation errors than do workers.

Errors made in extrapolating the sun's azimuth angle while navigating could also cause drifting errors for either workers or drones. The azimuth angle of the sun increases during the morning, is at its greatest around noon, and decreases until sunset. Theoretically, (in a south-facing row), when the rate of change of the sun's position is increasing (in the morning) the bees should undercompensate for its movement and drift should be greater towards the west. When the rate of change of the sun's azimuth angle is decreasing (in the afternoon), then the bees should overcompensate and drift should be greater towards the east (Appendix 3).

If errors in extrapolating changes in the rate of the apparent movement of the sun cause drifting errors they should occur more frequently with drones on longer flights than with drones on shorter ones. Therefore, drones that drift as a result of making errors in extrapolating the rate of change of the sun, should do so after longer flights than those drones that drift for other reasons. It is of interest that drones that drifted west, did so after longer flights than did drones that drifted east (Table 5, queenright pairs).

Drones flew (Figures 18-21) and drifted (Table 4) only in the afternoon when the rate of the sun's azimuth angle was decreasing (Appendix 2). More drones drifted towards the west than towards the east (Figure 11). This is opposite to the direction that would be predicted by Gould's theory (1982) if the bees extrapolated the change in azimuth angles of the sun (Appendix 3). However, if the combination of change in azimuth and change in zenith angles of the sun are considered together, then the rate of change in the position of the sun appears to increase at about 15:45 (Appendix 2) which is the time of day when most drones drifted (Table 4). Gould (1982) studied the mechanism that workers use to compensate for the sun's movement by preventing workers from foraging for periods of two hours and then determining how their orientation to food sources was affected. He may not have considered possible effects of the combination of azimuth and zenith angles of the sun because his experiments took place around noon when the rate of change in the zenith angle of the sun is relatively small.

If the combined effects of the change in the sun's zenith and azimuth angles are considered together, then the sun's position appears to increase at a faster rate later in the day (Appendix 2), and the westward tendency of drift is consistent with what would be predicted if bees extrapolated to compensate for the movement of the sun but under-estimated its rate of movement (Appendix 3).

Worker bees fly throughout the morning and the afternoon, when the relative position of the sun is changing at decreasing and increasing rates respectively. Therefore, workers might be expected to show a less consistent westward tendency for drift than drones, which fly only in the afternoon (not considering any effects that the position of the sun might have on drift). The movement of workers in west-facing rows does in fact show only a slight tendency for westward movement along rows (Jay 1968, 1971). However, the tendency for westward movement of workers along rows is greater at latitudes near the equator (Jamaica) and in the southern hemisphere (New Zealand). It is not known at present what length of time workers fly before drifting in different directions, or what time of day most workers drift at various latitudes.

The drifting of drones is probably influenced to some extent by both the position and the apparent movement of the sun. The proportion of drones that drifted towards the direction of the sun was not significantly lower for older drones. The direction of drone drift is consistent with that which would be expected if drones could not compensate for the apparent movement of the sun. However, it is also consistent with Gould's hypothesis that bees can extrapolate the rate of the sun's movement throughout the day if the extrapolation is based upon the combined effects of the sun's azimuth and zenith angles. Given that workers are able to compensate for the apparent movement of the sun (Frisch 1967) and that the pattern of worker drift is similar to that of drones, the latter hypothesis is more tenable.

## Factors Affecting the Proportion of Drones Attracted to, and Retained by, Colonies with Different Queen Types

The proportion of drifting drones attracted to (Figure 12) and retained by (Figure 13) the treated colonies differed on different dates. On 9 September, 1983, a high proportion of drones was retained by the treated colonies (Figure 13) yet attraction of drones to the treated colonies was still high (Figure 12). The treated colonies consisted of 2 colonies that were queenright, 2 colonies that were queenless and 8 other colonies that were queenless but differed with respect to queen state. In periods of nectar dearth, queenless colonies are more tolerant of drones (Free and Spencer-Booth 1961; Woyke 1977). In the present tests the honey flow was over by 9 September and the treated colonies had no laying queens. Therefore, workers in the treated colonies may have been more tolerant of drifting drones than those in the queenright colonies. Fewer drones may have appeared to drift from the queenless colonies to the queenright colonies in September, either because more were rejected by guard bees at the hive entrance of queenright colonies or because more drones were evicted from the queenright colonies.

Queenless (treated) colonies retained more drones on 9 September, 1983, (Figure 13) than on other dates, but the relative numbers of drones attracted to, or retained by, colonies undergoing each treatment did not vary significantly on different dates. Therefore, although seasonal effects influenced drone drift, they did not affect the relative numbers of drones attracted to colonies of different queen types.

## The Relative Attractiveness of Different Queen Types to Drones

In colonies with different queen types containing drones of different age groups, the direction that drones drifted was most consistent in pairs of hives that faced south (Figure 10 and 11). Drift in south-facing pairs was also less influenced by the rejection of drifting drones by workers (Figure 9, see previous discussion, page 94). Therefore, it was decided to use south-facing pairs to determine if different queen states or pheromones could attract drones from neighboring hives.

In south-facing pairs with the same queen state, the proportion of drones that drifted towards the east was lower than the proportion that drifted west, probably because of an apparent sun effect (see previous discussion, page 95). The positions in which the treatments (i. e. different queen states) were placed, were analysed separately because the westward tendency for drift (Currie 1982, and Figures 10 and 11) might either mask, or increase any effects that were due to different queen states or pheromones.

Therefore, to determine if colonies with virgin queens attracted drones from neighboring colonies, and to determine if that attraction was due to the queen's pheromones, the treated colonies were placed on the east side of pairs that faced south (see Block 2, Figure 2). Thus it was expected that drones drifting to treated colonies would drift in the opposite direction to that of the apparent sun effect.

Although Butler (1939) and Levenets (1951) had reported that colonies with virgin queens do not attract or retain drones. However, Currie (1982) observed that colonies with virgin queens appeared to attract large numbers of drones from neighboring colonies. Three hypotheses could explain more drones being found in colonies with virgin queens than in other colonies (i. e. queenright or queenless controls): (1) more drifting drones may be evicted between sampling periods in the controls than in the colonies with virgin queens, (2) more drifting drones may be repelled by guard bees at the hive entrances of the control colonies and then drift back to their original hives, or (3) more drones may drift to colonies with virgin queens because they are attracted by some factor related to the presence of a virgin queen in the hive.

Differences in drift that might have resulted from differences in the rate of rejection between queenless and queenright colonies were not apparent. Eastward drift of drones to queenless colonies did not vary significantly from drift to queenright colonies (Figure 15). Free and Spencer-Booth (1961) showed that drift of both workers and drones is greater from queenright to queenless colonies than from queenright to queenright colonies. They proposed that this occurs because the guard bees in queenless colonies are less inclined to repel intruders than the guards in queenright colonies. Queenless colonies are also less inclined to evict drones than queenright colonies (Free 1957; Woyke 1977). Therefore, in the present study differences in the number of drones evicted between sampling periods in queenright colonies, queenless colonies and colonies with virgin queens might have occurred. This could have been the cause of the observed differences in drift between treatments. Colonies that had caged virgin queens, caged mated queens or pheromone might have been as tolerant, or more tolerant, of drones than either queenless or queenright colonies.

If drones were attracted to colonies with virgin queens, then drift to those colonies should be higher than the drift to queenless colonies. More drones drifted to colonies that had virgin queens than to either queenless or queenright colonies (Figure 15). Virgin queens produce a mating pheromone that attracts drones while in flight (Gary 1962). The major component of the mating pheromone is _trans_-9-oxodec-2-enoic acid (Butler and Fairey 1964; Blum _et al_. 1971; Boch _et al_. 1975). The attraction of drones to colonies with virgin queens is presumably a result of the virgin queen's pheromones. Bioassays that used a component of the virgin's mating attractant pheromone, _trans_-9-oxodec-2-enoic acid, in concentrations that are equal to or greater than those found in older virgin queens, confirmed that drones are also attracted to colonies containing the queen's pheromone in lieu of a queen (Figures 14 and 15).

It could be argued that the effects observed in Figure 15 were due to differences in the rate of rejection of drones by the workers in colonies with virgin queens or pheromone. Queen pheromones can influence the physiological state of worker bees (Voogd 1955; Butler 1957; Velthuis et al. 1965) and queen pheromones may be one of the factors that influences the acceptance of drones by workers (Free 1977). It is possible that virgin queens (or their pheromone) might have influenced the behaviour of workers towards drones, thus making colonies with virgin queens (or pheromone) more tolerant of drifting drones than either queenless or queenright colonies. To test this possibility the drifting behaviour of drones was observed in pairs of queenright, and queenless and colonies containing virgin queens.

Few drifting drones were rejected by workers at hive entrances (only 1.3 %), and there appeared to be no differences in the behaviour of guard bees towards drifting drones in any of the treatments. The colonies in which drifting drones were observed were also examined on the following day to determine if the drones that had drifted were still present. It was found that drones that had drifted during the day were not rejected by the colonies' workers before the next sampling period. Therefore, differences that were observed between treatments could not have resulted from differences in the rejection of drones by colonies with different queen types, either by their guards at the hive entrance, or by eviction of the drones between sampling periods.

Drones can pursue and mate with queens while the queens are returning to their colonies (Gary 1971b; Ruttner 1966; Dixon 1979). Thus, the drifting of drones to colonies with virgin queens could be caused by the drones following virgin queens when the virgins return to their colonies from mating flights. However, this could not occur in the present study because the virgin queens were caged.

The drones that were attracted to the colonies with virgin queens, drifted after long flights (34 minutes) (Table 5). Therefore, the drones that had drifted to colonies with virgin queens must have been attracted to those hives after returning from mating flights and not just as they left the hive.

The proportion of drifting drones that was attracted by the virgin queen's pheromone increased in the older drones (Figure 15). It is not known if higher proportions of older drones are attracted to virgin queens on their mating flights. However, Zmarlicki and Morse (1963b) found that drones that were attracted to mating lures were 9-23 days old. Gerig and Gerig (1976, 1982, 1983) sampled drones using radio-controlled aircraft and found that higher proportions of sexually mature drones were found closer to the ground. It is not clear if pheromones, or virgin queens, were used in conjunction with the aircraft studies in Gerigs' experiments in 1982 and in 1983, as they were in 1976. However, if pheromones were used, then the sexually mature (older) drones may have been found at the lower heights because they are more responsive to the pheromone.

Drones may not respond to the queen's pheromone until they are sexually mature (i. e. older). Increased attraction of drones to colonies with virgin queens coincided with the age when drones become sexually mature (Figure 15), between 8-23 days of age (Jaycox 1961;

Woyke and Jasinski 1978; Kurennoi 1953 b; Ruttner 1966). The highest concentration of sperm in a drone's vas deferentia and seminal vesicles occurs when they are 8-9 days old (Jaycox 1961), but the highest number of sperm enter the queen's spermatheca when queens are mated with 14 day-old drones (Woyke and Jasinski 1978). Drones will evert their genitalia when greater than 10 days old but most evert when between the ages of 13-23 days (Kurennoi 1953b; Ruttner 1966).

As drones aged, the attractiveness of older virgin queens increased relative to that of young virgin queens. The number of drones attracted to lure queens is proportional to the quantity of trans-9-oxodec-2-enoic acid in the queen's mandibular glands (Boch et al. 1975). Very little trans-9-oxodec-2-enoic acid is found in newly emerged virgin queens and the quantity of pheromone increases to a maximum level when the virgin queen is from 5-10 days old (Pain et al. 1960; Butler and Paton 1962). Higher proportions of older drifting drones were attracted to the older virgin queens than to the young virgin queens probably because of the quantitative differences in the production of their pheromone.

Higher proportions of drones were attracted to the colonies with trans-9-oxodec-2-enoic acid than to those with either young or old virgin queens. The blocks containing pheromone were replaced daily so that similar concentrations of pheromone were administered over each day. However, the pheromone was mixed in batches and used over several days. Due to evaporation of the solvent the concentration of pheromone may have been higher than 100 µg, the level that would normally be found in an older virgin queen (Butler and Fairey 1964).

Mated queens produce the same quantity of trans-9-oxodec-2-enoic acid as do older virgin queens (Butler 1961; Butler and Paton 1962), and they attract as many or more drones to mating lures than do virgin queens (Pain and Ruttner 1963; Butler and Fairey 1964; Boch et al. 1975). However, the number of drifting drones that were attracted to colonies with mated caged queens did not differ from the number attracted to colonies with mated laying queens or to queenless colonies (Figure 15). The numbers of drifting drones attracted to colonies with virgin queens was significantly higher than the number attracted to colonies with caged mated queens. Differences in the attractiveness of mated and virgin queens for drifting drones may be caused by qualitative differ-ences in the queen's pheromone, perhaps with respect to relative contents of trans and cis isomers.

The cis isomer of 9-oxodec-2-enoic acid is 400 times less attractive to drones than the trans isomer, but can be photoisomerized to the trans isomer if exposed for sunlight for prolonged periods (Doolittle et al. 1970). Possibly mated queens have mostly the cis isomer and therefore do not attract drones within the hive. If the cis isomer is converted to the trans isomer when the queens are exposed to sunlight this might explain the attractiveness of mated queens to drones when they are placed in mating lures.

The queen's mandibular gland secretion consists of at least 32 different compounds (Callow et al. 1964; Simpson 1979). Many authors believe that other attractants or synergists than trans-9-oxodec-2-enoic acid must be present in queen substance (Gary 1962; Pain and Ruttner 1963; Strang 1970; Boch et al. 1975). Winston (1982) found that the role of different enantiomeres of (E)-9-hydroxy-2-decenoic acid have different effects on the swarm clustering of worker bees and he proposed that their effects on drone attraction should be re-evaluated. Other qualitative differences in pheromone production between virgin and mated queens occur (Butler and Fairey 1963, 1964), and some odours and glands have been identified in virgin but not mated queens that may function in the mating process (Renner and Baumann 1964; Boch et al. 1975; Grandperrin and Cassier 1983).

More research is required to determine how the various components of virgin queen's pheromones influence mating behaviour of drones. Quantitative and qualitative differences in pheromone production between virgin queens of different ages and between virgin and mated queens appeared to influence the drifting behaviour of drones. However, the age of queen, type of queen and age of drones are usually not considered in studies that use queens to attract drones to mating lures . Therefore, when designing bioassays, careful consideration should be given to the types of queens that are used to attract drones to mating lures. The relative attractiveness of these differerent queen types to drones in flight should be re-evaluated.

When treated hives were placed on the west side of south-facing pairs of hives the combined effects of attraction to the queen's pheromone was masked to some extent by the apparent sun effect and differences between treatment effects were more variable (Figure 14). Drift to the queenright controls was as high, or higher than to the colonies with virgin queens or pheromone (Figure 14). The drifting of drones to colonies with virgin queens and to colonies with pheromone was still greater than the drift to queenless colonies. Drifting of drones to colonies with mated laying queens did not vary significantly from drifting to colonies with caged mated queens. However, the drift of drones to queenright colonies or to colonies with caged mated queens was higer than drift to queenless colonies. This was in contrast to the finding of Free and Spencer-Booth (1961), that drifting of drones from queenright colonies was higher to queenless colonies than to queenright colonies. Free and Spencer-Booth (1961) conducted their experiments in England from mid August to September when guard bees at the hive entrance may have been more aggressive. In the current study the rejection of drones by guard bees at the hive entrance did not occur. It was expected that westward drift to queenright colonies would be high, but it is not known why fewer drones drifted to queenless colonies.

Drones from queenright colonies returning from flights were attracted to neighbouring colonies with virgin queens (see previous discussion, page 104). Therefore, it was expected that the drones from colonies with virgin queens would also be attracted to their own colonies when they returned from mating flights. Thus colonies with virgin queens should retain their own drones. Unexpectedly, the colonies that had either virgin queens or pheromone did not retain significantly more drones than either the queenright or queenless colonies (Figures 16 and 17). However, this result does agree with the observations of Butler (1939) and Levenets (1951).

Although drifting drones are attracted to neighboring colonies with virgin queens, it appears that they are not attracted by virgin queens from their own colonies. When both members of a pair of colonies had the same queen type, the proportion of drones that drifted between pairs with virgin queens did not vary significantly from that occurring between queenless colonies (Figure 9).

Flight activity may be required to activate drones to search for queens. Drones are not attracted to virgin queens while they are in the hive (Pain 1973) and only respond to the virgin queen pheromone while in flight. They do not detect differences in concentration gradients of the queen's pheromones, but use anemotaxis to locate the queen (Butler and Fairey 1964). Drones that drifted to colonies with virgin queens were attracted to neighboring colonies with virgin queens when they returned from longer flights (Table 5) as would be expected if drones search for the queen's pheromone only while in flight. However, the drones that came from colonies with virgin queens should also have been responsive to the virgin's pheromone when returning from flights and should not have drifted away from those colonies. Therefore these data cannot be fully explained by this hypothesis alone.

Although the drones from colonies with virgin queens were not retained by those colonies, high proportions of them (48 %) drifted back to the colonies with virgin queens (from queenright colonies) on subsequent flights the same day (Table 6). The number that drifted back to colonies with virgin queens was significantly greater than the number of drones that drifted back to either queenright or queenless colonies (Table 6).

Drones may become habituated to a virgin queen's pheromones. Habituation is a process through which the responsiveness of an animal to innocuous stimuli becomes temporarily or permanently eliminated (Marler and Hamilton 1976). The process of habituation is thought to be mediated by the central nervous system and recovery from habituation can take minutes, hours or days. A different form of habituation called sensory adaptation that occurs as a result of repeated rapid stimulation of sensory receptors can also occur, but recovery occurs within seconds (Thorpe 1963). Habituation to chemical stimuli such as kairomones (Waage 1979; Weseloh 1980) and defensive pheromones (Wohlers 1981) does occur in insects.

If drones that were in colonies with virgin queens were habituated to the queen's pheromone they would not be attracted to those colonies when returning from flights and could drift to a neighbouring colony. If habituation does occur the recovery time is probably short because high proportions (48 %) of the drones that drift away from colonies with virgin queens are attracted back on the same day (Table 6).

If drones mate with queens from their own colony the brood viability would be reduced by about 50 percent (Page and Laidlaw 1985). Thus natural selection should favour queens that do not mate with drones from their own colonies. Although drones were attracted to colonies that have virgin queens (Figure 15), drones are not known to pursue virgin queens from their own colony when the queen leaves on its mating flight. The mechanism that prevents drones from mating with queens from their own colony is not known.

It was formerly thought that drones did not respond to the queen's pheromones at heights below 10 m (Ruttner 1957; Butler and Fairey 1964; Jacobson 1972) and that could act as a mechanism to prevent drones from inbreeding by chasing queens from their own colonies as they left on flights. However, drones have been reported to be attracted to virgin queens at heights as low as 1-2 m (Gerig and Gerig 1982; Tribe 1982), and several authors have observed drones pursuing or mating with virgin queens returning to their hives from mating flights (Gary 1971b; Ruttner

1966; Dixon 1979). Drones returning from flights were even attracted to hives that had virgin queens in the present study (Figure 15). Therefore, the height at which attraction occurs would not be an effective mechansim for the prevention of inbreeding.

It is possible that drones search for virgin queens only after a certain length of time in flight, and/or that drones habituated to the virgin queeen's pheromone from their own colony and thus cannot detect virgin queens that leave on mating flights from the drones' own colony. Habituation to sexual stimuli is a common behavioural mechanism for terminating bouts of sexual behaviour (Marler and Hamilton 1976). Animals that have been habituated to a stimulus will often respond strongly if presented with a new stimulus (*e. g.* a different mate), or if the stimulus is presented in a new situation. If drones can be habituated to the queen's pheromone they would not be able to detect the presence of the queen through her pheromones when she left on mating flights. Thus habituation to the virgin's pheromones might act as a mechanism for preventing drones from mating with queens from their own colonies.

More research is needed to determine, how frequently drones mate with queens from their own colonies, if drones can become habituated to the queen's pheromone and if this can act as a mechanism to prevent inbreeding in honey bees.

# CHAPTER VI

## SUMMARY

A technique for marking drones with individually numbered tags of up to 8 different colours was developed. Gluing tags onto drones with nail polish or Testors plastic model cement, did not reduce the longevity of caged worker bees. When the number of tags that was retained by marked worker bees was considered, the number of bees marked with tags that survived did not differ significantly from the number of bees marked with Areo Gloss dope. Nail polish was effective for gluing tags onto the thoraces of drones. When commercially made tags were used for marking drones, more tags were retained when tags were attached with nail polish than when the glue supplied with the tags was used.

The acceptance of marked drones that were introduced into colonies was highest when drones were confined within the colony overnight by placing a queen excluder between the bottom board of the hive and the bottom box. Acceptance was higher when drones were introduced into colonies in the afternoon using excluders than when drones were introduced in the evening or on the following morning. Introduction of drones, using this technique, did not reduce the population of drones already present in the colony. The highest acceptance of marked drones occurred in the evenings if drones were not confined within the colony (*i. e.* no excluders were used).

The optimal number of drones for introduction into single storey colonies was 50. The number of drones accepted by a colony after a period of five days did not vary significantly when either 95 or 50 drones were introduced, but was greater than when only 25 drones were introduced. The optimal number of drones to introduce into a colony probably varies with factors that influence worker tolerance of drones such as the amount of forage available, the colony's queen type, the colony size, or environmental conditions.

There were no significant differences in the proportion of drones that drifted between pairs of hives facing different directions when colonies were all queenright. When colonies were queenless, or had virgin queens, then the proportion of drones that drifted between pairs that faced east or west was higher than the proportion that drifted between hives that faced north or south. In pairs that faced north or south there were no significant differences in the proportion of drones that drifted between queenright colonies, queenless colonies or colonies with virgin queens. In pairs of hives that faced east or west the amount of drift between queenless colonies did not differ significantly from colonies with virgin queens, but was higher than the amount that drifted between queenright colonies.

In pairs of hives that faced north or south a higher proportion of drones tended to drift towards the west. A higher proportion of drones tended to drift towards the south in pairs that faced east or west. However, these differences were significant only in south- and east-facing pairs. These trends varied only slightly with the colony's queen state and with the different age-groups of drones. The direction in which drones drifted was most consistent in south-facing pairs.

The proportion of drones that drifted east did not vary significantly from the proportion that drifted west, in the average flight of the day after which drones drifted, in the average time of day that drones drifted, or in the number of times that drones drifted per day. However, drones that drifted west did so after longer flights than did drones that drifted east.

The directions in which drones drifted in pairs of hives that faced N, S, E, and W, were correlated with the position and apparent movement of the sun and indicated that drones may use the sun as an orientation cue, and that they may be able to compensate for the apparent movement of the sun. The proportion of drones that drifted between pairs did not vary significantly between groups of drones that were 5-10, 10-15, 15-20 and 20-25 days old, but tended to decrease with the age of the drone.

A higher proportion of drones were attracted to colonies with virgin queens or to colonies with lures containing a component of the virgin queen's pheromone (trans-9-oxodec-2-enoic acid), than to either queenless or queenright colonies. Higher proportions of older drones were attacted to the colonies with virgin queens. More older drones were attracted to older virgin queens than to younger ones. This may have been caused by quantitative changes in the amount of pheromone produced by virgin queens of different ages.

Drones were not attracted to colonies with caged mated queens even though mated queens produce the same quantity of trans-9-oxodec-2-enoic acid as do older virgin queens. Qualitative differences in the pheromones produced by virgin and mated queens may be responsible for these differences. Drones were attracted to neighbouring colonies with virgin queens when returning from flights that averaged about 30 minutes in length.

The attraction of drones to colonies with virgin queens or pheromones was masked when drones drifted to treated colonies that were placed to the west because the westward tendency of drift in the queenright control colonies was already high due to the apparent sun effect. Drift to colonies with virgin queens was lower than drift to queenright colonies, but was still higher than drift to queenless colonies.

Although colonies with virgin queens attracted drones from other colonies they did not retain their own drones; evidently the virgin queens did not attract drones from their own colonies when those drones returned from mating flights. However, after drones from colonies with virgin queens had drifted to a neighbouring queenright colony a higher proportion (48%) drifted back to the colonies with virgin queens on subsequent flights (that same day) than to queenright (10%) or queenless (19%) colonies. The reason for this may be that drones search for queens only after taking flight, or become habituated to the pheromone of virgin queens from their own colonies. This would prevent them from pursuing the queens from their own colony when they are leaving, on or returning from flights. The possibility that drones may become habituated to the pheromone of virgin queens from their own colony, and the possible role that this might have as a mechanism for preventing inbreeding in honey bees requires further investigation.

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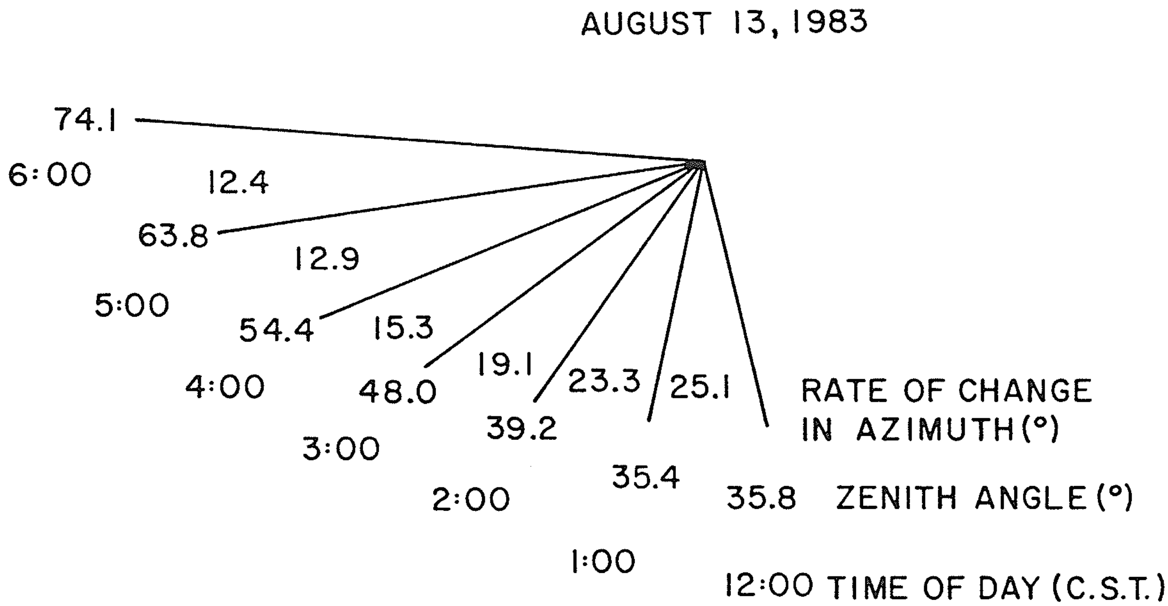
# APPENDIX 1

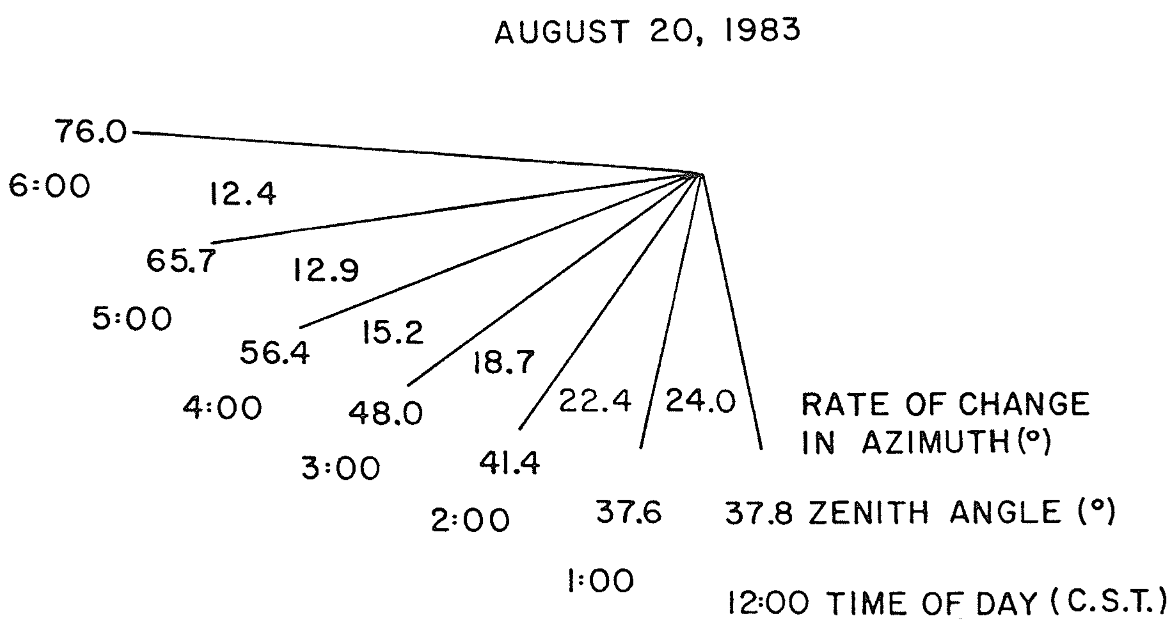
Weather data[1] for the dates during which experiments on the acceptance of introduced drones took place.

| Date that drones were introduced | date | Precipitation (mm) | Temperature °C Max | Min | Mean | Sunshine insolation(h) |
|---|---|---|---|---|---|---|
| 09/06/83 | 09/06/83 | 1.8 | 26.5 | 4.0 | 15.3 | 13.6 |
| | 10/06/83 | 0.0 | 32.0 | 12.0 | 22.0 | 10.3 |
| | 11/06/83 | 0.0 | 32.5 | 17.0 | 24.8 | 4.1 |
| | 12/06/83 | 14.5 | 25.0 | 18.0 | 21.5 | 4.3 |
| 13/06/83 | 13/06/83 | 5.0 | 16.5 | 13.3 | 15.0 | 0.0 |
| | 14/06/83 | 10.0 | 23.0 | 8.0 | 15.5 | 7.0 |
| | 15/06/83 | 0.4 | 11.5 | 7.5 | 9.5 | 0.0 |
| | 16/06/83 | 0.0 | 19.5 | 3.5 | 11.5 | 15.0 |
| 17/06/83 | 17/06/83 | 0.0 | 24.5 | 6.0 | 15.3 | 15.2 |
| | 18/06/83 | 0.0 | 26.0 | 11.0 | 18.5 | 14.5 |
| | 19/06/83 | 0.5 | 25.0 | 15.5 | 20.3 | 3.4 |
| | 20/06/83 | 3.0 | 27.5 | 18.0 | 22.8 | 5.8 |
| | 21/06/83 | 17.5 | 28.5 | 15.0 | 21.8 | 4.7 |
| | 22/06/83 | 0.0 | 24.0 | 15.5 | 19.8 | 8.6 |
| 04/07/83 | 04/07/83 | 0.0 | 15.0 | 12.5 | 13.8 | 5.9 |
| | 05/07/83 | 0.0 | 19.5 | 2.0 | 10.8 | 13.7 |
| | 06/07/83 | 0.0 | 28.5 | 10.0 | 19.3 | 13.0 |
| | 07/07/83 | 0.0 | 30.5 | 16.0 | 23.3 | 10.9 |
| | 08/07/83 | 0.0 | 32.0 | 15.5 | 23.8 | 11.9 |
| | 09/07/83 | 0.0 | 30.0 | 20.0 | 25.0 | 8.4 |
| 10/07/83 | 10/07/83 | 0.0 | 33.0 | 22.0 | 27.5 | 9.2 |
| | 11/07/83 | 0.0 | 23.0 | 14.0 | 18.5 | 5.9 |
| | 12/07/83 | 0.0 | 31.0 | 11.5 | 21.3 | 14.3 |
| | 13/07/83 | 0.0 | 31.0 | 17.5 | 24.3 | 12.4 |
| | 14/07/83 | 0.0 | 34.0 | 18.0 | 26.0 | 14.3 |
| | 15/07/83 | 0.0 | 32.0 | 22.0 | 27.0 | 9.3 |
| 12/08/84 | 12/08/84 | 0.0 | 28.5 | 16.0 | 22.3 | 10.0 |
| | 13/08/84 | 1.5 | 33.5 | 16.5 | 25.0 | 13.1 |
| | 14/08/84 | 0.0 | 31.0 | 20.0 | 25.5 | 5.6 |
| | 15/08/84 | 0.0 | 24.0 | 11.0 | 17.5 | 12.6 |
| | 16/08/84 | 0.0 | 30.0 | 12.0 | 21.0 | 7.8 |

[1] Environment Canada Weather data for Glenlea Manitoba.

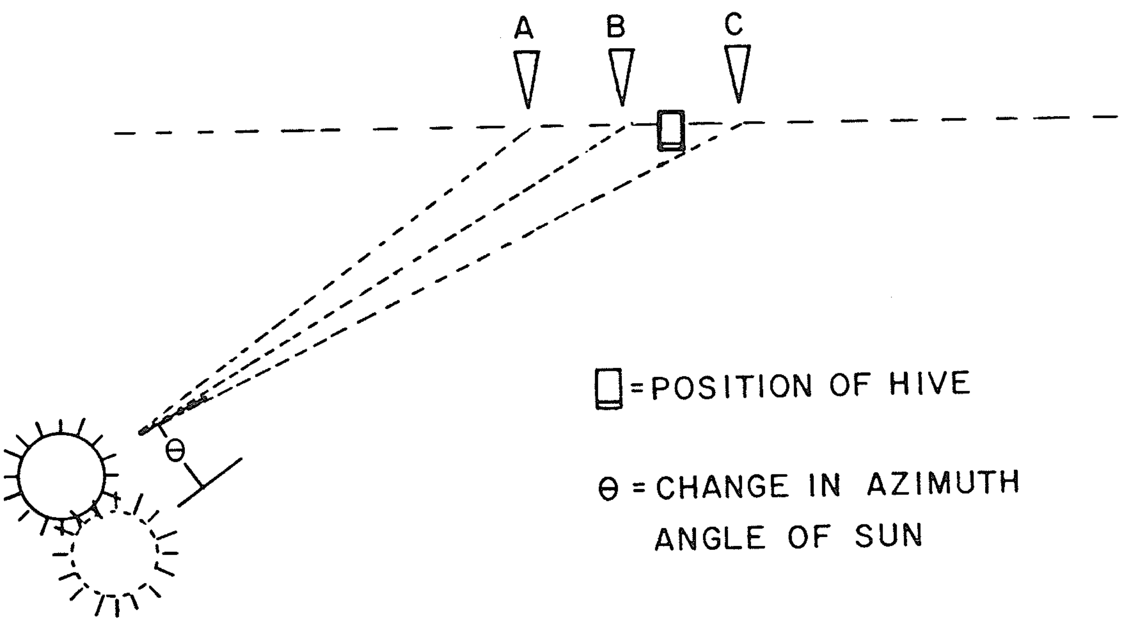
APPENDIX 2




The change in the zenith and azimuth angles of the sun throughout the day on the dates that the experiments on the effects of the position and apparent movement of the sun, were replicated.

APPENDIX 3




The theoretical direction in which more drones should drift if: (A) drones did not compensate for the apparent movement of the sun; (B) if drones under compensated; (C) if drones overcompensated.