Nesting Biology of Bombus ruderatus Fab.
(Hymenoptera: Apidae)

A thesis presented in partial fulfilment of
the requirements for the degree of
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Thirty day old Bombus ruderatus colony

To the bumblebees,
whose cooperation made the project
possible.

ABSTRACT

Most natural nests of Bombus ruderatus were found in underground locations. Underground domiciles were highly attractive to queens of B. ruderatus and B. terrestris, these two species occupying 93% of 45 domiciles. A design modified to simplify inspection and colony removal yielded 62% occupation by B. ruderatus only in 13 domiciles. These percentages compare favourably with overseas results (various other Bombus spp.) and surpass those obtained previously in New Zealand.

Colonies were transferred from domiciles to observation hives for study of their development. The observation hives were designed to provide the colonies' needs for warmth, ventilation, sanitation, and brood-comb support. The total number of bees produced per colony in both natural sites and observation hives ranged from 230 to 750 (mean = 420).

Egg production in B. ruderatus post-incipient colonies is regulated by the quantity of fresh cocoons, one egg clump being made per five (approx.) substrate cocoons. Egg clumps consist of 1 - 5 (mean = 1.5) egg cells, each cell containing 10 - 19 (mean = 14.3) eggs. Egg mortality, especially in multi-celled clumps, appeared to be high but could not be quantified.

Larval rejection caused the loss of 13% to 36% of the larval populations of five colonies in observation hives. The role of larval rejection appeared to be the control of adult size. It was very prevalent during colony decline when food shortages coincided with queen production.

B. ruderatus differs from most other "pocket makers" in having a clear size distinction between workers and queens. Larvae which became queens consumed at least twice as much pollen as most worker larvae. In most colonies there was an interim period of male-only rearing between worker and queen production. Colonies varied in their time of initiating male production, earlier male production being associated with less worker production and lower overall colony productivity. It is suggested that future research should attempt to elucidate the causes of male production in Bombus colonies.

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CHAPTER 1 INTRODUCTION

This project arose from a personal interest in the life of bumblebees. In particular, the subject of colony growth and decline matched my interests as well as being relevant to the management of bumblebees for crop pollination.

Long-tongued bumblebees are considered to be ideal pollinators of red clover (Trifolium pratense L.)(Free, 1970). Short-tongued bumblebees and honey bees (Apis mellifera L.) are sometimes effective pollinators of diploid varieties of red clover (Forster & Hadfield, 1958; Palmer-Jones et al., 1966), but the recently released tetraploid varieties are less likely to be pollinated by these species (Åkerberg & Stapel, 1966).

Bumblebees were introduced to New Zealand from Great Britain in 1885 and 1906 (Hopkins, 1914). Four species became established. They included three long-tongued species; Bombus ruderatus Fab., B. hortorum L., B. subterraneus subsp. latreillelus Kirby, and one short-tongued species B. terrestris L. B. ruderatus is the most widespread of the long-tongued species (Gurr, 1964), and is the subject of the present study. The purpose of the introductions was to improve the seed yields of red clover, which had been negligible when only honey bees were present. There was a spectacular increase in seed yields following establishment of the bumblebees, but the yields subsequently declined, possibly because the bees could not cope with the increased acreages of red clover which were enthusiastically planted (Gurr, 1975).

What limits bumblebee numbers? There are two components to social insect populations; the number of colonies and the number of individuals per colony.

Bumblebee colonies are established in the spring (about November for B. ruderatus in New Zealand; Cumber, 1954) by individual overwintered queens. The number of colonies which can be established is probably limited by the availability of spring bloom and nesting sites (Gurr, 1957; and others). Most bumblebee colonies are started in the deserted nests of small rodents (Free & Butler, 1959). During the present study I obtained information on the features of B. ruderatus nest sites.

One method often employed to gain bumblebee colonies for research or for pollination is to put artificial nesting sites ("domiciles") into the field for occupation by colony-founding queens (Sladen, 1912; Fye & Medler, 1954a; Hobbs, 1967; and others). I designed domiciles using my knowledge of natural nest sites and the physical needs of captive colonies. They proved to be a reliable source of B. ruderatus nests, which were then used for observations of colony growth.

The total number of bees produced in Bombus colonies may range from a few tens to several hundred (Sladen, 1912). The relative colony sizes for the three long-tongued species in New Zealand does not seem to be known, but it would be desirable to determine this, so that management attempts can be aimed at the most prolific species. I have provided figures for the populations of both natural and artificially-housed colonies of B. ruderatus. There is considerable intra-specific variation in colony size however. Cumber (1953) recorded a five-fold range in the sizes attained by wild colonies of B. agrorum Fab. Hasselrot (1960) and Holm & Haas (1960) have shown that the sizes of artificially-reared colonies are similarly unpredictable.

In order to understand the population dynamics of Bombus colonies, some knowledge of their natural history is necessary. The following outline is taken mainly from Sladen (1912), Free & Butler (1959), and my own observations. On her own, the foundress queen rears about eight to sixteen workers. These take over the foraging and most of the "household" duties, and successive batches of workers are reared. The workers are small females. Female size in bumblebees is highly variable and appears to depend on the quantity of food received during the larval stage. The larval food intake is affected by the relative numbers of larvae and workers, the amount of food in the nest, and the behaviour of the workers. Worker numbers increase due to the rate of production being greater than the mortality rate. After a while however (several weeks perhaps), worker production is slowed or halted by a change to male production. Males arise from unfertilised eggs. Then queen production occurs, and continues (usually with concurrent male production) until the colony dies out, often at mid-summer. The males and young queens contribute little to colony maintenance, and soon fly out to mate. The queens return to the nest and feed copiously to build up their fat reserves; then they leave to hibernate.

Cumber (1953) suggested that an abundant food supply could result in small colonies due to queen production being induced prematurely. This suggestion was quoted without dissent by Free & Butler (1959) and Holm (1966), but was criticized by Plowright (1966) on the grounds that larval food intake was not necessarily related to a colony's food supply. Gurr (1957) differed from Cumber by stating that ample food would permit colonies to grow larger. Holm & Haas (1960) found that colony size was related to the time of initiation; earlier started ones reaching the greatest size. Differences in colony sizes could also result from other factors, such as genetic variation between queens with respect to fecundity or foraging efficiency.

The main purpose of my project has been to observe the undisturbed development of B. ruderatus colonies, especially with respect to the growth and survival rates of the juvenile stages. Very little has been published on the intra-colonial biology of this species before. For B. agrorum, Cumber (1949) and Brian (1951) calculated that less than 50% of the eggs laid survived to adulthood. But neither author established the main cause of mortality[1]. A source of juvenile mortality which has particularly interested me, and which has been little studied, is the rejection of living larvae from the nest. Such rejection might be associated with larval undernourishment, because Plowright (1966) found the larval rejection occurred whenever his captive B. terricola Kirby or B. perplexus Cresson colonies ran out of pollen. However, it is difficult to define larval "undernourishment" in bumblebees, because adult size and hence larval nourishment is so variable. Perhaps larval rejection is concerned with queen

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[1] Cumber suggested egg-eating, and Brian suggested larval canabalism, but no evidence has been found for either of these behaviours in B. agrorum (Alford, 1975).

production (when adult size generally becomes uniform), and serves to prevent the production of undersized queens. This view was supported by Röseler (1967) who considered that the presence of male brood stimulated larval rejection such that the subsequent female larvae would each have enough food to reach queen-size. Plowright (1966) found that B. terricola colonies sometimes rejected larvae even when ample pollen was present. In every case premature queen production followed, although the converse was not always true. Both Röseler and Plowright studied artificially fed colonies. I wanted to know the prevalence of larval rejection in naturally foraging colonies.

The whole practical approach to this investigation has been based on ensuring that the bumblebee colonies being observed would develop in a natural manner. Observation hives were designed to closely mimic natural nest conditions, yet allow various aspects of colony development to be monitored with the minimum of disturbance.

The findings fall into two categories. Firstly, some whole-colony features of B. ruderatus ecology have been described. These include nest-site preferences and colony sizes under both artificial and natural conditions. Secondly, detailed information on colony growth has been obtained. This shows B. ruderatus to be an atypical member of the bumblebee sub-group to which it belongs. The significance of larval rejection and other forms of brood mortality are discussed. The timing of male production was found to be a major factor affecting colony size. This phenomenon is poorly understood at present, and I suggest that future studies on the subject would be rewarding.

CHAPTER 2 METHODS

During preliminary studies (1973 - 74 season) I located seven *B. ruderatus* nests and transferred them to observation hives. Some of the colonies were discovered and transferred while at an early stage. Their subsequent development seemed normal and they produced queens at a similar time to undisturbed colonies. Therefore I decided to retain the basic scheme of transferring naturally-started colonies to observation hives.

For the following season I set out field domiciles as nesting sites for overwintered queens. The domicile design used information from the previous season on the features of natural nest sites, and the requirements of artificially-housed colonies. Colonies which were initiated in the domiciles were transferred to an improved type of observation hive and various techniques for observation and data-recording were tried.

During the 1975-76 season a new type of domicile was tested. The observation hive design received minor refinements, and data for five colonies was recorded in detail.

2.1 OBTAINING COLONIES

2.1.1 Wild colonies

It is very difficult to find bumblebee nests. This is because the foragers arrive at and leave from the entrance in a quick unhesitating manner and are easily overlooked. Only when there is a continuous stream of foraging traffic does the location become obvious, but few nests ever have that many foragers. Most nests are found only by the chance siting of a bee entering or leaving a nest entrance. A more objective way of finding bumblebee nests is to follow the flight path of homing foragers. Alford (1970a, b) located incipient nests of several British species by noting the flight direction of pollen-laden queens. This was effective mainly where there was a high density of surface-nests.

My attempts to find B. ruderatus nests were made on the farm of the late Mr. H.S. Barrow. This 400 ha property is about 10 km inland from the township of Waverley, just south of the Taranaki land-district boundary. Searches for nests were made mainly during the 1973-74 season, on one half of the farm which is hilly and covered with areas of bush, scrub, and rough pasture. About half of the nests found, were discovered more or less by accident. However, some were found by a more objective process. I induced foragers to fly home to their nest, by giving them a capacity load of honey solution. This was achieved by placing about 0.5 ml of 50% (v/v) honey/water solution on or in the nectary of a flower on which a bee was beginning to feed. Different tactics were required for the two flower species used; foxglove (Digitalis purpurea), and Scotch thistle (Cirsium vulgare). As soon as a bee entered a foxglove flower, I pierced the base of the corolla with the bluntened needle of a hypodermic syringe and injected about 0.5 ml of honey solution. When bees were foraging on Scotch thistle, the honey solution was merely dripped onto the flower surface with a bulb pipette. Foxgloves had the advantage that a bee inside a tubular flower could not see the experimenter and so rapid movements did not frighten it away. However, bees spent only a few seconds in each foxglove flower so there was little time in which to introduce the extra "nectar". The reverse was true of thistle flowers, i.e. the bees could see the experimenter, and were easily frightened away, but if not disturbed, they spent more time on each flower. When its proboscis touched the honey solution, the bee would remain imbibing for a minute or more. The homeward flight was always preceded by a few minutes of rest and/or cleaning movements, and began with a few circuits of the area before departing in a specific direction. I would keep it in sight for as long as possible. Sometimes an assistant waited at the place where a bee went out of sight, and I fed a second bee (hoping they both came from the same nest). This method would probably be enhanced if several sharp-eyed assistants were available.

Descriptions of B. ruderatus nest sites (located by various methods) are given in section 3.1.1.

2.1.2 Colonies from Field Domiciles

A domicile needs two qualities in order to be a suitable nest site for bumblebees. It must be attractive to searching queens, and it must provide adequate physical conditions for colony growth (henceforth called "protection").

Natural B. ruderatus nests were usually in disused burrows of small mammals. These sites were presumably both attractive and protected. The physical requirements of small mammals while rearing their young, are probably similar to the requirements of bumblebee colonies. They both need the warmth of insulatory nest material and both require drainage for liquid excretions. The metabolic rate of a small mammal is likely to be similar to that of a colony of bumblebees (calculated from Heinrich, 1974), thus even the requirements for dissipation of carbon dioxide and water vapour may be similar. This implies that so long as queens are able to identify subterranean mammal nests, they do not need the capacity to judge all the physical properties of the site, because these will have been already tested by the mammalian occupant. But in a man-made domicile, the relation between attractiveness and protection might be different. Queens might be attracted to some domiciles which are physically unsuitable, while others providing optimal conditions for colony growth, might be ignored by searching queens.

In this project attractiveness of domiciles was considered more important than protection, because colonies were going to be transferred to the laboratory at an early stage, and some physical requirements (such as ventilation) may be less crucial for small colonies. Although natural colonies were nearly always found in mammal nests, the attractive stimulus was not necessarily mammalian scent. Evidence against mammal scent came from an experiment in which I found insectary-housed B. hortorum queens to prefer nesting in upholsterer's cotton than mouse nests (see appendix 1). This agrees with the findings of Hobbs (1967) on Canadian species. However, the possibility of queens responding to a general mammalian scent should not be excluded, because many of the nest materials accepted by bumblebees have been handled by humans and may contain human scent. This applies particularly to used carpet underfelt, which probably contains human foot-smell!

Observations made prior to 1974 on colonies in observation hives, suggested some physical requirements of domicile design. I had been impressed by the quantity of liquid faeces voided by even small colonies. If a colony was housed in a non-porous (e.g. styrofoam) container with nesting material, the bees usually defaecated between the nesting material and the hive-wall, or in the entrance tube. In a subterranean burrow, the excess moisture would soak into the surrounding soil, but in a non-porous enclosure the faecal moisture either soaked back into the nest material (impairing the insulation), or collected in sticky puddles. Therefore, I decided to give all my domiciles free-draining earthen floors, and preferably porous walls as well. I wanted them to have porous or earth-floored entrance tunnels too, even though Hobbs (1967), used plastic hose tunnels and did not report a moisture problem.

2.1.2.1 The 1974-75 season: The main type of domicile used was an underground type shown in figure 2.1. Although I would have preferred to use a porous material to form the cavity, no reasonably-priced and sufficiently large receptacle seemed available. The slab of wood over the entrance contained an inverted "V"-shaped notch, which left a hole about 5 cm high, 2 cm wide at the top, and 4 cm wide at the bottom. This slab served to prevent the entrance caving in, and to make the hole more conspicuous. Fifty of these domiciles

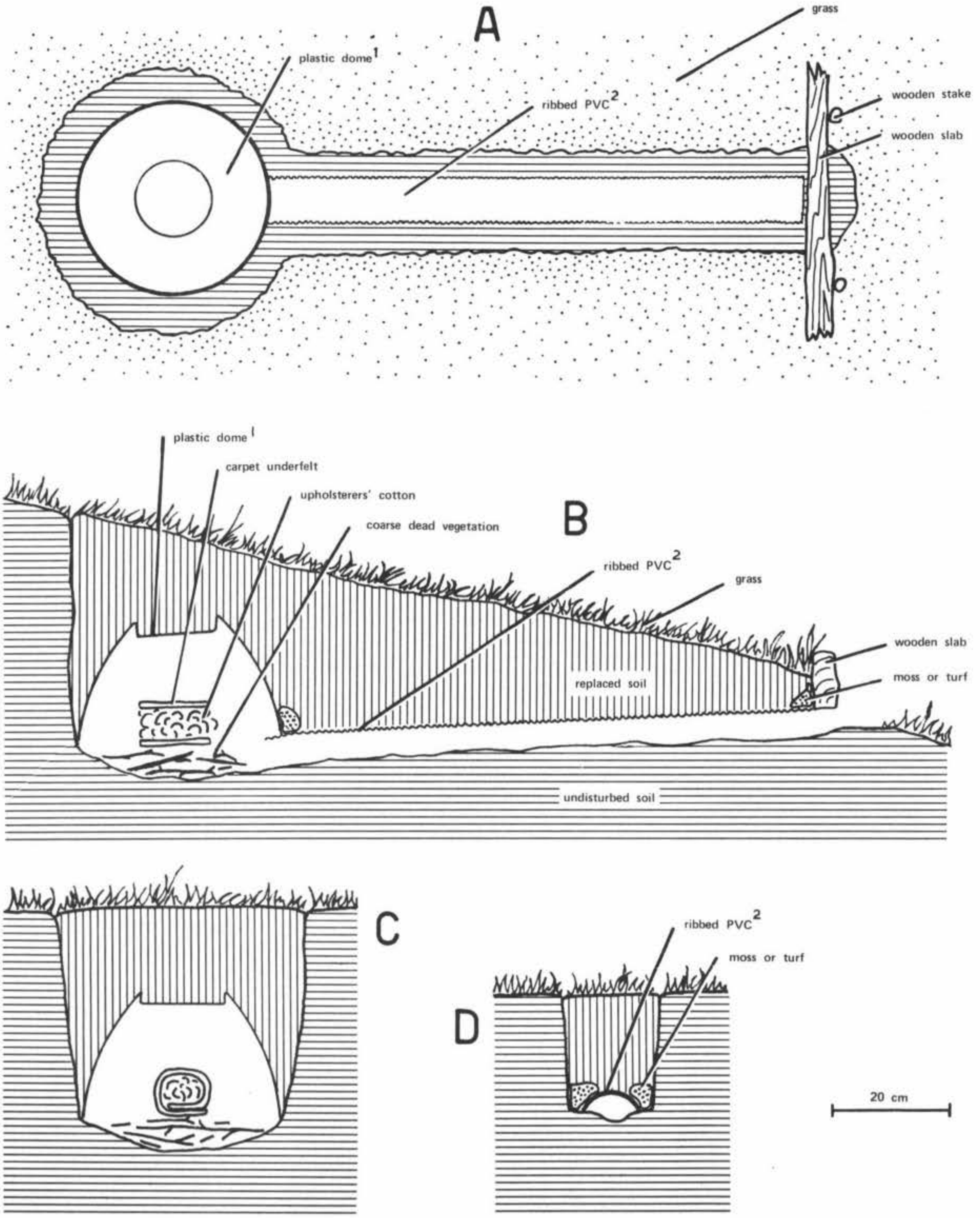

Figure 2.1. Underground domicile. (A) Vertical view of domicile in position, before being covered. (B) Installation complete: mid-vertical longitudinal section. (C) Vertical-transverse section through cavity. (D) Transverse section through tunnel.

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[1] ABS plastic; outer portion of salon hair-dryer hood; manufactured by Ralta Ltd. of Palmerston North.

[2] Strip (1/3 circumference) cut from 10cm diam. Novaflo® land drainage pipe; manufactured by Novoplast Ltd. of Porirua.

were constructed during August 1974 in the same area in which natural nests had been found the year before (see section 2.1.1). They were nearly all installed in sloping ground, damp hollows being avoided in favour of drier ridges. Wire-gauze grilles were tacked over the entrance holes at the time of installation to prevent the entry of the earlier-nesting B. terrestris (the only other bumblebee in the area). The grilles were removed on 13 October, 1974.

A sudden availability of used bricks inspired the design of an above-ground type. (A brick chimney was being dismantled, and I recalled observing a nest searching B. ruderatus queen examine the gaps between the bricks of the same chimney the year before.) The construction of this type is shown in figure 2.2. Twenty-three of these were placed in similar situations to the underground ones, during the last week of October, 1974.

Occupation rates and a discussion of these domiciles are given in section 3.1.2.

2.1.2.2. The 1975-76 season: The moderate success of the surface brick domiciles, suggested that enough colonies for my study could be obtained without further use of the underground type, from which the identification and removal of occupants was very time-consuming. I hoped to improve on the brick type by designing a domicile which was more rain-proof, but which would allow internal moisture to evaporate through porous walls.

The more newly designed domicile is shown in figure 2.3. It was made from lightweight, porous concrete. This material is lighter and has a much higher insulation value than brick

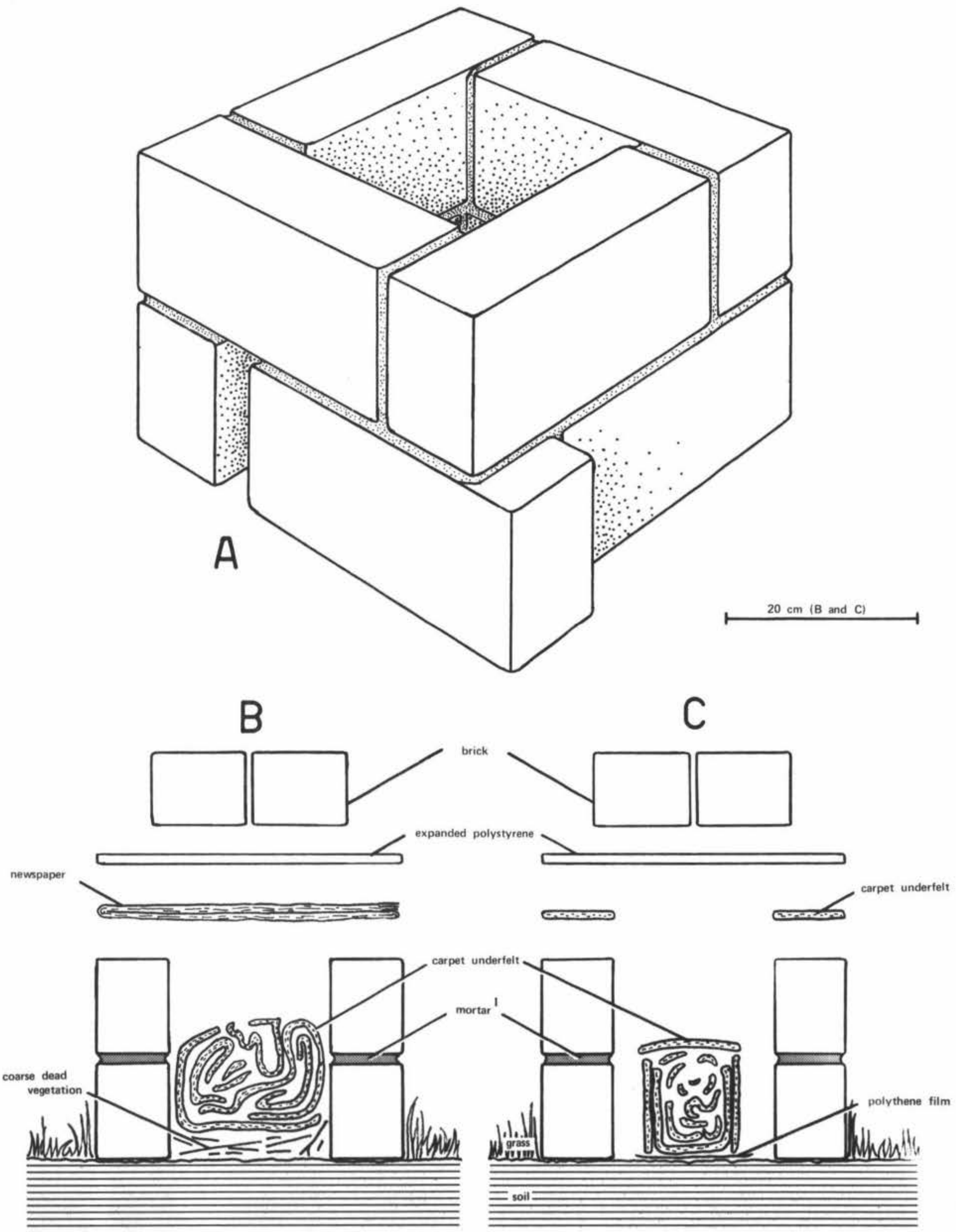

Figure 2.2. Brick surface domicile. (A) Perspective view without lid, showing arrangement of bricks. (B) Mid-vertical section with lid parts raised for clarity; 1974-75 season. (C) Same; 1975-76 season.

[1] Mortar not used in all domiciles.

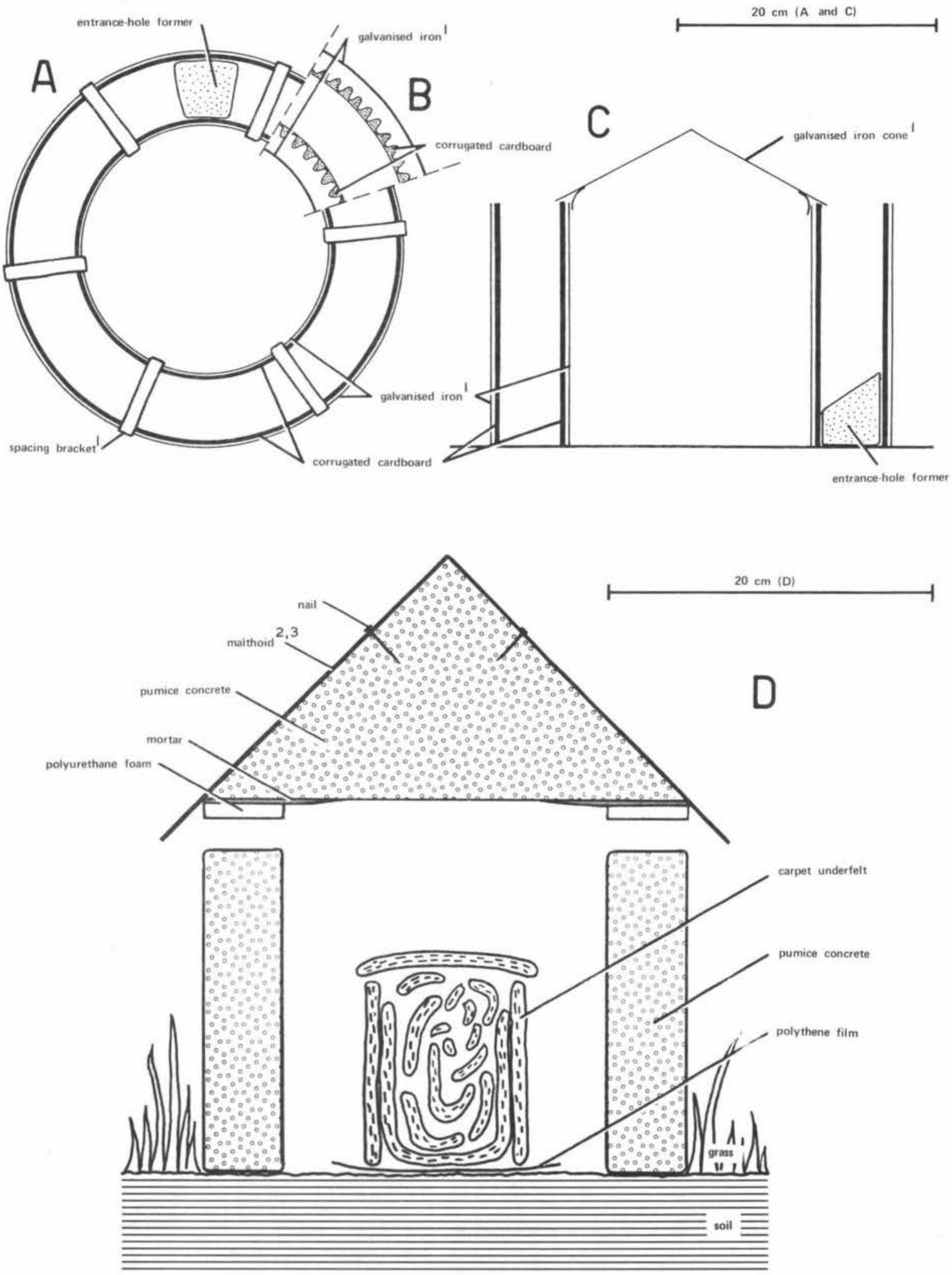

Figure 2.3. Lightweight-concrete surface domicile. (A) Vertical view of wall-mould. (B) Magnified portion, showing cardboard corrugations (gap between cardboard and iron greatly exaggerated). (C) Mid-vertical section of wall-mould. (D) Mid-vertical section of domicile with lid raised for clarity.

[1] Metal parts removed and re-used after mould filled.

[2] Painted silver on outside with bitumen-based aluminium paint.

[3] Malthoid® is a flexible, bitumen-coated, roofing and damp-coursing material.

(Short and Kinniburgh 1968). The aggregate was horticultural pumice with particle sizes in the range from 1.5 to 7.0 cm. This was mixed with Portland cement at the ratio of 6.5 : 1 (pumice : cement) by volume. Calcium chloride ($CaCl_2 \cdot 3H_2O$) was added to the cement at the rate of 2% by weight, to speed the setting of the concrete. The water : cement ratio cannot easily be specified when using porous aggregates, because variable amounts of water are absorbed by the aggregate depending on its previous moisture content (Short and Kinniburgh 1968). I added water in small quantities while mixing, until the pumice particles became coated with a uniform and glistening film of cement slurry. The mould for the walls is shown in figure 2.3 (A, B and C). The metal parts were made of 26-gauge galvanised iron. The corrugated cardboard was waterproofed with a 50/50 mixture of motor oil and petrol. During filling, the mix was compacted with a wooden rod. After filling, the metal parts were slid off and used for the next mould, the cardboard sleeves alone being adequate to support the concrete while it set overnight. The lid required no mould. Concrete mix was simply poured into the inverted cone of malthoid. The nails were pushed through the malthoid first and provided anchorage for the concrete. The concrete was kept moist in polythene bags for three weeks to allow proper hardening. Then the concrete surfaces were scrubbed with detergent and warm water to remove oil residues (originating from the cardboard mould) and soluble alkalis formed during setting. Finally, they were hosed with clean water and air-dried.

The number of brick domiciles (mostly still in position from the previous season) was increased to 25. The concrete domiciles numbered 25 also and were installed in similar places to the brick ones, on 3rd October, 1975.

The fifty surface domiciles were inspected at night time, once a week, from 25 October to 26 December, 1975. I examined some of the underground domiciles too, although the only nest material in them was the remnants from the previous season.

2.1.2.3 Transfer of colonies to the laboratory: The domiciles were 130 km away from the laboratory at Massey University. Nests were always removed from the domiciles after dark. During the first season (1974-75) they were taken to the laboratory on the same night. In the second season, however, I stayed at the farm overnight. This enabled me to re-check in the morning those domiciles from which colonies had been removed, in case any foragers had returned after being out overnight. I supplied each colony with honey solution and a lump of pollen, to compensate for preventing them from foraging. The journey to Palmerston North was made by train. The bees were installed in the observation hives by mid-afternoon, but kept shut in (and fed) until the following morning.

2.2 OBSERVATION-HIVE DESIGN

Observation of natural bumblebee colonies is prevented by the envelope of nesting material which surrounds them. If the covering is opened, the main behaviour patterns observable are the bees' attempts to restore the covering and repel the creature that opened it. If colonies are to be regularly observed the nest material needs to be replaced by something which serves the same functions but does not obscure the bees from sight. If these modifications require the nest to be in a laboratory, then the functions of the surrounding soil and vegetation need also to be duplicated. It is possible, of course, that the physical properties of the nest-surroundings are not all relevant to life of the bumblebees. But departures from the natural situation should not be made without at least considering how the natural microhabitat affects the bees. Thermal insulation, ventilation, physical support, and drainage are functions of various features of natural sites which should be duplicated in observation hives.

2.2.1 Temperature

Bumblebees normally maintain their nests above ambient temperature (Himmer 1933; Cumber 1949; Hasselrot 1960; Wojtowski 1963a and others). It is generally agreed that populous colonies with adequate nectar stores have a temperature of about 30° to 33°C, regardless of the ambient temperature. Authors differ, however, in the degree of temperature stability they report for small colonies. The differences are probably related to the nutritional status of the colonies, and to the amount of disruption caused by the insertion of the temperature measuring devices. Hasselrot (1960) and Wojtowski (1963a) have shown that small colonies (i.e. with about ten workers) of various Bombus species often have nest temperatures 10°C or more above ambient. I obtained similar figures with a small B. ruderatus colony (1 queen, 9 workers, and 3 males) in the underfelt nest material from a domicile. It was found to have a nest temperature of 28° to 30°C (9° to 11°C above ambient) on several occasions. When the colony was put into a refrigerator for nearly an hour its inside temperature levelled out at 23°C, which was 16°C higher than a control cavity (empty ball of underfelt) at 7°C. I consider it likely that B. ruderatus nests in underground situations are able to be maintained at about 30°C from an early stage.

Heinrich (1972) has shown that much of the heat produced in bumblebee nests probably comes from bees incubating brood clumps. Incubation involves "shivering" the flight muscles, and conducting heat into the substrate from the ventral surface of the abdomen. This activity results in the warming of the whole nest cavity, heat loss being reduced by the close-fitting envelope of nest material (usually plant fibres gathered by a rodent), which is often lined with a thin layer of wax. If the nest material is removed to allow observation, the nest-temperature will fall, and as Heinrich (1974) has shown, lower temperatures cause incubating bees to expend more energy and consume more nectar. For example, incubating B. vosnosenskii queens expended twice as much energy at 20°C as at 30°C. Such an energetic drain on my colonies would have been unacceptable, especially if as Free (1955) suggested, nectar collection takes priority over pollen collection. This could result in a pollen shortage in nests where the bees were trying to keep up with the nectar demand for the increased "heating bill". And I didn't want artificially-induced pollen shortages when I was trying to monitor "normal" larval rejection and colony growth.

Obviously the ideal observation hive would provide as much insulation as a natural nest envelope without obscuring the colony from view[1]. Brian (1951, 1952) and Katayama (1971) have insulated their observation hives, but the amount of heat retention was probably less than that experienced by colonies with intact nest envelopes. Brian's hive was a glass-topped wooden box, containing a cylindrical wall of cardboard around the colony. The cardboard cylinder was uncoiled to make it wider as the colony grew. The space between the cardboard and the wood was filled with cotton wool. There was no nest material within the inner cylinder. It is difficult to envisage many improvements to Brian's design other than the use of a double-glazed or perspex lid, and the substitution of glass wool for the cotton wool. However, Brian (1952) reported that her colonies experienced greater temperature fluctuations than those reported by Cumber (1949) and Himmer (1933) for the same species (_B_. _agrorum_) _in_ _situ_. Katayama's design consisted of two unequal-sized oblong wooden boxes, one inside the other,

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[1] Less insulation might be satisfactory in a heated laboratory, but mine was unheated and the ambient temperature fluctuations were similar to those in the field.

with the intervening gap filled with cotton wool. The lid was double-glazed and had a wooden cover. His photograph shows that the hive contained a lot of unoccupied space to allow for colony growth, unlike Brian's expandable-chamber arrangement. Katayama (1973) recorded an inverse relation between egg-stage duration and external air temperature, for colonies housed in his type of hive. This suggests to me that the hive provided little protection from fluctuations in the ambient temperature. Unfortunately, the temperatures inside the hive were not reported. I experimented with insulated observation hives without much success. The main problem appears to be the very much greater wall area for heat loss in artificial rigid-walled containers, compared to the compact dome-shaped covering of natural nests, which fits snugly, and is pushed outwards as the colony grows. Brian's design was about as compact as possible, but I doubt whether any observation hive could duplicate the insulation efficiency of most natural nest envelopes. In Appendix 2 I have described an experiment on the heat retention of various rigid containers and domes of nest material, which supports the above opinion.

Horber (1961), Plowright (1966), and Röseler (1970), solved the temperature problem by placing uninsulated hives in an incubator or heated room at about 30°C. These authors maintained their colonies in total captivity however. With freely foraging colonies as I was using, there were difficulties in ducting exit tubes out of controlled temperature areas, and adequate spacing between the exits would have been difficult to arrange.

The final option was to build partially-insulated hives with an internal heat source to assist the bees in raising the temperature. Various amounts of supplementary heat would be needed, depending on the size of the colony and the ambient temperature, so a thermostat was provided in each hive to switch off the supplementary heat when 30°C was attained.

Early trials (pre 1973) used low voltage foil-covered light bulbs, in series with fish-tank thermostats. The bulbs caused locally-intense heating, which resulted in some parts of each hive being too hot while other parts (even a few cm away) were too cold. During the 1973-74 season each hive was equipped with a 12 cm-square metal plate, which was warmed with electric blanket element (insulated resistance wire) glued to its underside. This heat source was quite satisfactory. The fish-tank thermostats had their glass covers replaced with wire gauze to speed the response-time. They did an adequate job of temperature control but were unduly bulky and seemed to annoy the bees. Individual workers often behaved aggressively towards the thermostats and this behaviour did not subside with time, suggesting the bees were not merely responding to "strangeness" of odour. This would be interesting to investigate further.

The most recent design is shown in figure 2.4. The colony-development results obtained in this project came from colonies kept in this type of observation hive. It was constructed from sheetmetal (for the reason explained in section 2.2.3), so no extra warm-plate was needed. Two parallel strands (each 57.3 cm long) of electric blanket element (148.5 ohm/m) were taped to the underside of the hive. A layer of fibreglass insulation was placed over the

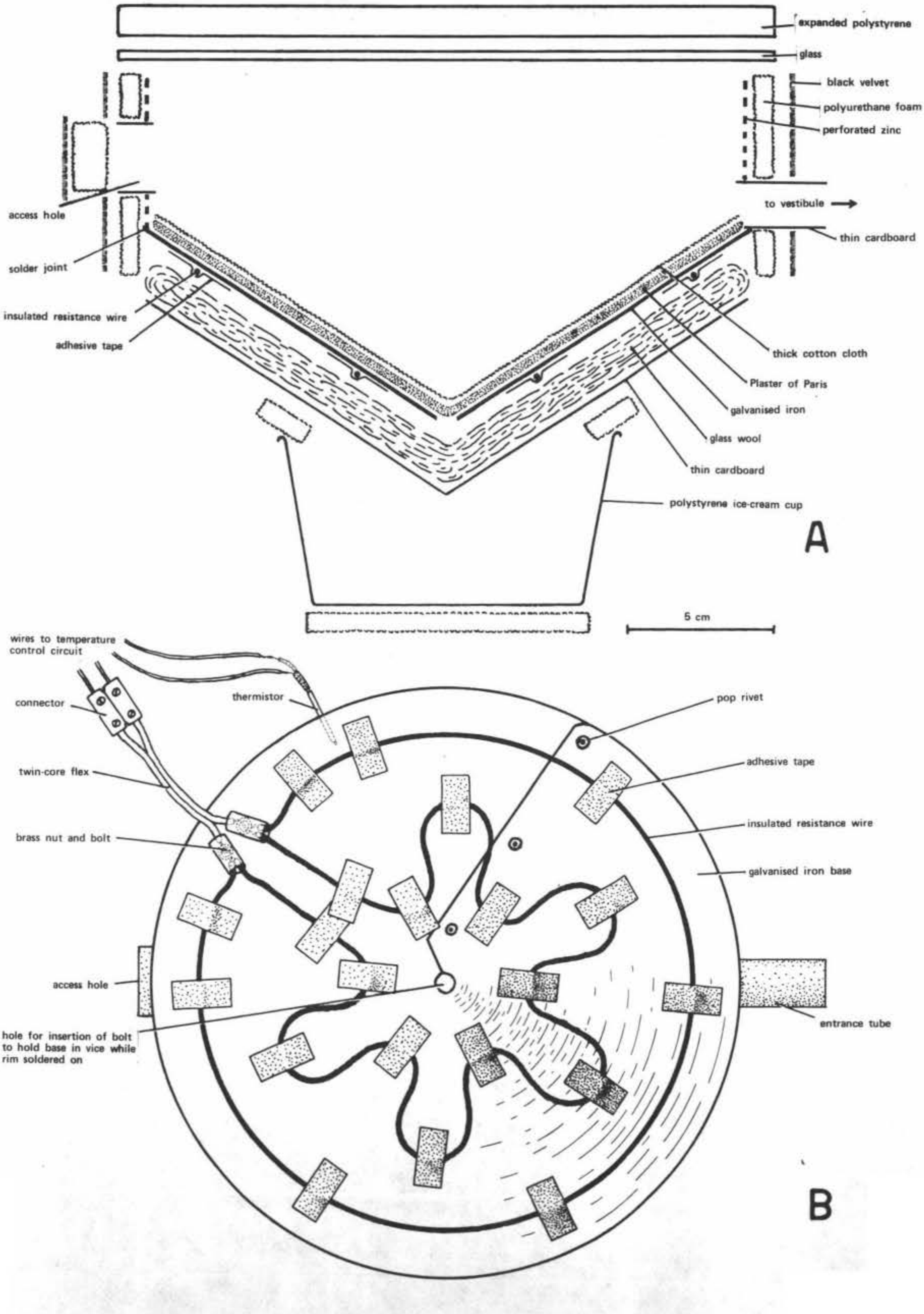

Figure 2.4. Nest-chamber of observation hive.
(A) Vertical-longitudinal section, with layers parted slightly.
(B) View of underside, with outer layers removed, showing heating wires.

wires to direct the heat into the hive. The power source was 12 volts AC. Thermostatic control was obtained via an electronic circuit (designed and built by Mr. M.D.K. Schmidt) which used a thermistor to sense the hive-temperature. The circuit enabled the temperatures of up to ten hives to be controlled independently. The on-off temperature differential was small (less than 0.5°C) and fine temperature control was thus achieved.

2.2.2 Ventilation

Both the amount of ventilation provided by natural sites, and the amount needed by bumblebee colonies, are difficult to estimate. Hasselrot (1960) and Wojtowski (1963a) have both found that the relative humidity (R.H.) in bumblebee nests is just as stable as temperature, and inferred that the bees controlled the humidity. However, relative humidity is a temperature-dependent quantity. I converted some of Hasselrot's R.H. values into absolute humidity (A.H.). This showed that the moisture-content of the nest atmosphere was nearly twice as great as that of the outside air, and that the absolute humidities inside and outside fluctuated by similar amounts. The A.H. in the nest seemed to be affected both by the nest temperature and the A.H. outside. (Wojtowski's data cannot be used to calculate A.H. because he did not record the temperature outside the nest material where he measured the R.H.). Thus the apparent humidity control reported by the above authors could well have been merely the R.H. passively following the temperature. However, observations by Plath (1934, p. 71) and myself (detailed in appendix 3) suggest that abnormally high quantities of carbon dioxide or water vapour may release fanning when high temperatures (the usual cause of fanning) are not prevailing. The existence of homeostatic ventilating behaviour is a sure sign that high humidity and carbon dioxide concentration are detrimental to the bees or their brood (which is not unexpected of course).

The usual way of ventilating an observation hive is by a gauze-covered hole in the wall (Free and Butler 1959; Alford 1975). However, hives with big holes are difficult to heat uniformly. Also, the bees are inclined to cover the hole with a layer of wax. My 1973-74 hives had two 25 sq. cm holes which were covered with a pad of glass wool to reduce heat loss, but the bees covered these ventilators with wax and built wax canopies over nearby areas of comb. In order to reduce the bees' reaction to ventilators, I have since tried to disperse the ventilation over a larger area. The present design (as shown in figure 2.4) has its whole wall area (315 sq. cm) made of perforated zinc, with 133 three-millimeter diameter holes per 100 sq. cm. The perforated zinc is covered on the outside with polyurethane foam which is quite porous but reduces heat loss. The rate of water vapour diffusion through this surface was found to be similar per unit area to the diffusion through a layer of underfelt (i.e. a "natural" nest envelope; see appendix 3), and the surface-area of this wall was similar to the nest-envelope area of a medium-sized colony.

2.2.3 Shape

Natural bumblebee colonies grow upwards and outwards, the peripheral parts being supported by the nest material. When colonies are kept in flat-bottomed observation hives without nest material, the peripheral larval clumps sometimes topple on to the floor. Katayama (1973) described the tendency of B. ignitus larval clumps to fall on to the floor of his observation hive, and mentioned that such clumps were often neglected by the adults. I have noticed clump-toppling when keeping B. ruderatus also in flat-bottomed hives. I wanted to avoid it happening in the present study because toppled clumps often had disorientated pockets and were not fed properly. Even worse, there was the possibility of such a larval clump rupturing as it fell. Then the exposed larvae would be rejected and would interfere with measurements of normal larval rejection. The problem was largely solved by providing a sloping floor. As shown in figure 2.4, the base of the observation hive was a cone of sheetmetal. A cone is in fact the simplest type of volume which can be made from a flat flexible sheet. This aspect of the design was thus a simplification of the traditional cuboidal shape rather than a complication. To help the bees secure a foot-hold on the sloping surface, I stuck a sheet of thick cotton cloth onto it with a layer of Plaster of Paris.

2.2.4 Drainage

Bumblebees always defaecate outside their nest covering and often some distance along their entrance tunnel. As mentioned earlier (section 2.1.2, domicile design) the faeces from natural nests soak into the surrounding soil, but if colonies are housed in impermeable containers, troublesome amounts of moisture often accumulate. Sladen (1912) advocated the use of a "vestibule", an extra chamber between the nest cavity and the entrance tunnel. The vestibule contained a layer of earth, and Sladen found that faeces were deposited there, provided there was not too much empty space in the nest chamber itself. Free and Butler (1959) suggested the use of absorbant paper instead of soil. Whatever is used to absorb faecal moisture, it will become wet and need frequent renewal, especially with large colonies. I have found that faeces are often deposited in the entrance tunnel too. The most suitable material for the vestibule floor (see figure 2.5) and the entrance tunnel seemed to be stiff nylon gauze. Bees usually forcibly discharge their faeces through the holes. Absorbant paper was placed under the vestibules and entrance tunnels. When colonies became large the nylon gauze in the vestibules usually became clogged and wet unless lined with absorbant paper which was changed daily. Even the entrance tunnels sometimes accumulated deposits of faecal sludge, and in such cases they were disconnected (after dark) and washed out with hot water.

<u>2.2.5</u> <u>The Exit Hole</u>

The underground nests of <u>B</u>. <u>ruderatus</u> described in section 3.1.1, had tunnels ranging from 0.6 to 3.75 metres in length. The observation hives used in this project had 2.5 metre tunnels. This length permitted them to be converged at one point for photographic foraging recording (see

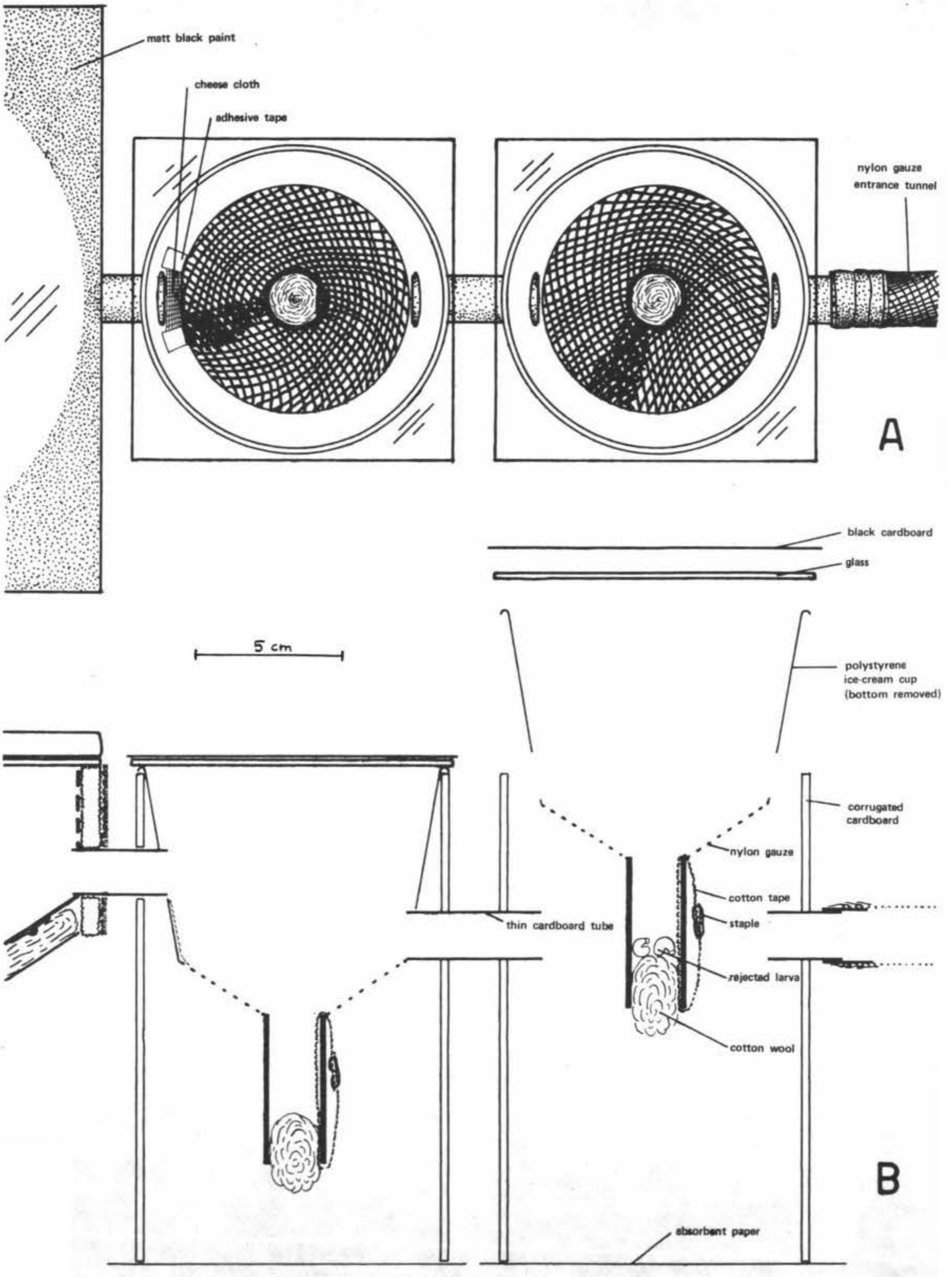

Figure 2.5. Vestibules (defaecation and rejected-larva retrieval chambers) of observation hive. (A) Vertical view, with covers removed. (B) Vertical-longitudinal section, with one chamber raised.

section 2.3.3), then diverge again to widely spaced exit holes.

Natural bumblebee colonies are usually so far apart that foragers are unlikely to return to the wrong one. Where colonies are artificially crowded, however, considerable confusion can arise. Bumblebees make orientation flights the first few times they leave the nest. On returning, their location of the entrance hole seems guided by the pattern of surrounding vegetation. If the vegetation is disarranged, they become disorientated, and may take a long time to find the entrance hole. If the vegetation and other landmarks near the entrance are obliterated, (such as by excavation) the bees become severely disorientated (i.e. lost), and search the ground within several metres, examining crevices and hollows. This suggests that if they cannot find their correct entrance, they generalise their search and look for any hole. This generalised hole-searching, and also social facilitation at entering the nest (Blacklith 1955), are reasons why lost foragers enter other nests if several are close together. Such mix-ups don't usually cause mortal combats between conspecific workers (unlike queens[1]) but they do upset colony growth data by supplementing the worker force of one colony at the expense of another. This phenomenon is called "drift" in apiculture.

Presumably drift can be prevented by ensuring that foragers accurately learn the nest-entrance features the first time they leave, and by making each entrance appear different (to a bumblebee). There is a dilemma concerning the conspicuousness of entrance holes. If all the holes are conspicuous, a bee is probably less likely to get lost but

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[1] "Spring" queens only fight; new or "Autumn" queens don't.

if it does, it would easily find and enter a wrong hole. Whatever the prominence of the holes, it should be equal for all of them, otherwise drift is likely to occur from colonies with inconspicuous entrances to those whose holes are easier to find. This happened with my colonies during the 1973-74 season when the exits were only 10 cm apart along a window-sill. A colony which had a conspicuous entrance appeared to recruit much of the foraging force from a nest newly installed at an obscure entrance.

A related problem with artificially housed colonies is that wind can hamper the orientation flights of bees leaving holes well above ground level on the sides of buildings. In the natural state, orientation flights begin close to the ground where the wind velocity is much lower.

To assist the bees' orientation when leaving the present observation hives, I terminated each exit tunnel inside the laboratory (see plate 1). This enabled the initial orientation flights to be performed inside the room, unaffected by wind (Massey University is in a windy district). The bees then flew out a window (pane removed) and orientated a second time to this much larger hole (about 40 cm x 50 cm) which was common to all colonies (see plate 2). This aperture was surrounded by a one-metre square sheet of yellow cheesecloth to make it look distinctive. The other windows in the room were covered with brown paper to prevent the bees from trying to fly out of them. The room was illuminated continuously with fluorescent tubes. Without this extra illumination the bees tended to walk a long way (50 cm or further) before beginning their orientation flight. On their return they alighted at the place from which they had flown, and tried

Plates 1 and 2 The Laboratory (1975-76 season)

1) 2) 3) 4) 5) 6)	Exit holes of observation hives
7	Nest Chamber
8	Inner vestibule
9	Outer vestibule
10	Black tent covering forager-photographing area
11	Tunnel
12	Power supply for electronics
13	Temperature-control circuits
14	Common exit window
15	Wires between nest chambers and temperature-control circuits


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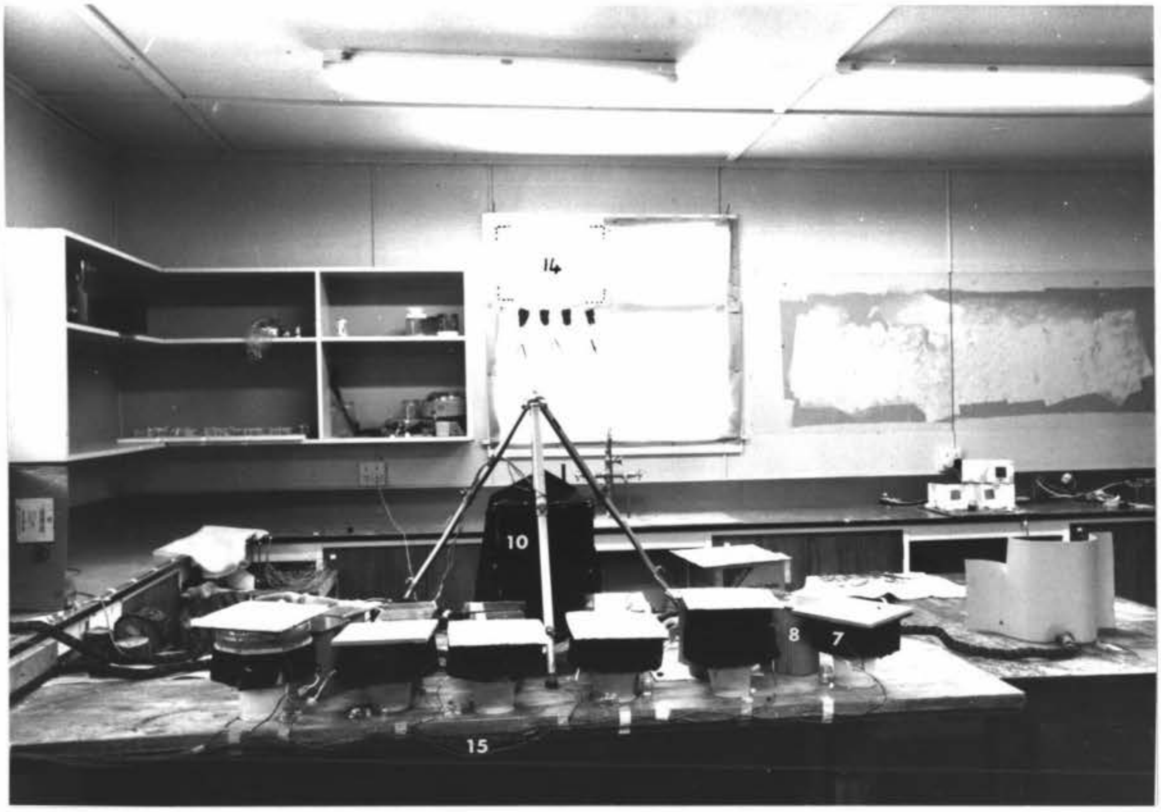
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to find their way back on foot, which they seemed to have difficulty in doing. With the extra light they did not walk as far as before flying, and found their way home more easily.

The arrangement described above was the end-product of three years of improvements but was still imperfect. Some foragers still returned to the wrong colony. This usually occurred within the first day or two of the bee beginning to forage; it subsequently returned always to the "foster" nest. New queens were especially liable to enter wrong colonies. The problem seems difficult to completely overcome. I suspect that drift is largely caused by bees following others into the nest entrance (social facilitation) as described by Blacklith (1955). He found that with B. lapidarius, new queens were more dependant upon social facilitation to pass through the nest entrance than workers were. This fits in with my observations of queens having a greater tendency to enter neighbouring nests.

2.3 DATA COLLECTION

This section describes the collection of information (e.g. photographs and measurements) and material (e.g. pollen samples and rejected larvae) which involved interaction with live colonies. Procedures not involving live colonies, such as weighing rejected larvae, measuring cocoons and studying photographs, are described in section 2.4 on data analysis.

2.3.1 Brood records

The state of the brood in the incipient colonies inhabiting surface domiciles, was noted weekly. The main records for each colony, however, were begun the day after it was installed in an observation hive, and concluded when it ceased to have any more live brood. This was done for six colonies (although one of them died prematurely due to the loss of its foundress queen) and involved a total time-span of ninety-four days (25 November, 1975 to 27 February, 1976). Data was recorded daily except for three missed days.

At 1030 ± 2 hrs each colony was photographed on 35 mm monochrome film. Prior to photographing each hive, I inserted labels (stuck to a length of wire) stating the date, time and colony-number; and a strip of card graduated in centimetres. The single lens reflex camera was set at f16 for maximum depth of field. The light source was an electroni flash unit. I tried to take the photographs at moments when brood clumps were least obscured by bees.

Written records were also kept. The aim was to record the developmental changes of each brood clump. A "clump" was defined as the group of individuals growing from a contiguous group (usually one to three) of egg cells. At the time I started detailed records, adults had already emerged from the first (incipient) clump, and the 2nd and 3rd clumps had often pupated. Clumps were numbered in the order of their formation, and their positions marked on the glass hive-roof with wax pencil (the glass was swapped with a clean sheet for photographing). As soon as the photographs were printed, they also had clump numbers written on them. Colonies were examined each day (directly after the photographs had been taken) and the dates of the following were recorded for each clump where possible:

egg-cell construction
egg-cell closure (i.e. oviposition)
construction of larval-clump pocket
completion of first cocoon
completion of final cocoon
emergence of first adult
emergence of final adult.

The adult emergence dates were sometimes not determined due to the cocoons being obscured by later brood clumps. The identification of the cocoon clump on which each new egg clump was built, was always recorded. The caste of the freshly-emerged bees (callows) was noted for each colony daily but I could not necessarily find which clump they had each emerged from.

Behaviour such as larval-feeding, egg-eating, egg-laying, larval rejection and inter adult aggression was noted whenever observed, but no attempt was made to systematically determine the frequency of such events.

A few workers from each colony were marked with honey bee queen-markers (convex-concave plastic discs of various shapes and colours, glued to the bee's thorax while under CO_2 anaesthesia) for foraging data (see section 2.3.3).

Six males and about ninety queens were marked when freshly emerged to see how long they stayed in the parent nest. Their presence was looked for each day while brood records were being taken, and sometimes after dark too. Adult censusing is difficult in large colonies because so many bees are able to hide in the lower parts of the nest.

2.3.2 Larval rejection

I wanted to know the number (or at least the relative frequency) of larvae rejected by each colony on a daily basis, and to retrieve them for examination of their size and sex. Collecting rejected larvae from naturally-foraging colonies is made difficult by the fact that workers carry some of these larvae all the way out the entrance tunnel and even fly away with them (Miyamoto, 1960; and my observations). However, I have observed that rejected larvae are often not taken all the way out of the nest by the same bee. Some (perhaps most) of those rejected are initially dropped a few centimetres from the nest-chamber (i.e. in the vestibule if one is present), and carried further when encountered by a second bee. To prevent larvae being carried further by successive bees, I designed a vestibule which would trap any larvae dropped in it, in such a way that they were unlikely to be discovered and carried further. A series of two of these vestibules were connected to each observation-hive to see what proportion of larvae were carried different distances (see figure 2.5).

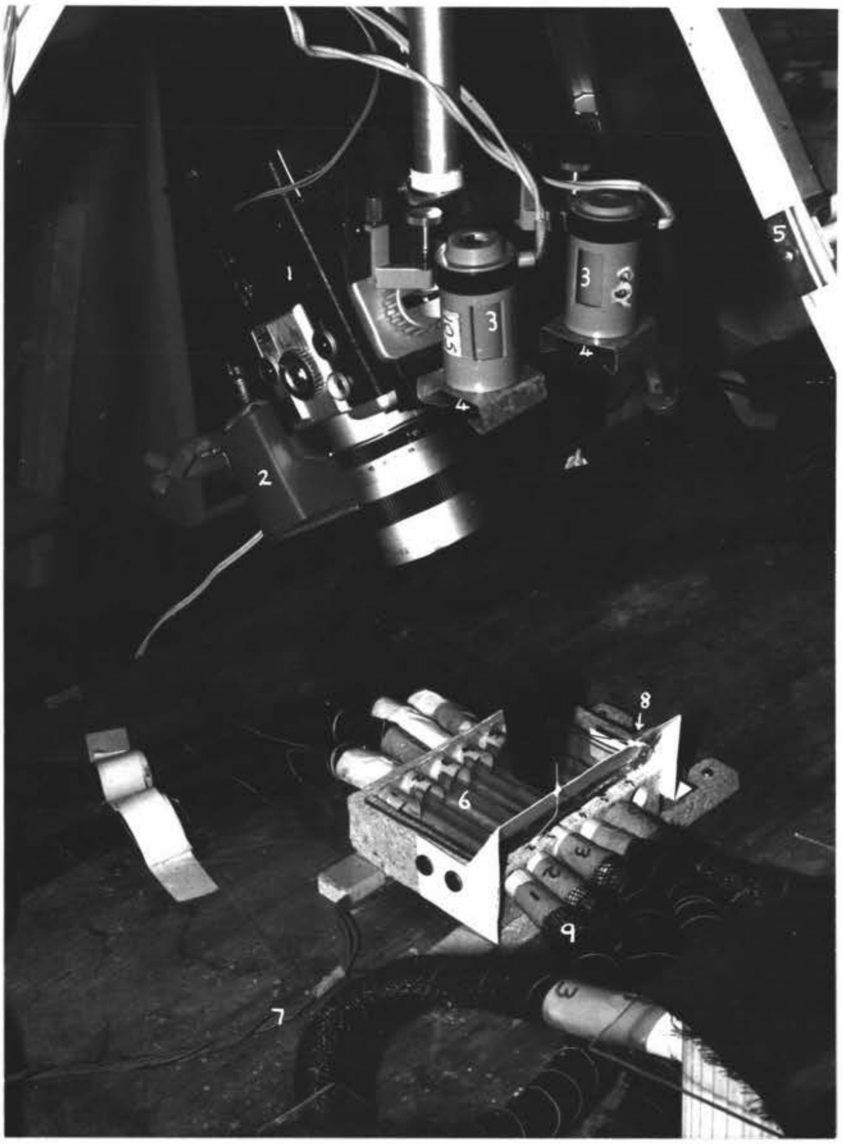
Larvae were taken out of the vestibules as soon as the photographs had been taken each day (i.e. about 1030 hours), and preserved in 70% ethanol.

2.3.3 Foraging data

Pollen is the only protein-source in the diet of bumblebees, and it is, therefore, a major factor in larval growth. Pollen intake to colonies was estimated by automatic photography of foragers in the entrance tunnels. The apparatus is shown in plate 3. An electronic circuit (designed and built by Mr. M.D.K. Schmidt) sensed bees breaking a light beam as they passed through the glass-topped chamber. To prevent them from trying to get out through the glass, the area was darkened with a black tent (removed for plate 3, but visible in plates 1 and 2). The light beam was emitted by a pair of microscope epiiluminators shining through sheets of red perspex. When a bee interrupted the red light beam the super-8mm movie camera fired a single frame (there were 3,600 frames per film), which included the bee, a wristwatch and a date label. An electronic flash unit provide illumination for the photographs, but it took 3 seconds to completely recharge, and photographs taken more often than every 1.5 seconds (approx.) were black. To prevent film wastage on such shots (which were often caused by the vacillation of one bee), a timing circuit was included which prevented the camera from being fired more often than once every 1.5 seconds. Although this system was able to provide detailed data on foraging, the film expense, and the time required to analyse it, prevented records from being taken daily. A total of 29 colony-days of foraging were filmed.

Plate 3 Apparatus for automatic photography of foraging bumblebees

1	Super-8 mm camera (Canon 814 auto-zoom)
2	Electronic flash unit
3	Microscope epiilluminator lamp
4	Red perspex
5	Tripod leg
6	Glass-topped channel
7	Wires to light dependant resistors
8	Bracket for wristwatch
9	Tunnel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This was done in seven sessions (sometimes filming for several days at a session) on five colonies at intervals between 9 December, 1975 and 3 February, 1976.

Sometimes, as a pollen-bearing forager passed through the photographing chamber, I inserted a numbered label into the camera's field of view. The label was photographed on the same frame as the bee, which was then captured and its corbicular pollen weighed. The bee and its pollen were returned to the appropriate hive. In this way I was able to determine the relationship between the weights of corbicular pollen lumps, and their size on the photographs. Twenty-four such pollen records were taken, each having two weights; one for each hind leg.

Other pollen samples were taken for a different purpose. I wanted to determine the number of grains per milligram for the main pollen species collected by the bees. This would enable me to convert the number of pollen grains found in the pupal meconium or the larval gut into actual weights of collected pollen. A few corbicular samples were taken of the common pollen types (judged by their colour). The pollen lumps were weighed, then stored in small vials of 70% ethanol for later dilution and counting.

2.3.4 Adult male and queen food consumption

This was a small-scale trial to determine the approximate amount of honey and pollen consumed by newly emerged males and queens. Two cocoon clumps (one with five male cocoons, the other with five queen cocoons) without brood on top, were placed in gauze-topped plastic jars in a 30°C incubator. The adults emerged from their cocoons satisfactoril without worker assistance. Small plastic vials (capacity about 1.7 ml) containing 50% (v/v) honey solution (S.G. = 1.15) and pollen (from an Apis-hive pollen trap) were securely plugged into holes in the bottom of each jar. Each day the food-vials were weighed, replenished and re-weighed. A control vial of honey solution was provided outside the jar to determine the weight loss by evaporation. The males were released five days after emergence, and the queens after seven days.

2.3.5 Destructive sampling of whole colonies

During the 1974-75 season when I had a surplus of colonies, I deep-froze four of them. Two were taken from field domiciles (one at an early stage, and a larger one at male production), and the other two were from observation hives (although initiated in field domiciles) and had reached queen production. The purpose of freezing these nests was so that their brood could subsequently be examined. In particular, I intended to examine the contents of egg cells and larval clumps which was not possible with live colonies.

2.4 DATA ANALYSIS

2.4.1 Brood development

The area of certain larval clumps was measured from daily nest photographs with a planimeter.

The larval clumps of frozen nests were measured, drawn, and dissected. The position of each larva in relation to the pollen mass and neighbouring larvae, was recorded. The larva was then stored in a vial of 70% ethanol. Later its head capsule width was measured under a dissecting microscope with an eyepiece micrometer. Then it was dried at 55°C for 1.5 days, and weighed. From these data, a relationship between the area of a clump, and its biomass was derived.

When there were no live adults or brood left in the observation hives, the clumps of empty (and unhatched) cocoons were dismantled and examined. The cocoons of most brood clumps could be identified from the nest-photographs. Clumps adhered only weakly to one another, but in order to separate the individual cocoons within each clump, I wetted them with detergent and water, and left them in a moist environment overnight. The positions of the individual cocoons could often be seen from the photographs. The diameter of each cocoon (except some badly mutilated ones) was measured by placing it upright on an overhead projector stage, and matching its shadow on the screen with a set of standard circles.

The amount of pollen consumed by larvae was determined from the quantity of empty pollen grains found in the faeces, which are voided prior to pupation and incorporated into the cocoon wall. Cocoons of known diameter were dissolved in

1 to 2 mls of 20% (w/v) KOH (24 hrs at room temperature).

After slight dilution with water, basic fuchsin was added to stain the pollen-grain coats, which were not harmed by the KOH. Then a few drops of detergent were added. (Without detergent the grains tended to clump together when diluted.) Finally, the mixture was diluted to 100 or 250 mls (depending on the size of the cocoon) and thoroughly shaken. About 1 ml of mixture was poured into a 1 mm deep counting cell. Fields of 0.277 µl were examined using a compound microscope with an eyepiece grid, at a magnification of 100x. The number of pollen grains of each main type (about three species usually comprised the bulk of the sample) were counted in several fields. I counted enough fields to obtain an S.E.M. for each main pollen type of $\leqslant$ 10%, up to a maximum of 35 fields. A similar procedure was done on monospecific corbicular pollen samples of known weight. This enabled the weight per grain to be calculated for the main pollen types counted from the cocoon walls.

2.4.2 Larval rejection

Each rejected larva had its head capsule width and dry weight determined by the methods described for frozen larvae (section 2.4.1). Their sex was also ascertained. The characters used for sexing were those described by Stephen and Koontz (1973) on the ventral surface of the abdoman. The blind pocket on the penultimate segment of the male could often be located with a human eyelash glued to the end of a seeker. The larvae whose sex could not positively and quickly be determined while intact, were re-examined by a different method after being dried and weighed. They were placed in vials of Essig's Aphid Fluid containing acid fuchsin, erythrosin and lignin pink (formula in appendix 4). The vials were capped with perforated lids (to reduce evaporation but prevent pressure build up) and incubated at 55°C for several hours. This treatment re-hydrated the larvae, partially dissolved the internal structures, and (most importantly) stained the cuticle. On removal from the fluid each larva was cut in half. The posterior half was placed on smooth paper and a wooden or glass rod rolled over it (moving anteriorly) to squeeze out the body contents. The empty skin was then placed in a watch-glass of alcohol under a dissecting microscope. The surface features looked for earlier could now be seen with greater clarity on the stained cuticle.

I wanted to know whether or not larval rejection was stimulated by pollen shortage as suggested by Plowright (1966). I did not want to experimentally modify the pollen intake to the colonies because the primary aim of the investigation was to observe normal colony development. So I devised two indirect methods of approaching the problem. The first was the morphometric comparison of rejected with non-rejected larvae, to see if as Plowright reported, rejected larvae were "underweight for their instar". The only non-rejected larvae I could examine were the frozen ones from the 1974-75 season. Two of the frozen colonies were from observation hives and I possessed preserved specimens of larvae rejected from both colonies during the weeks preceding their being frozen. Dry weight versus head capsule width was plotted for rejected and non-rejected specimens. The second way of investigating the effect of pollen supply, was by plotting the intake of pollen loads (on the days when it was recorded), versus the estimated larval biomass (calculated from areas on photographs) present that day. If pollen shortage caused larval rejection, then most larvae ought to have been rejected following days of lower than average pollen intake per unit larval biomass.

2.4.3 Foraging records

The super-8mm films of foragers in their entrance tunnels proved to be too time consuming to analyse to the extent originally planned. About two-thirds of one film (there were six altogether), representing five colony-days was fully analysed. The rest were scanned with a film editor and the number of pollen loads per colony per day, counted.

Full analysis involved examining each frame under a dissecting microscope (magnification 40x). The following data were recorded for each frame:

the time (from the wrist-watch)
the colony to which the forager belonged
whether it was leaving or returning
its label (if present)
the size of its corbicular pollen loads
    (if present).

The size of the pollen loads were measured as the length and breadth (in eyepiece micrometer units) of whichever lump was easiest to see. Most bees had similar amounts of pollen on each corbicula (from weight samples), but if an obvious difference between the two sides could be seen on a frame, the sizes of both were recorded.

CHAPTER 3 RESULTS AND DISCUSSION

3.1 COLONY ESTABLISHMENT

3.1.1 Natural Nest Sites

During the 1973-74 season seven B. ruderatus nests were found, including three using the forager-feeding method. These three were located at the intersection of a homing direction with a patch of trees. Five more nests have been discovered in other years, and these have been described, although not all the details of the earlier ones (pre 1973) were recorded. One of the earlier discoveries (no. 3) was near Richmond in the Nelson district. All the rest were found at the Waverley study area.

The features of each nest site are shown in table 3.1. Although most of the nests (nine out of twelve) were underground, the tunnel dimensions were quite variable. Diameters ranged from 3 to 12 cm, and lengths from 60 to 375 cm. All but one of the colonies seemed to be occupying the nests of small mammals (I was uncertain about no. 3). The species of mammal was tentatively identified by the diameter of its burrow (where present). The following categories were chosen:

less than 4 cm - mouse (Mus musculus)
4 to 8 cm - rat (Rattus spp.)
more than 8 cm - rabbit (Oryctolagus cuniculus).

According to these categories, nos. 7, 8 and 9, would be in mouse nests; nos. 2, 4, 6 and 12, in rat nests; and no. 5 in a rabbit nest. No. 11 was occupying what appeared to be a nest made by a mouse in a polythene bag of hay. The bag

TABLE 3.1 Natural Nest Sites of Bombus ruderatus

	Season 19..	Surrounding Features	Entrance Features	Tunnel:		Cavity depth cm
				length cm	width cm	
1	ca 60	sloping pasture	hole in ground	ca 100	ca 5	?
2	69-70	flat pasture near pond	hole in low clay bank	ca 70	ca 7	ca 30
3	72-73	debris beside woolshed	?	no tunnel, nest under debris		
4	73-74	flat pasture 1m from woodshed	depression under board	155	7	50
5	73-74	edge of native bush	(13x45)cm gap between tree roots	375	12	90
6	73-74	edge of native bush	slit (2.5x13) cm at base of tree	60	5	40
7	73-74	heap of logs on sloping pasture	small hole in bare ground under logs	90	3	20
8	73-74	rough hillside pasture	grass-concealed hole in low bank	210	3	20
9	73-74	rough hillside pasture	depression amongst long grass	300	3	25
10	73-74	scrub-covered hillside	gap in dead vegetation	no tunnel, nest amongst dead vegetation		
11	74-75	in concrete-floored shed	hole 12 cm sq. at base of door	1m walk along floor		on shelf 12 cm above floor
12	75-76	edge of native bush	fern-concealed gap (5x10)cm between roots in low bank	110	5-12	60

had a mouse-sized hole, and the hay around the bumblebee nest (which was found during its incipient stage) appeared more neatly arranged than would have been done by the queen. The surface colony no. 10, was in what appeared to be the nest of a hedgehog (Erinaceus europaeus) or a hare (Lepus europaeus).

The cavity of no. 6 contained the decaying remains of a B. ruderatus colony which had lived there the previous season. Several authors cited by Free and Butler (1959) (Hoffer 1886; Frison 1930; and Plath 1934) have seen overwintered queens examining old nest sites of their own species. I have seen overwintered B. ruderatus queens examining a window, through which queens had departed the previous summer from observation hives. I suggest that this might be a normal occurrence, and is a behaviour pattern which leads to overwintered queens re-using their maternal nest site if there is enough nest material remaining. If this is so, the trait offers possibilities for inducing naturally overwintered queens to occupy artificial nest sites simply by shifting a mature colony (or even part of one) to that site the previous Summer and allowing young queens to depart from it.

It is noteworthy that different types of nest sites were found by different "sampling methods". The only nest found by members of the public (no. 3) was at surface level. This nest came to my notice after a newspaper advertisement (a method recommended by Free and Butler, 1959) was published by the DSIR. Accidentally discovered bumblebee nests probably include a disproportionate number of above-ground ones, because these are more likely to get disturbed, and disturbed bumblebee colonies produce a buzz which is audible from several metres away! In fact I discovered nest no. 11 only by the sound produced by the single queen. All the nests which I found accidentally or by random searching, were either in or near frequented buildings, or in open (i.e. unshaded) farmland. However, the three nests found by the more objective method were all in the shade of trees. I suggest that B. ruderatus might commonly nest under trees, but that such nests are likely to be overlooked.

3.1.2 Domicile occupations

The occupation rates of the domicile types over the two seasons is shown in table 3.2.

During the first season several occupied domiciles of both types contained dead queens which had presumably been killed in battles over nest site possession. I also observed searching queens enter already-occupied domiciles, then hastily retreat, on two occasions. One was a B. ruderatus queen which had entered the domicile of another B. ruderatus with larval brood, and the other was a B. terrestris queen which had gone into a B. ruderatus nest containing a few workers. These incidents showed that death did not necessarily result for queens which entered previously occupied sites. Unless the unoccupied domiciles were unsuitable for some reason, such as dampness, I would have expected queens to occupy them rather than risking death trying to usurp a resident queen. Some, but not all, unoccupied brick domiciles were in fact noticeably damp, and I suspected this was making them unattractive to searching queens. Several authors (including Sladen 1912; Frison 1926; and Fye and Medler 1954a) have stated that dampness

TABLE 3.2 Domicile Occupation Results

Season and type	Number available	Number occupied(2) (percentage in brackets)			
		B. ruderatus	*B. terrestris*	Unidentified	Total
1974-75					
Underground	45(1)	23 (51)	7 (16)	12 (27)	42 (93)
Surface-brick	23	6 (27)	1 (4.5)	0	7 (32)
1975-76					
Surface-brick	25	4 (16)	0	0	4 (16)
Surface-concrete	25	1 (4)	1 (4)	0	2 (8)

(1) Fifty were put out but five became lost or damaged.

(2) "Occupied" means that a broody queen was present. Some brood was always also present in surface domiciles but this was not always checked for in underground ones.

TABLE 3.3 Relative Dryness of Brick and Concrete Domiciles Early in the 1975-76 Season (6 November, 1975).

Subjective Category	Number (percentage in brackets)	
	Concrete	Brick
Reasonably Dry	23 (92)	9 (36)
Slightly Damp	1 (4)	7 (28)
Moderately Damp	0	5 (20)
Wet	1 (4)	4 (16)

can be a problem in bumblebee domiciles.

The lightweight concrete type of domiciles used during the second season stayed drier than the brick ones (as shown on table 3.3), and I hoped this would enhance their attractiveness to searching queens. However, as table 3.2 shows, the drier type of domiciles were not accepted by any more queens than the brick ones were. In fact the lightweight concrete domiciles were occupied by only half as many queens as the brick type, although due to the small numbers involved, the difference was probably insignificant. One lightweight concrete domicile contained a dead B. ruderatus queen as well as the live occupant. The brick domiciles were more sparsely occupied in the second season. Free and Butler (1959) emphasise that bumblebee numbers fluctuate annually and that domicile results are similarly unpredictable. However, it is quite possible that I unwittingly reduced the attractiveness of the brick domiciles by some of the modifications I made. In particular, the removal of the newspaper layer may have allowed light to enter (polystyrene is translucent) although the two bricks on top should have stopped most of the light; and the change of shape of the nest material may have made it less attractive.

A most interesting and significant finding during the second season was that many of the underground domiciles were occupied although the nest material in them was about one year old, damp and decaying. As mentioned in section 2.1.2.2, I had decided to discontinue the use of the underground type, a decision apparently not shared by the bumblebees! Examination of twelve of the underground type revealed 3 B. terrestris queens and 3 B. ruderatus queens (the grilles to exclude the earlier-nesting B. terrestris had not been used). Three of the six unoccupied domiciles were excavated and found to have no nest material. I removed three of the incipient colonies (1 B. terrestris, 2 B. ruderatus) and replenished the nest material, before the end of the nest-founding season. These three domiciles yielded a second colony each (all B. ruderatus).

To summarise: the underground domiciles were highly attractive to B. ruderatus (and B. terrestris) queens. Two types of surface domicile attracted fewer queens, and the reason for this was not wholly due to dampness. In fact, queens appeared to prefer damp underground domiciles to dry surface ones. The 93% occupation rate (76% of the identified occupants were B. ruderatus) of 45 underground domiciles in the 1974-75 season, exceeds the best recorded by Hobbs (1967); 90% of 19 underground domiciles in the Rocky Mountain foothills.

3.2 BROOD DEVELOPMENT

This section describes the development of individual brood clumps, as a prelude to later descriptions of seasonal changes on a per-clump basis.

3.2.1. Eggs

Apart from the first (incipient) egg cell, nearly all were built on top of unhatched cocoons. (One batch of eggs was laid in the pollen pocket of a larval clump, but they failed to develop.) Egg cells on cocoon clumps may be seen in plate 4. Over 95% of the egg cells were formed on cocoons which were less than three days old. Frequency distributions of the substrate cocoon ages for each colony are shown in figure 3.1.

Each egg cell is a cup, 4 to 6 mm in diameter, made of wax-pollen[1] (which I shall call wax). Most egg cells are built by the foundress queen, and she lays the eggs in them. Workers sometimes lay eggs too: this is discussed in section 3.3.1.2. Sladen (1912) states that B. ruderatus prime their egg cells with pollen, but I have seen a number of them to be closed without being primed, especially early in the season. Egg cell priming is done by foragers which unload their pollen into the cell. Reasons for early cells not being so primed may be due to their being slightly smaller and considerably thinner-walled (both probably due to wax shortage). Later-made egg cells are more robust, and may

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[1] Wax-pollen is a mixture of wax and pollen. It is a soft material, of similar consistency to plasticine; in fact, I have found that B. terrestris will readily use plasticine, if provided, for structures normally made of wax-pollen. B. ruderatus seems much less inclined to use plasticine. All of the mid-brown structures in plate 4 are either made of, or covered with, wax-pollen.

Plate 4 Fifty day old *Bombus ruderatus* colony in observation hive (about natural size)

1 Foundress queen
2 Marked worker
3 Male (note long antennae)
4 Newly emerged worker (about 12 hours old)
5 Worker eating pollen from pollen pocket
6 Worker regurgitating food to larva
7 Wax honey pot
8 Honey in vacated cocoon

	Brood:	Age (days since eggs laid):
9	Egg cell	0.5
10	Egg clump (3 egg cells)	2
11	Larval clump with partially-built pocket	5
12	Larval clump at "smooth" stage	7
13	Larval clump at "bumpy" stage	8
14	Larva at silk-spinning stage (f = faeces)	11
15	Fresh cocoon	13


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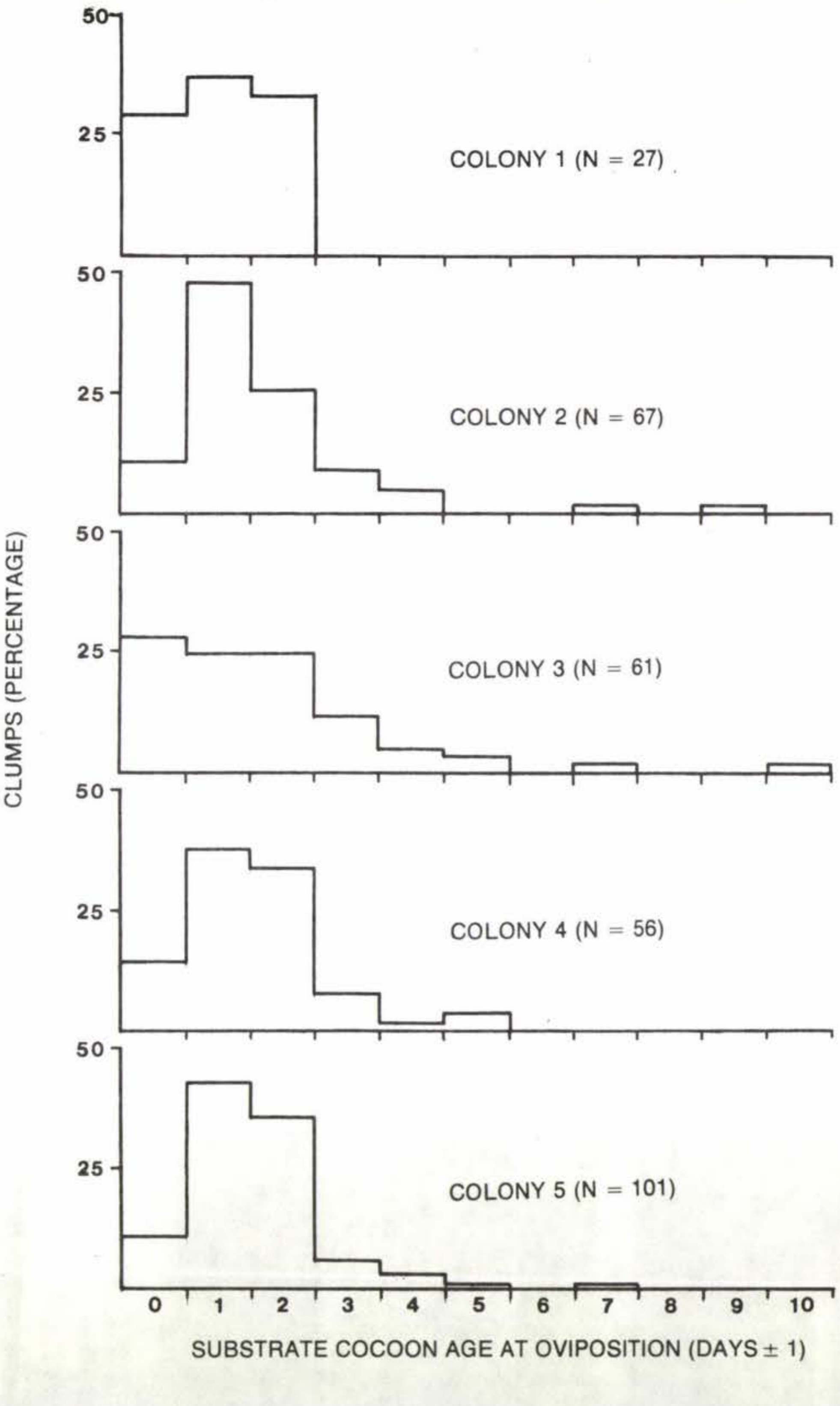

Figure 3.1 Percentages of brood clumps formed on cocoons of various ages.

provide the same stimulus as a pollen-pocket (which is where pollen is usually unloaded), to a returning forager.

The act of egg-laying is shown in plate 5. The sting protrudes each time an egg is laid, and by observing this, the number of eggs laid at one "sitting" may be gauged. From such observations, and by dissecting frozen nests, the number of eggs laid per cell was found to be 14.3 ± 3.0 (standard deviation (s.d.)) (N = 23), with a range from 10 to 19. Queen-laid eggs were 2.85 ± 0.11 (s.d.) mm long and 0.84 ± 0.04 mm wide (N = 17). Four worker-laid eggs were found to be about 10% shorter than queen-laid ones, but just as wide. Egg cells are closed immediately after the eggs are laid. Egg cell closure is shown in plate 6.

Often two or more egg cells are made in a line or group. Frequency distributions for the numbers of cells per group are shown in figure 3.2.

The time taken for eggs to hatch could not be determined from watching normal live colonies or from dead ones. During the 1974/75 season I fixed a clear acetate dome (4 mm wide) over a cell of newly-laid eggs before the queen could cover them. The eggs were seen to have hatched in slightly less than 4 days, at an observation hive temperature of 30°C.

3.2.2 Larvae

3.2.2.1 The larval instars: Figure 3.4 shows the frequency distribution of B. ruderatus larval head capsule diameters. Four main peaks are obvious. These correspond to four of the instars, but Stephen and Koontz (1973) state that there are five in the genus Bombus. Few first instar larvae were found, and this stadium is probably brief. The

Plate 5 Foundress queen laying eggs
Note the sting projecting over
the rim of the cell.

Plate 6 Egg cell being closed

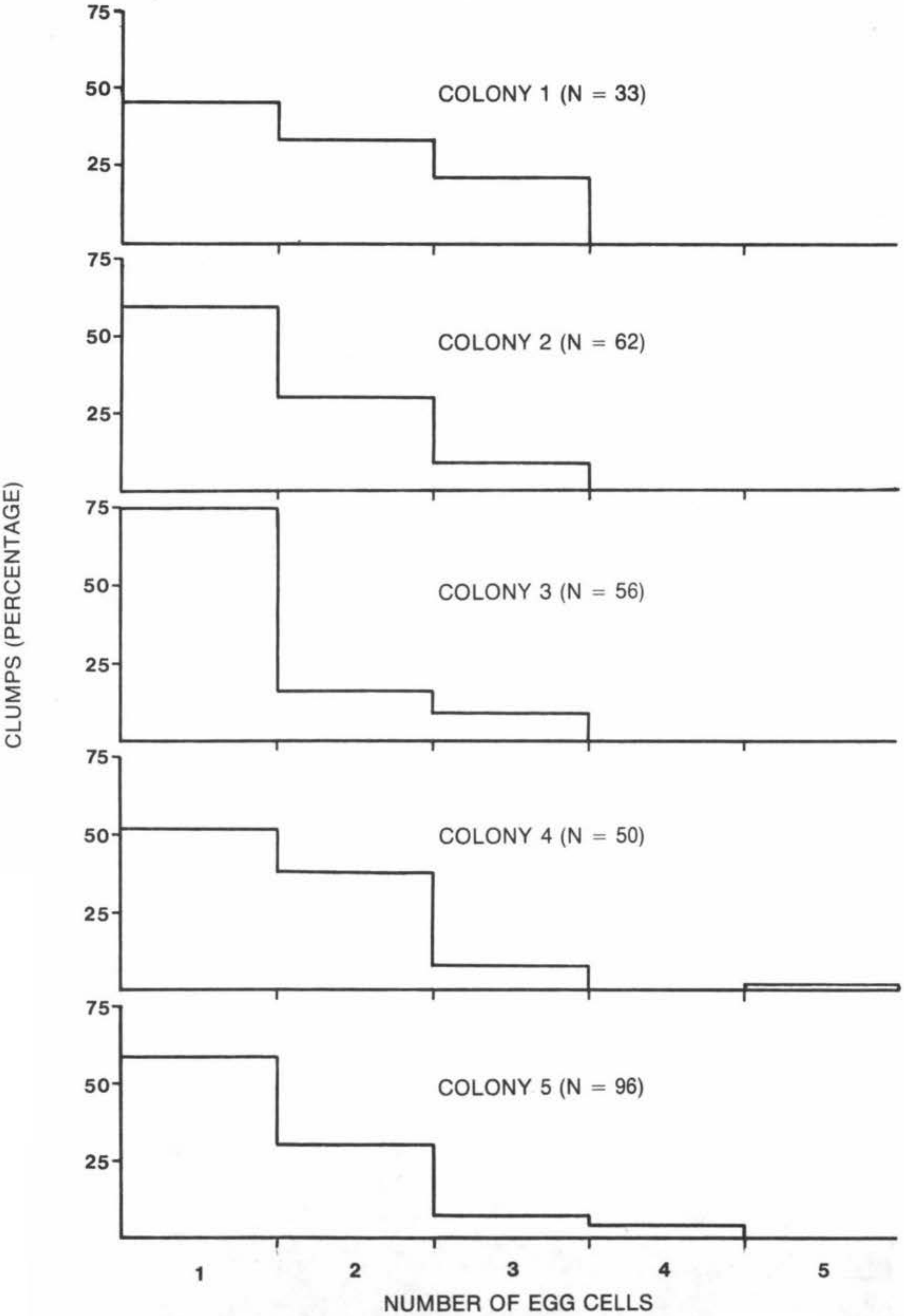

Figure 3.2 Number of contiguous egg cells per brood clump. (About 14 eggs are laid in each cell.)

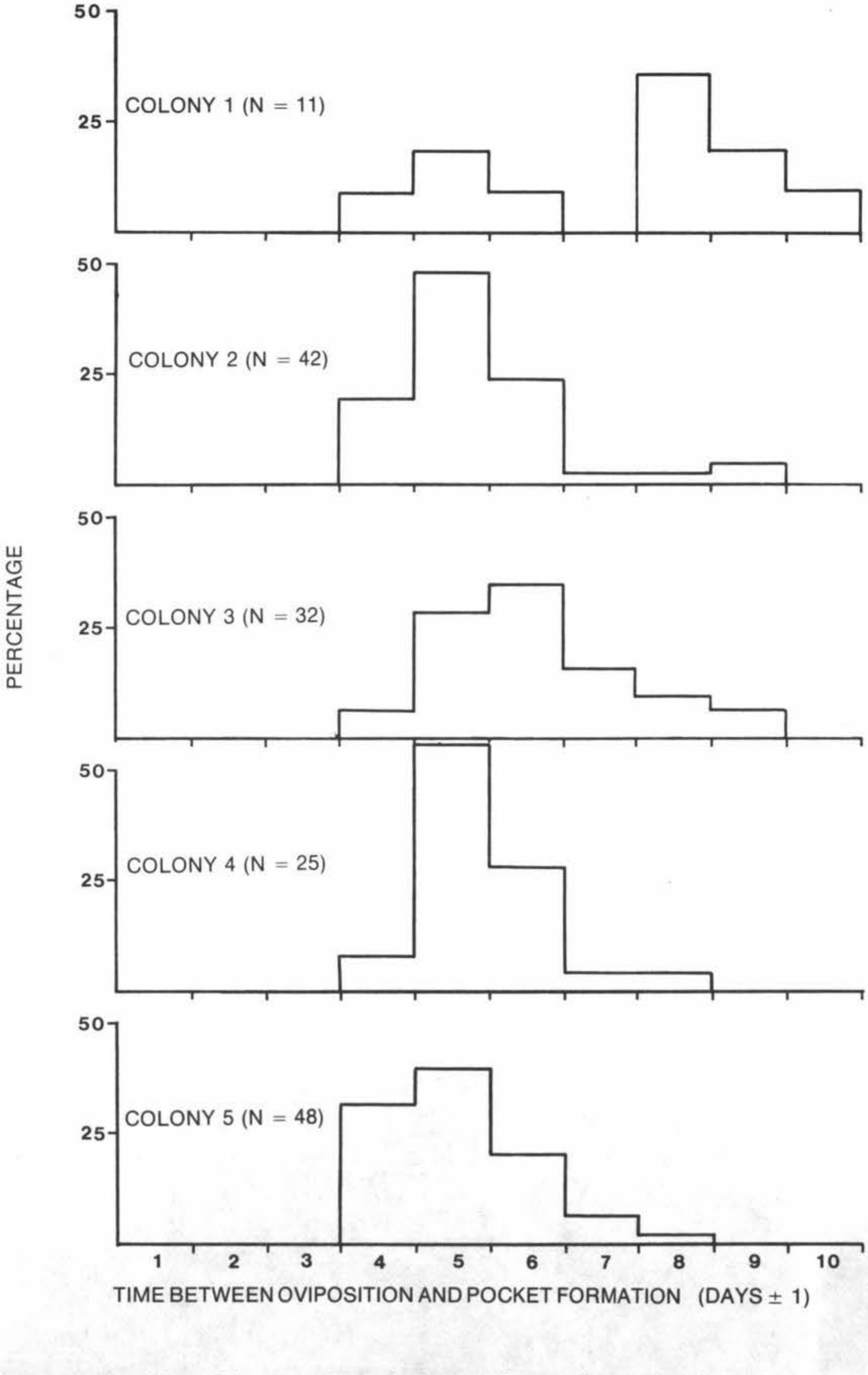

Figure 3.3 Age of brood clumps when pollen-pocket formed.

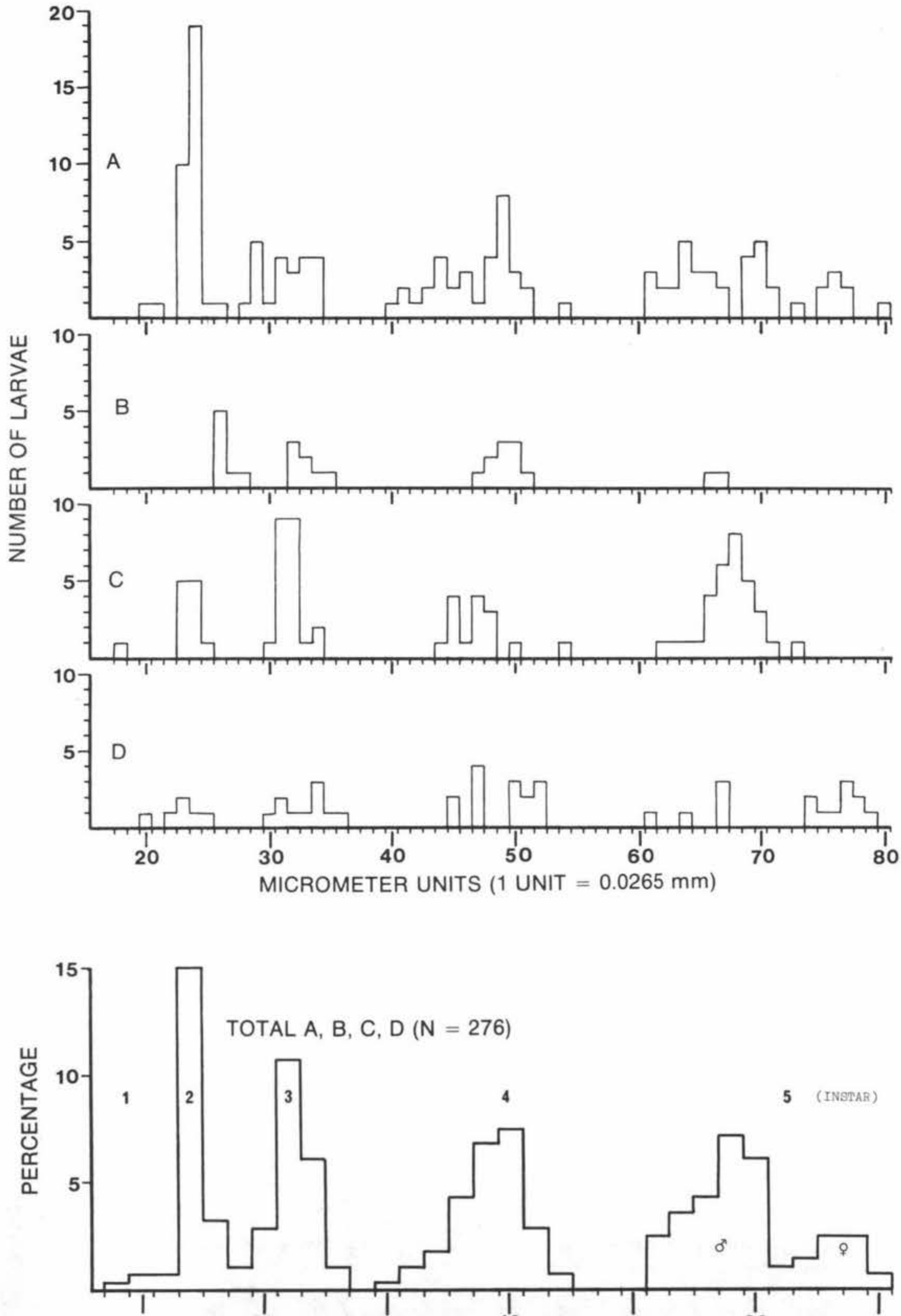

Figure 3.4 Frequency distribution of larval head capsule diameters. Colonies A and B were from field domiciles (frozen 6 Jan. 1975), C and D were from observation hives (frozen 18 and 19 Jan. 1975).

distribution of head widths was continuous over the first three instars, but was discontinuous between the third and fourth, and between the fourth and fifth. The fifth instar shows a bimodal distribution. Most of the larvae with narrower heads were males, and most with wider heads were females.

3.2.2.2 Physical conformation of larval clumps: The first change observable on brood clumps was usually the construction of a pollen pocket (see plate 4 and figure 3.5). The pocket was usually built on the side nearest to the periphery of the nest. The construction of these pockets is a well known characteristic of certain Bombus species, termed "pocket makers", while other species store their pollen separately from the larval clumps, and are called "pollen storers" (Sladen, 1912).

The pollen deposited in the pockets forms a bed on which the larvae feed (see figure 3.5), although the larvae are also fed by direct regurgitation from the adults. (Larval nutrition is described in more detail in the next section.) Although pollen pockets were involved with only one of the two methods of feeding larvae, the appearance of a pocket usually coincided with the beginning of clump growth. This suggests that pocket formation is a reasonable guide to the time when food begins to be invested in a clump of larvae.

Figure 3.3 shows frequency distributions of brood clump ages at the date of pocket formation. (A minority of clumps had their eggs laid over a period of two or more days, and these have been excluded from figure 3.3, because no single oviposition date could be assigned to them.) The histograms show pocket formation to most frequently occur on the fifth

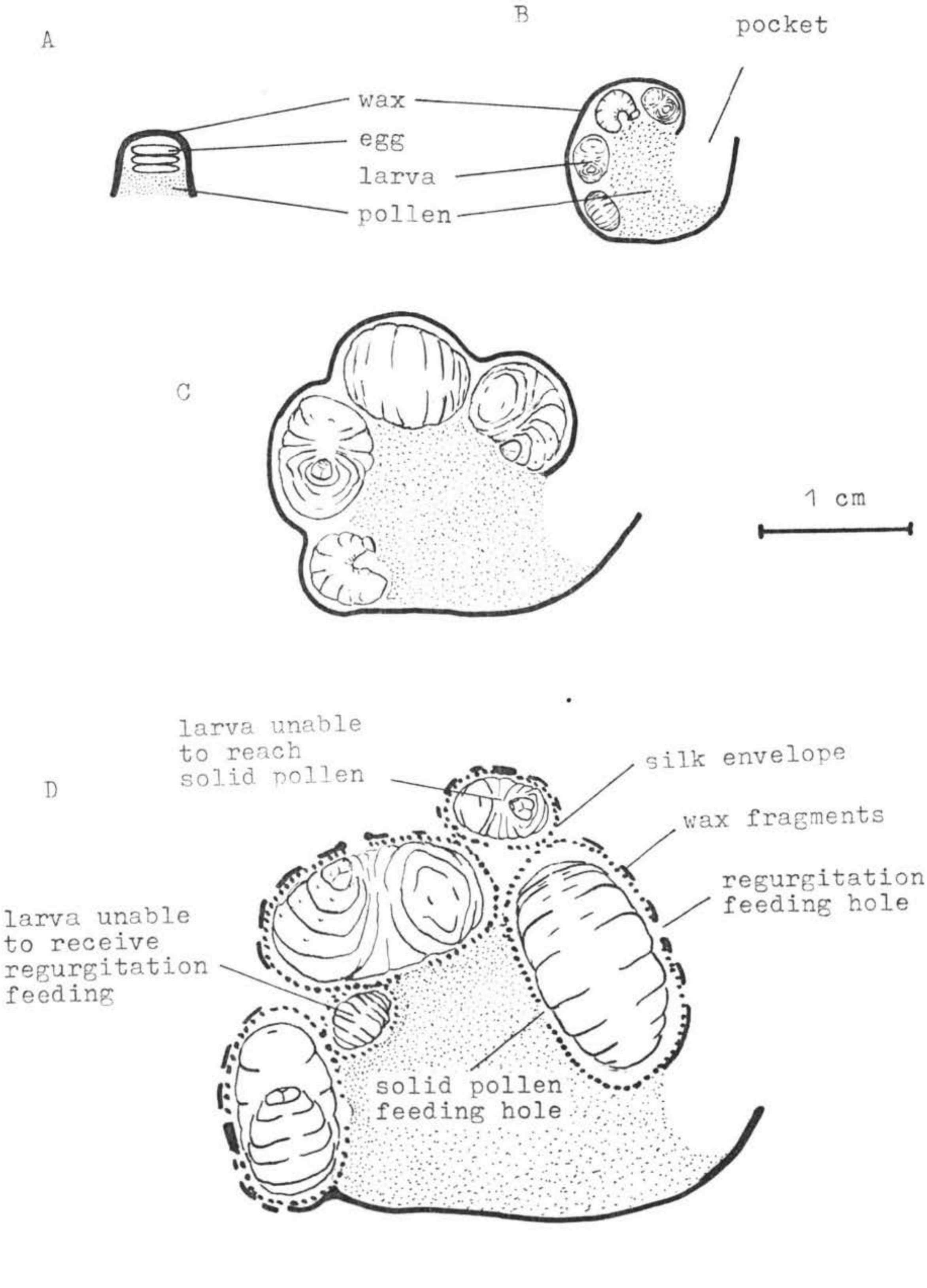

Figure 3.5 Diagrams of developing brood clump (mid-vertical sections, through pocket). (A) Egg cell, 0 - 4 days after oviposition (d.a.o.). (B) "Smooth" larval clump, 5 - 9 d.a.o. (C) "Bumpy" larval clump, 7 - 10 d.a.o. (D) Silk-spinning larval clump, 8 - 15 d.a.o.

day after oviposition; which is to be expected if the eggs hatch in three to four days, and then about one day is occupied with the first larval stadium which does not feed according to Stephen & Koontz (1973). However, pocket formation was sometimes delayed, as often occurred in colony 1, which was the least prolific. I do not consider that delayed egg-hatching was involved, because temperatures were kept stable. It is possible that shortages of food or labour might delay pocket formation and larval growth. The most prolific colonies (2 and 5) had many of their pockets built promptly, i.e. on the fourth day after oviposition.

After the pocket is built, the clump continues to expand for the next one to four days, retaining a smooth rounded appearance. At this stage much of the clump's volume may be taken up by the mass of pollen. It is sometimes said that the pollen placed in the pocket is "forced under the larval clump" (Alford 1975), but I consider that the larval clump grows over the pollen. That is to say, the relative positions of the larvae and the pollen mass, are explainable in terms of the larval clump expanding in the direction of the pocket. I found larvae of the first three instars inside "smooth" clumps. Much more clump expansion and larval growth occurs during the third stadium than during the first two. Larvae in smooth expanding clumps were found to be normally arranged in a single layer, so that they all had access to the pollen.

About two to four days after pocket formation, the larval clump becomes bumpy in appearance. Each "bump" or bulge, represents the position of a larva, which subsequently remains unchanged. Most of the larvae found in "bumpy" clumps were fourth instar.

During the fifth stadium, larvae spin a thin layer of silk around themselves. Little wax seems to be added to the clump after this, and as the larvae grow, the clefts between them become quite deep. This is shown in plate 4. The silk envelope has a hole about 2 mm wide, through which adults regurgitate food to the larvae. The hole is often, but not always, kept covered with wax between feedings. There is also a gap in the silk underneath the larva, through which it feeds on the bed of pollen. Sometimes larvae grow so fast that neither their silk envelope, nor the wax provided by the adults, keeps them completely covered, and gaps appear (distinct from feeding holes) through which the larvae can be seen. An example of this is shown in plate 7. Defaecation occurs during the latter part of the fifth instar, the faeces being spun into the wall of the silk envelope. Although most authorities (e.g. Free and Butler 1959; Michener 1974) state that bumblebee larvae only defaecate after the cessation of feeding, this does not appear to be so in B. ruderatus. Faeces are visible in the silk envelopes of two larvae in plate 4 which are probably still at a feeding age. Several times I have removed defaecating larvae from nests, and they still readily consumed a liquid honey/pollen mixture if it was provided.

About ten to fifteen days after oviposition, the larva ceases feeding, secretes much more silk than previously, closes its feeding holes and spins an oval cocoon.

3.2.2.3 Mode of nutrition: B. ruderatus larvae feed from the bed of pollen on which they lie, and are also fed by regurgitation from the adults.

Plate 7 A rapidly-growing clump of queen larvae.

1 Rupture in wax covering

2 Feeding hole

Plate 8 Egg clump broken open. There are two egg cells. Note the shrivelled appearance of some eggs.


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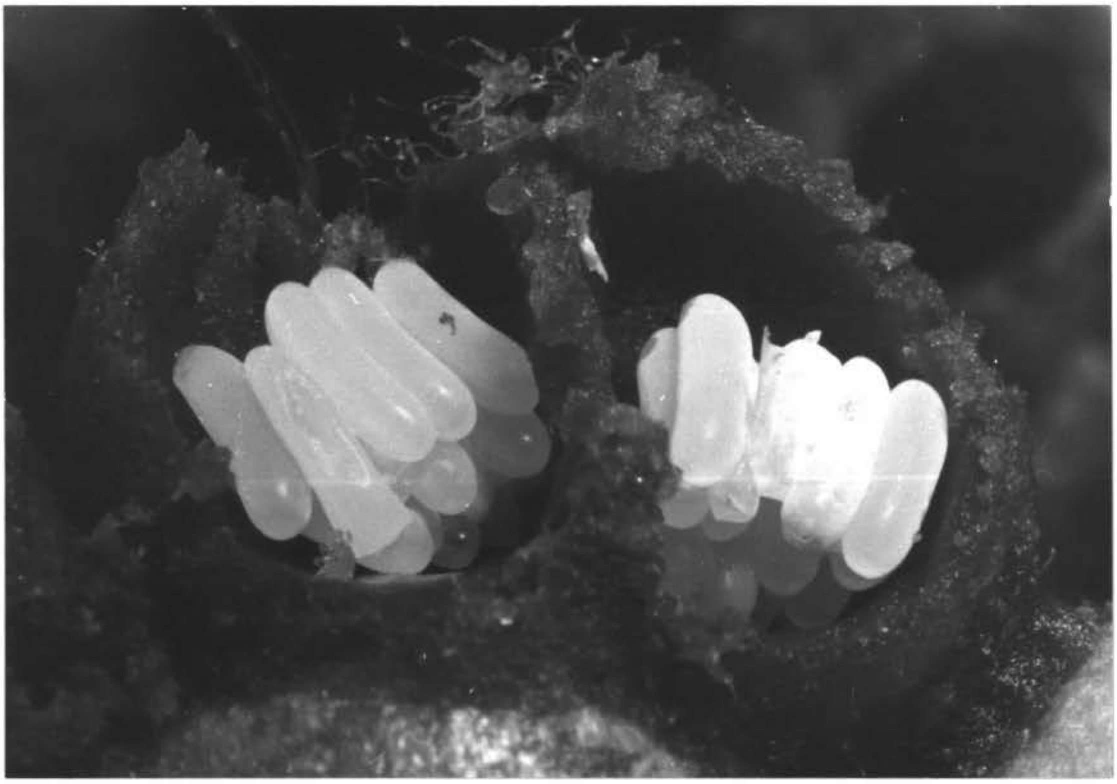

Nearly all larval clumps, irrespective of caste, had pollen pockets. The pockets were sometimes removed before the larvae had finished growing, but in such cases, there may still have been pollen remaining underneath the larvae. When larval clumps (from frozen nests) were dissected, depressions underneath each larva could often be seen on the pollen mass. The depressions appeared to have resulted from the larvae eating the pollen. As mentioned above, fifth-instar larvae left a hole in their silk envelope, over the pollen. This hole must have allowed them to eat the pollen. Sometimes, rapidly growing male and queen clumps had two pollen pockets, either at each end of the clump or side by side.

Regurgitation feeding was observed for larval clumps of all stages, and (apart from the incipient period when colonies were not observed) throughout the season. The regurgitated food, on the few times it was examined, proved to be a liquid mixture of honey (or nectar) and pollen. Three samples of the mixture were analysed (by dilution and counting the pollen grains), and found to contain 0.91, 1.34, and 0.93 milligrams of pollen per microlitre. The pollen required for regurgitation was ingested by the adults from pollen pockets or from primed (unclosed) egg cells. Eating it involved an alternation of abdominal contractions with bouts of nibbling at the pollen surface. The impression I gained was that the abdominal contractions served to regurgitate honey (or nectar) onto the pollen surface, and that the nibbling served to mix the honey and pollen into a soupy consistency, which was then imbibed. Plate 9 clearly shows how the pollen in a pocket becomes wet and pitted, as a result of adults eating it. Plate 4 shows a worker in a typical pollen-eating position, and another worker in the act of regurgitating food into a larval clump. When feeding a larva, the adult bee makes a hole (about 2 mm diam.) in the wax covering of the larval clump, contracts its abdomen to regurgitate the food, and reseals the hole. As mentioned above, some fifth instar larvae have a feeding hole which remains open. When individual bulges were visible on a clump, feeding was always done at the centre of a bulge, i.e. larvae were fed individually. The degree of abdominal contraction at different feedings varies, and so presumably does the amount of food discharged. In general, large larvae seemed to receive larger feeds than small larvae.

The relative importance of solid pollen and regurgitation feeding in pocket makers is by no means clear. Sladen (1912) said, "... I have no doubt that the larvae of the pocket-makers do partly feed on the pollen placed in the pockets, at least during the earlier stages of their growth." Michener (1974) apparently regarded regurgitation feeding as unimportant saying, "In pocket-making species, larvae that are to become workers (and males) are left largely to their own devices in obtaining food from stores in the pockets." He excluded queens because most North American pocket-makers do not build pockets on clumps of queen larvae (Hobbs 1964). Hobbs (1966a, b) pointed out that some North American pocket-makers fed fifth instar larvae by regurgitation only, even though the same clumps had pockets when younger. Although some authors seem to have considered regurgitation feeding of little importance when pollen pockets are present, Plowright and Jay (1968) found that pocket-makers can rear their larvae entirely by regurgitation feeding.

Plate 9 Worker about to feed larva. The tip of the worker's left antenna is in the feeding hole. The pocket contains red-clover pollen. Note the wet and pitted appearance where adults have been eating the pollen.

Plate 10 Worker carrying rejected larva

Although B. ruderatus larvae of all ages and castes seemed to be fed by both methods, the importance of each was not quantified. Of the thirty larval clumps dissected, all the pre-fifth instar larvae seemed to have access to the pollen mass. The positions of the spinning (fifth instar) larvae were fixed and could be seen more accurately. Some of these larvae had plenty of access to the pollen, but due to being overlain by adjacent larvae, had no access to regurgitation feeding. Two such larvae were found, and at the time of sampling were the lightest in their clump. This suggests that they were handicapped by not receiving regurgitation feeding. On the other hand, a few larvae were fixed in positions above their neighbours, and separated from the pollen mass. These, which had (around the time of sampling) been relying on regurgitation feeding, were also smaller than the mean for their clump, but were not the smallest. On one occasion, a larva was seen in such a position in an observation hive. It became displaced upwards, and almost certainly lost access to the bed of pollen. When first noticed (11 days after oviposition; early spinning stage) it was smaller than its neighbours, and remained so for the next two days. However, it seemed to catch up to the size of the others on the third day, and pupated about the same time as them, forming a cocoon no smaller than the average for the clump. Larvae which form cocoons underneath a clump usually form small ones. These larvae, I suspect, suffer more from lack of regurgitation feeding than lack of access to the pollen mass, which presumably lies on top of them.

The question of whether queen larvae receive food which is qualitatively different from that fed to worker larvae, has been raised by Roseler and Roseler 1974, and Plowright and Pendrel 1976. I had a brief look at this question by assessing the quantities of pollen used to rear larvae of different sizes. Cocoon diameter was used as representing the size attained by the larva. As shown in figure 3.9(B) cocoon diameter correlates closely with adult linear measurements (correlation coefficient = 0.9914 for radial cell length). But I decided the cube of the cocoon diameter would be more proportional to the biomass of the bee, although this relation was never verified due to there being major difficulties in objectively assessing adult bee biomass. The relation between cocoon diameter^3 and the pollen content in the faeces is shown in figure 3.9(A). The lower portion (workers and possibly some males) is linear, but the one large worker and the five queens seem to have consumed somewhat less pollen for their size, than the small larvae. This result is open to several possible inaccuracies. Perhaps the main problem might be that progressively larger bees might spin more roomy or thicker cocoons so that cocoon diameter^3 might not be linearly proportionalto biomass. But if it is true that large larvae consume relatively less pollen for their weight, two explanations spring to mind:

(1) small larvae might receive proportionately less carbohydrate (nectar) and may have to deaminate pollen proteins for their energy requirements, or

(2) queen larvae might receive some of their protein requirement from a source other than pollen, e.g. from glandular secretions of the workers.

3.2.2.4 Larval growth: Assuming an egg-stage duration of four days, larval durations ranged from six to twelve days. These figures are shorter than those quoted by Brian (1951) for B. agrorum, and Katayama (1973) for B. ignitus, in unheated observation hives; but are similar to the times given by Hasselrot (1960) for various European species with nesting material, and Röseler & Röseler (1974) for B. hypnorum and B. terrestris heated to 30°C.

There was no strict relation between the time larvae spent growing, and the size they eventually attained. For example in worker clumps the first cocoon formed was sometimes the smallest, and sometimes the largest. However, in clumps containing a mixture of males and queens, the males nearly always pupated one day earlier than their larger sisters.

There was a seasonal change in larval durations, but this will be discussed in section 3.4.

The above-mentioned independence of larval-duration and size-attained implies of course that larvae grew at different rates. I wanted to know whether the different growth rates were associated with the whole larval period, or whether, say, they all grew at the same rate until the final instar. Unfortunately, the growth of individual larvae could not be measured directly, due to them being enclosed in a wax canopy. However, I was able to derive a relationship between larval clump area, and the biomass of larvae inside, from frozen colonies (see figure 3.6). It seemed logical to relate biomass to clump volume, rather than area, so I multiplied the area by the square root of itself. The graph is shown in figure 3.6. The relationship is roughly

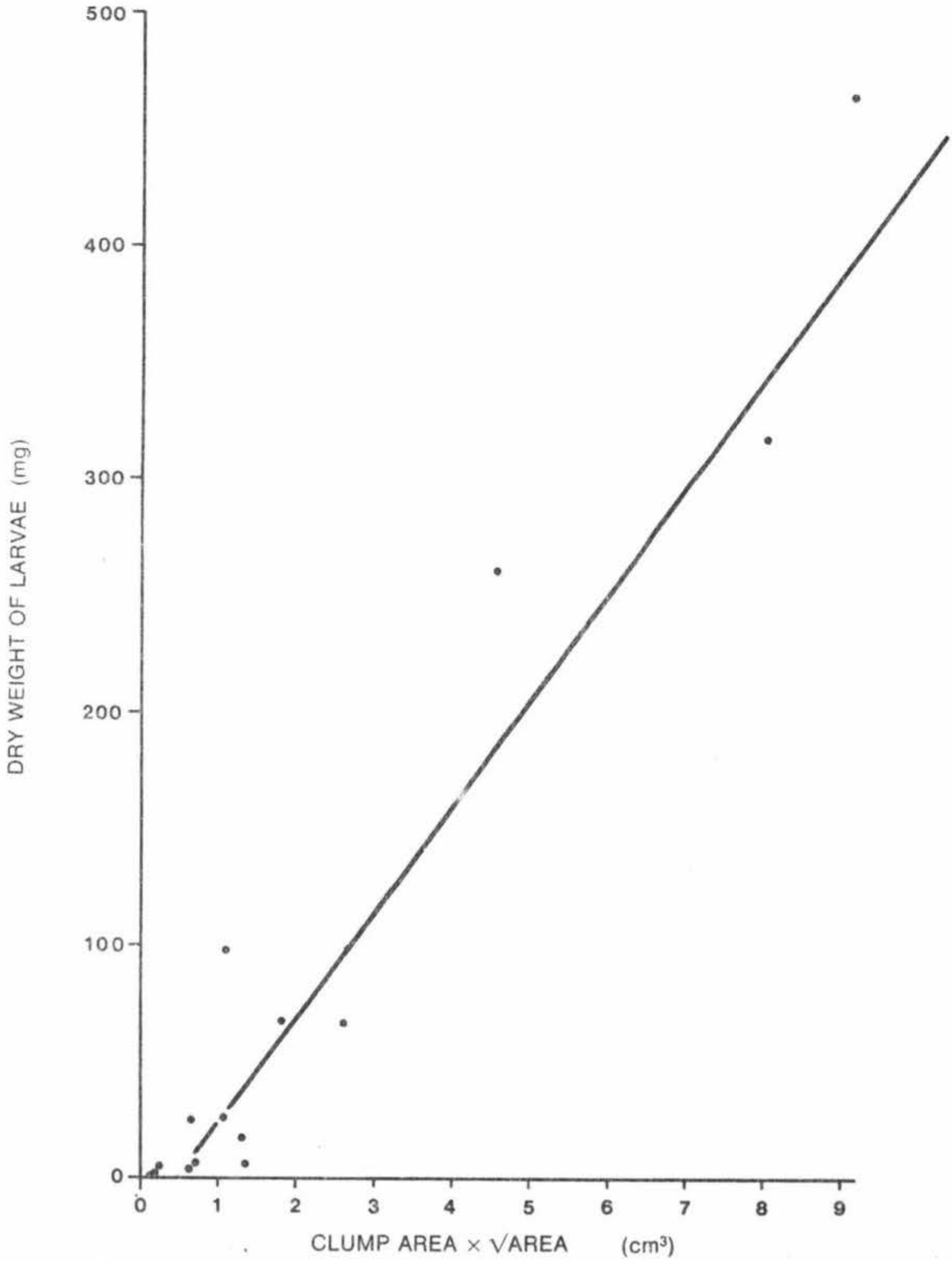

Figure 3.6 Relation between larval clump area (as viewed from above), and the biomass of larvae inside. Same colonies as in fig. 3.4.

linear for the larger clumps but the smaller ones are disproportionately light. This is probably due to young larvae occupying a thin layer over the mass of pollen which takes up much of the clump's volume. Imperfect though it was, I applied the linear regression equation to the larval clump areas measured from daily photographs, then to estimate the growth of individual larvae I divided the weight by the number of cocoons formed in the clump. I had not got a clear sequence of photographs for any pure-worker or pure-queen clumps for any one colony, so I chose mixed clumps of workers + males and queens + males. The resulting curves are shown in figure 3.7. The graphs show that all six clumps had similar shaped sigmoid growth curves, and that the clumps which produced queens and males had faster growth rates throughout their larval periods than the clumps which produced workers and males. Although the curves drawn were probably typical, it must be remembered that there was considerable variation in larval durations. In general however, larvae which became workers had longer durations than those which became queens.

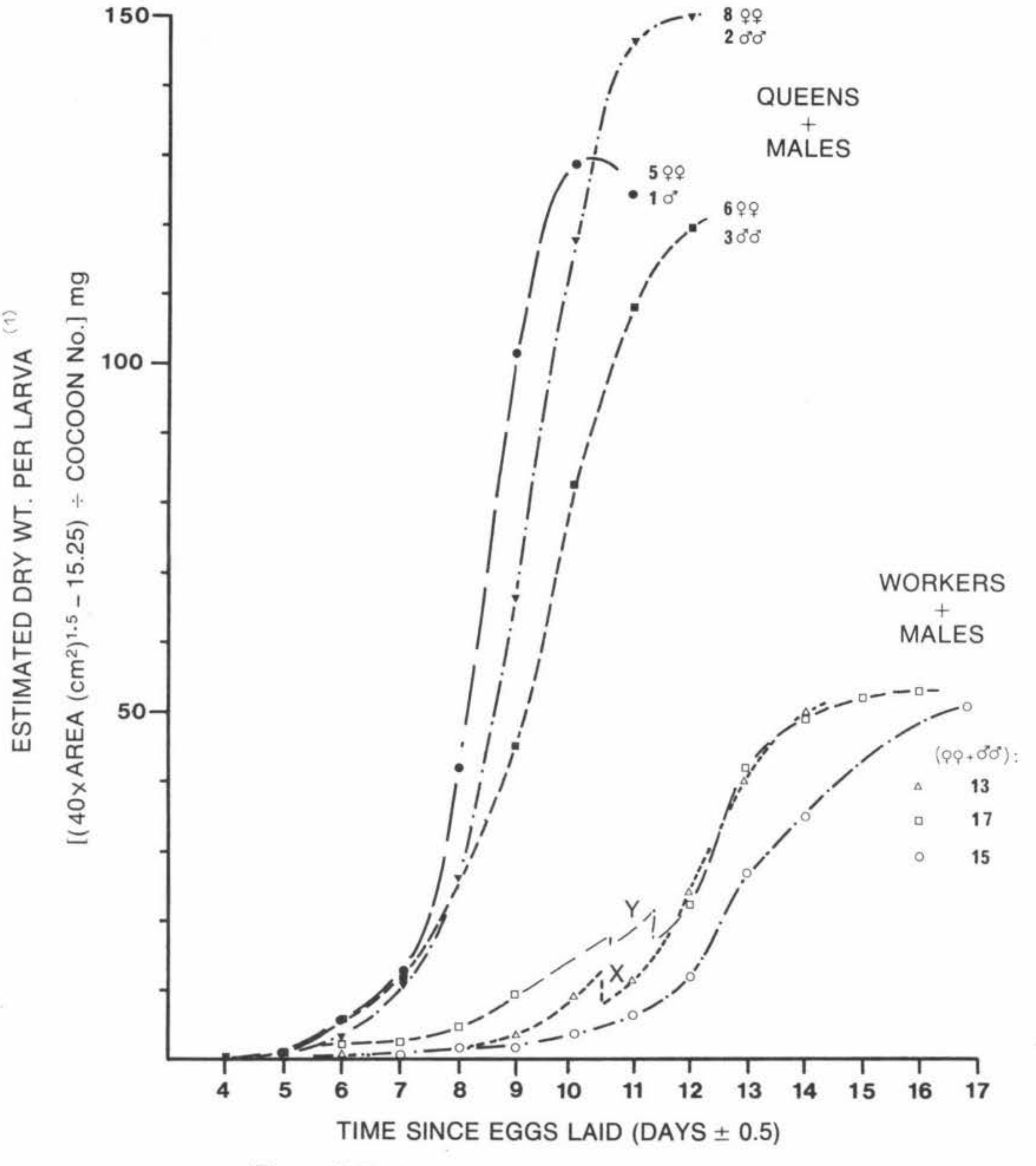

Figure 3.7 Larval growth rates; estimated from areas on photographs, area/biomass relationship of dissected nests (fig. 3.6)(1), and the cocoon number. The "step" drawn at X represents a biomass reduction due to larval rejection. The "steps" at Y also represent larvae rejected from the colony, but I was uncertain whether they came from that clump.

(1) FOR EASE OF CALCULATION, THE (AREA x $\sqrt{\text{AREA}}$): (BIOMASS) RATIO (IN FIG. 3.6) WAS TAKEN AS LINEAR, ALTHOUGH THAT APPEARED TO UNDERESTIMATE THE BIOMASS OF MATURE CLUMPS, AND OVERESTIMATE THE BIOMASS OF YOUNG CLUMPS. THEREFORE, THE ABOVE LARVAE PROBABLY ATTAINED GREATER WEIGHTS THAN SHOWN, AND THE GROWTH RATES WOULD HAVE BEEN EVEN FASTER THAN INDICATED. THIS, HOWEVER, SHOULD NOT ALTER THE RELATIVE GROWTH RATES OF QUEENS AND WORKERS.

3.2.3 Pupae

Pupal development times are shown in figure 3.10. There is a linear relationship between pupal duration and cocoon diameter. One of the advantages of producing small workers early in the season could be due to their short pupal durations, and this would enable worker numbers to be built up more quickly than if the pupal duration was longer. Wilson (1971, p. 442) emphasises the importance to social insects of building up their worker force quickly.

The sizes of the adults produced over a whole season can be estimated from cocoon diameters. In figure 3.8 I show histograms of the numbers of cocoons of various sizes in five B. ruderatus colonies. A very small proportion of the cocoons in each colony was too mutilated to measure. The histograms include unhatched cocoons (i.e. where the occupant died), because these represent a food investment by the colony regardless of their fate. The peaks between 8.5 and 10.0 mm are mostly composed of males. The larger workers also fall in this range, but the total number of males produced (except possibly in colony 5) was usually more than the number of workers. The few individuals in the range 10.5 to 11.5 mm were females of intermediate size between typical queens and typical workers. A number of these cocoons failed to hatch (see section 3.3.3) but those that did, usually yielded workers (which were constant foragers).

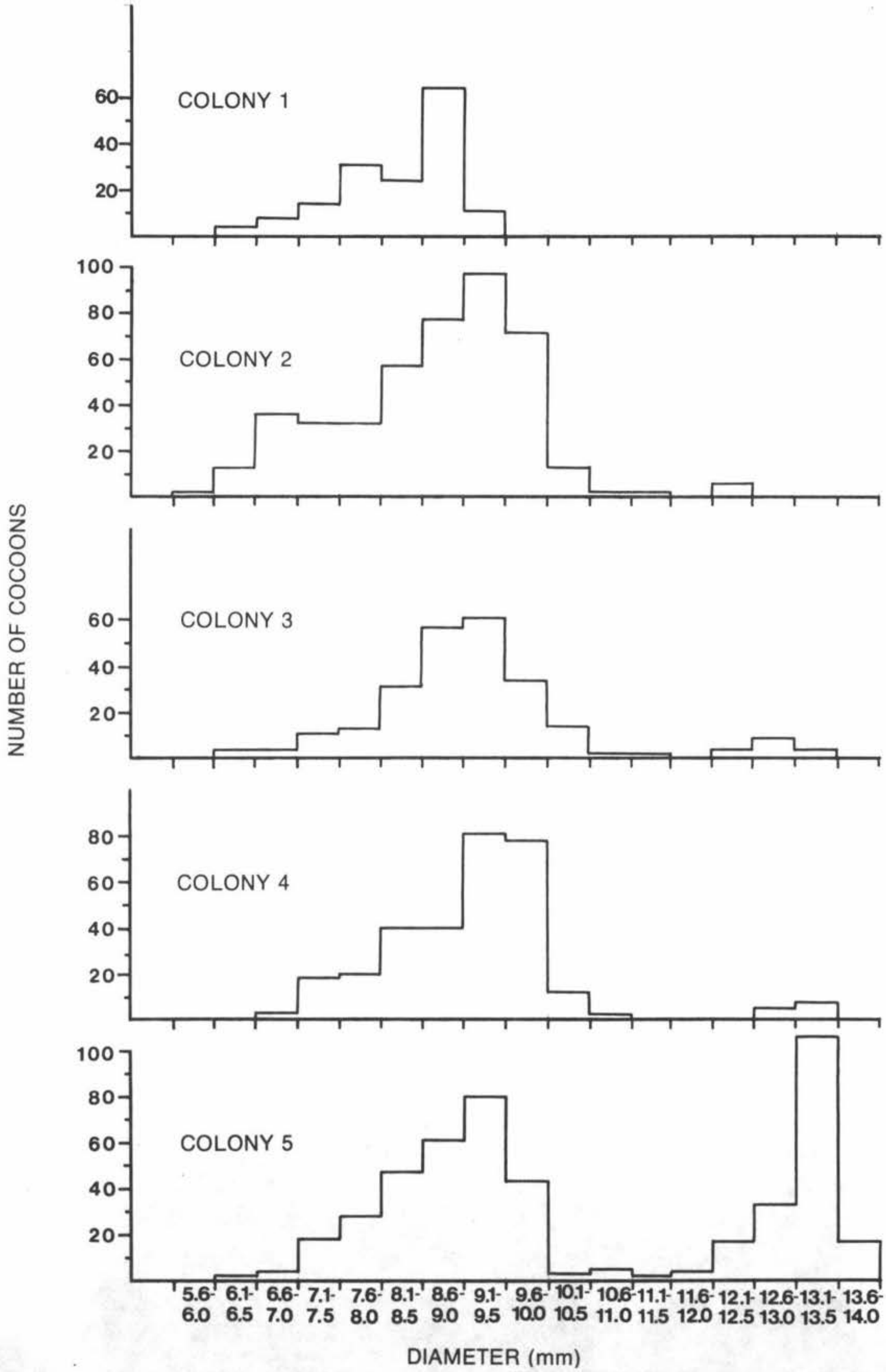

Figure 3.8 Frequency distribution of cocoon diameters.

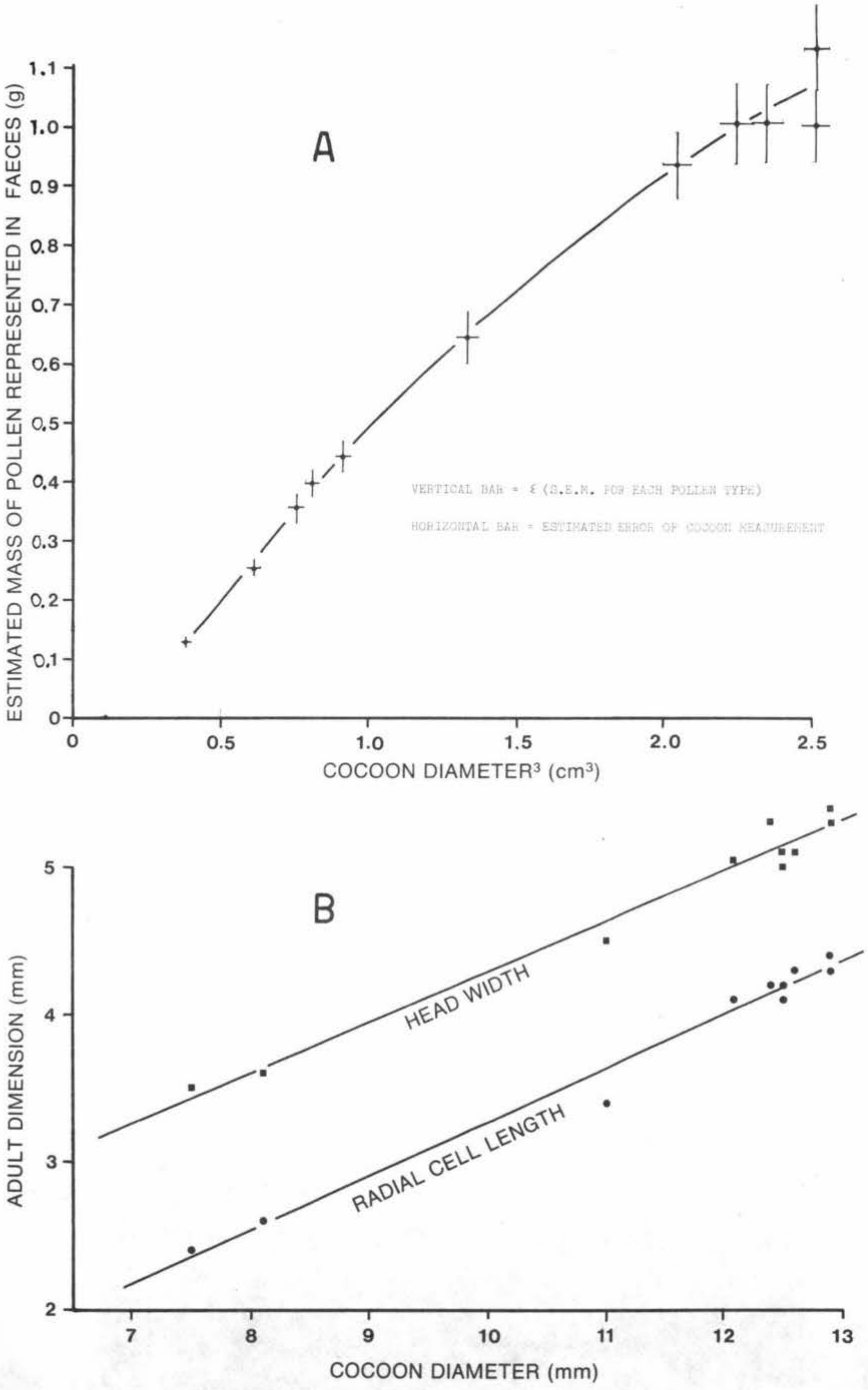

Figure 3.9 (A) Relation between cocoon "volume" (diameter³) and quantity of pollen consumed during larval stage. (B) Relation between cocoon diameters and dimensions of adult females emerging from them.

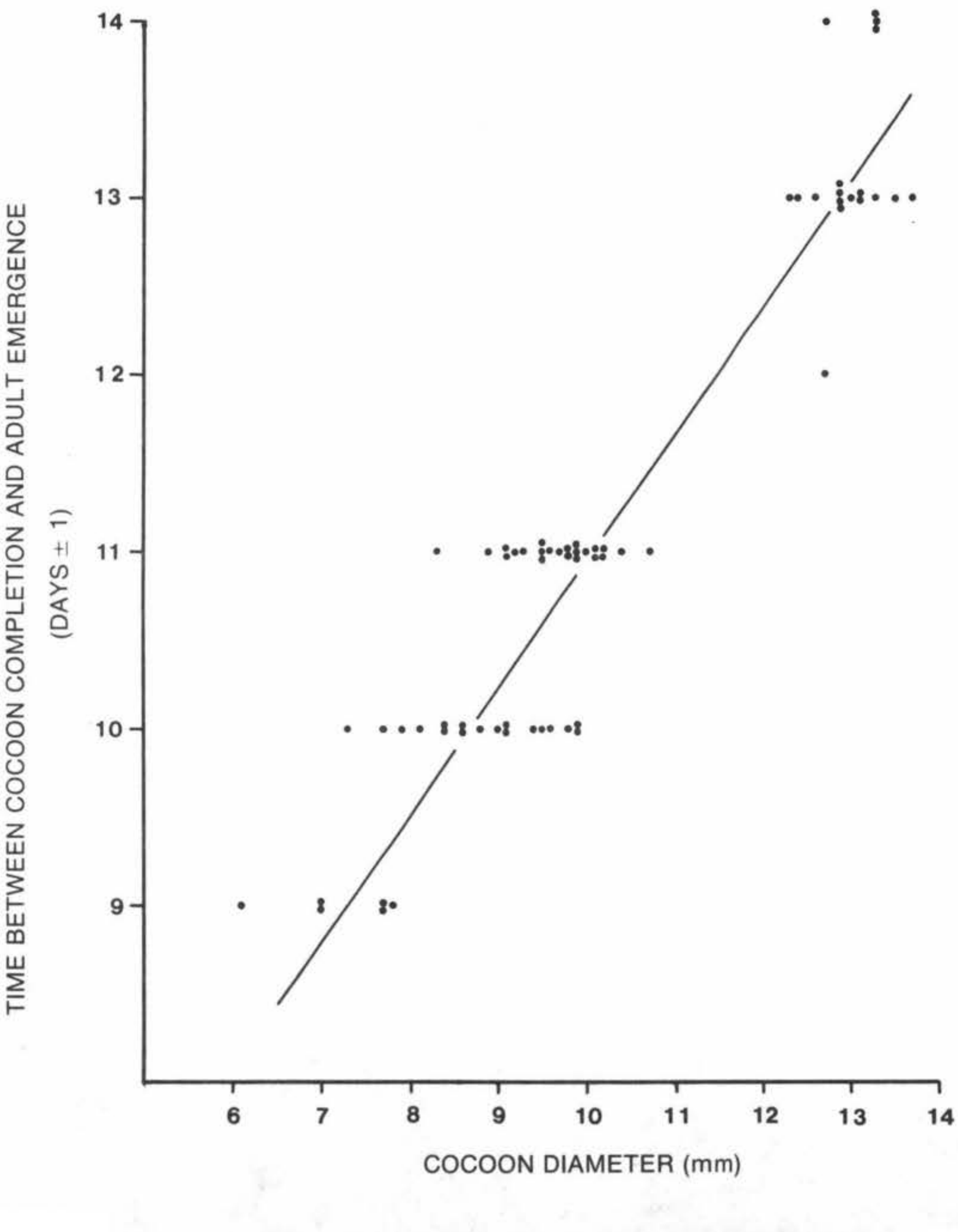

Figure 3.10 Relation between cocoon diameter and pupal duration. Line fitted by eye.

3.2.4 Adults

The final stage of "juvenility" is the maturation of the adults during the first few days after emerging from a cocoon. Several recently-emerged bees can be seen in plate 4. Sladen (1912) and several authors since, have reported that bumblebees drink from a honey-pot as soon as they emerge from their cocoon, and I also observed this for B. ruderatus. On one occasion I removed a newly-emerged male from the nest before it had reached a honey-pot, and weighed it. Then I allowed it to feed on some 50% honey solution (S.G. $\doteqdot$ 1.16) and re-weighed it. The bee, in one draught, imbibed 0.0278 g of solution, which was 4.3% of its own body weight.

A small-scale food consumption trial showed males and queens to have similar patterns of food intake except that queens ate and drank twice as much as males. Queens were about twice as heavy as males, however, so that both consumed similar amounts on a per-weight basis. The rate of honey consumption remained fairly constant throughout the experiment, but the rate of pollen consumption declined with time, most being eaten during the first two days (for queens). Over the five-day period, the total amount of pollen consumed per queen was about 0.3g, which is nearly one-third of the quantity each probably consumed during its larval stage (see figure 3.9A). Proportionately, males consumed a similar amount.

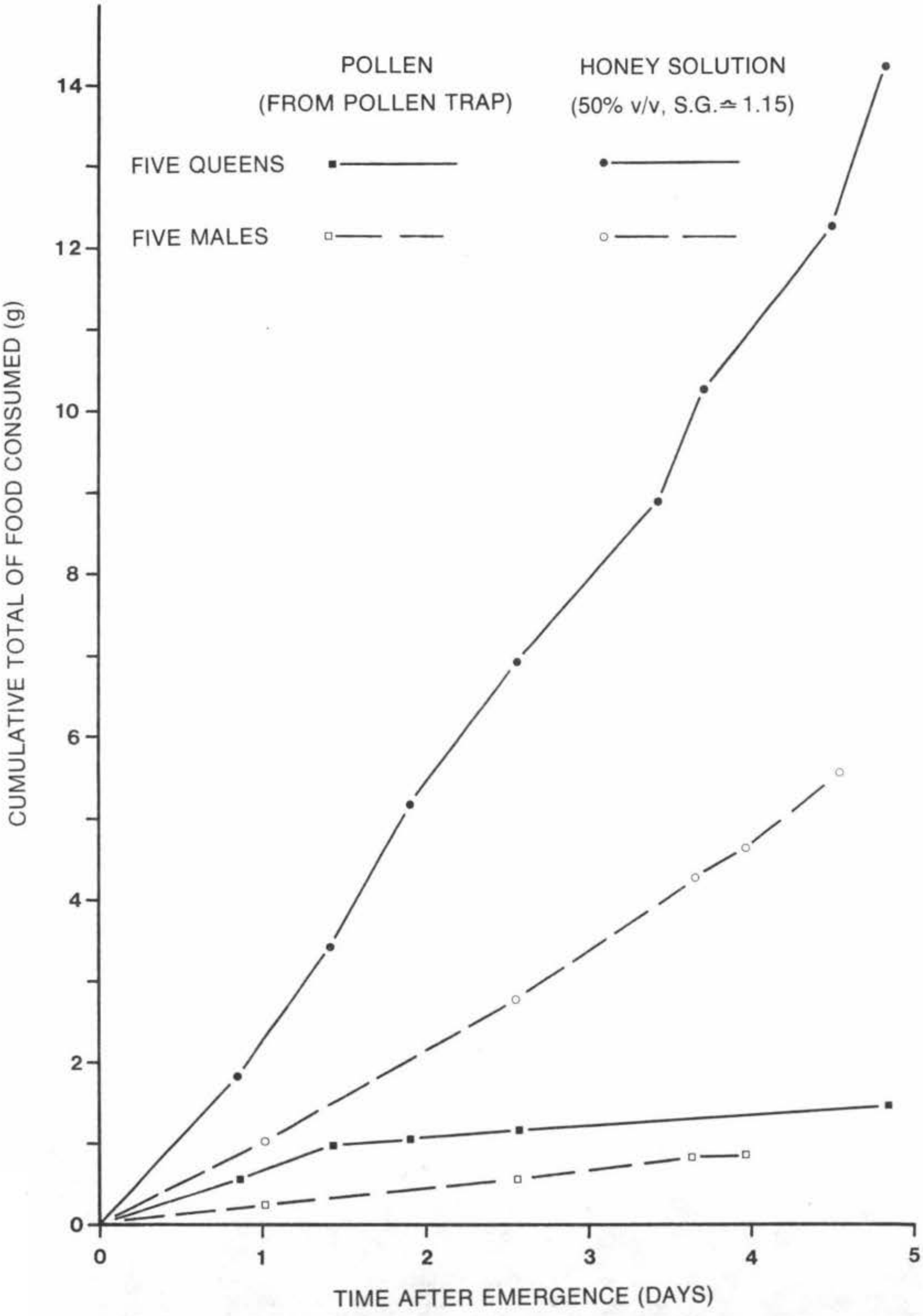

Figure 3.11 Food consumption by newly emerged queens and males.

3.3 BROOD MORTALITY

In this section I am mainly concerned with non-pathological brood mortality, i.e. deaths which are a normal feature of colony development, and especially those attributable to adult behaviour.

3.3.1 Egg Mortality

3.3.1.1 Hatching failure: Shrivelled, apparently dead eggs were sometimes found among normal turgid-looking ones in the egg cells of frozen nests. Several can be seen in plate 8. The cause of this was not determined. The wrinkled remains of dead eggs (different from empty chorions) were sometimes also seen in young larval clumps. This could possibly have arisen from the latest-laid egg batch in a group of contiguous egg cells being crowded out by the rapidly growing larvae of the earliest-laid eggs of the group.

3.3.1.2 Oophagy: I have observed eggs being eaten by queens and workers in _B_. _ruderatus_ colonies. This is only the second pocket-making species in which oophagy has been recorded. The other species is _B_. _fervidus_ (Plath, 1934). Egg-eating is probably associated with worker ovary development, but although some workers in the nests of most _Bombus_ species lay eggs near the peak of colony development, egg-eating only occurs in a few species (Free and Butler, 1959).

In my observations of _B_. _ruderatus_, workers only succeeded occasionally, in eating eggs laid by the queen, and even then it was only a few eggs from any cell which were successfully consumed. Usually the queen interrupted egg-eating attempts by displacing the workers, and recovering the cell. Occasionally a queen was seen to rush at, and butt a worker which was touching an egg cell. Queens were seen successfully eating worker-laid eggs on several occasions. Overall, the queen seemed to be much more successful at destroying worker eggs than they were at destroying hers. Similar behaviour in *B. lapidarius* has been described by Sladen (1912) and Free, Weinberg and Whiten (1969). *B. ruderatus* egg-eating behaviour seems qualitatively very similar but less frequent or vigorous, than in *B. lapidarius*.

When interpreting the various levels of cooperation and animosity in *Bombus* societies, it may be useful to consider a theory which has been put forward by Hamilton (1964), and evaluated at length by Wilson (1971). Hamilton's theory is based on the principle that altruistic traits will be selected for, if the sacrifice undergone by the altruist is compensated by an increase in the reproductive success (R.S.) of a related individual. The boost to the relations' R.S. must be greater than the reciprocal of the degree of relatedness (i.e. the fraction of genes shared) between the altruist and recipient. In most animals the degree of relatedness between siblings with the same (diploid) father and mother averages $\frac{1}{2}$. In such cases a female would have to more than double the R.S. of one of her sisters for there to be any advantage in evolving the loss of her own reproductive capacity. However, the hymenoptera are a special case, due to their haplodiploid mode of sex determination (fertilised eggs become diploid females, unfertilised eggs become haploid males). For them the degree of relatedness between sisters is $\frac{3}{4}$[1]. This close bond of kinship (which is rare elsewhere in nature) is a result of sisters each having 100% of their father's genes, because he was haploid and only had one set of genes to perpetuate. However, the kinship bond is weak between sisters and brothers, being only $\frac{1}{4}$ (because the brothers have none of the "fathers" genes). The degree of relatedness between mother & daughter, and mother & son, is the usual $\frac{1}{2}$. Due to the asymmetrical kinship between male and female siblings, we could expect bumblebee workers to cooperate with the foundress queen in the production of more female offspring (workers and/or young queens) but not in male production. Hamilton's theory suggests that there is an adaptive advantage to workers in competing with one another and with the foundress, to produce their own sons (who carry 50% of their genes, instead of the 25% in brothers).

Is oophagy an example of competition for male production? The findings of Free *et al*. (1969), that in *B*. *lapidarius* egg-eating and associated aggression are at least partly related worker ovary development, supports the idea. So does the observation that egg-eating is usually confined to later parts of the season when at least some of the queen's eggs are usually male (unfertilised). What we do not know, however, is whether workers actually discriminate between male and female eggs.

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[1] This assumes that queens mate only with one male, which was true for 8 out of 9 European *Bombus* species tested by Röseler (1973).

3.3.2 Larval Mortality

3.3.2.1 Larva-eating: I observed a worker eat a larva on one occasion, when I was looking at young larval clumps through a dissecting microscope. The worker made a hole in the young larval clump (which had a freshly-made pocket) as if to feed the larvae. However, when an opening had been made, one of the larvae was seen to be leaking clear fluid, which the worker imbibed. The worker then pulled the larva out of the cell and ate most of it, except for a discarded fragment which was eaten by another worker. Although I did not see it happen, I suspected that the "leak" was caused by the worker biting the larva. The larva appeared to be at the second instar and was about 5 mm long. This was the only occasion on which larva-eating was observed, but there were few occasions when live colonies were examined microscopically.

3.3.2.2 Larval rejection: Before being rejected from the nest, larvae had to be removed from their clump. The uncovering procedure involved similar wax-manipulating movements to the formation of a feeding hole. But whereas feeding holes were no more than 3 mm wide, larva-removal holes were eventually made to the diameter of the underlying larva. The hole was usually neat and circular. Sometimes, the bee which had begun uncovering the larva would walk away, leaving it partially exposed. In such a case, the larva would usually soon be encountered by another worker which would either close the hole or enlarge it. The apparent differences of "opinion" between workers over whether or not a larva should be rejected agrees with the observation of Oster (1976) that, "a task performed by one individual is likely to be immediately undone by another." When the larva was well-exposed, bees encountering it would bite at it. However, the larva was sometimes too smooth and turgid for the workers' mandibles to easily grip it. Attempts at grasping the larva would alternate with enlargement of the hole, until the larva could be lifted out. Plate 10 shows a worker carrying a newly rejected larva out of the nest. Removal of the larva from the nest chamber was usually done very promptly, in contrast to the gradual process of uncovering it.

Figures 3.15.1 to 3.15.5 show the daily totals of rejected larvae recovered from the vestibules of each hive. About three times as many larvae were found in the inner vestibules (i.e. closest to the nest) than in the outer ones. This suggests that most of the rejected larvae were not carried far. As a further indication of the success of the vestibules for trapping rejected larvae, only two larvae were photographed by the forager-recording camera after being carried out past the vestibules, while over the same period thirty larvae were recovered from in the vestibules. During their period in observation hives the percentage of larvae rejected from each colony, i.e.:

$$\frac{\text{number of larvae rejected}}{(\text{number larvae rejected}) + (\text{number of cocoons formed})} \times 100$$

was 21%, 24%, 36%, 13% and 16%, for colonies 1, 2, 3, 4 and 5, respectively.

At the times when both sexes of brood were being reared, both sexes were usually present among the rejected larvae. One exception was observed however. When colony three was raising its first clump of queen larvae, no female larvae were rejected but eleven male larvae were (over a five-day period). The significance of larval rejection to the overall colony development will be discussed in section 3.4.

Two approaches were used to check the effect of nutrition on larval rejection. The first was to compare the estimated larval food demand with the amount of pollen available on various days, to see if larvae were rejected following days of low pollen input per larva. The larval food demand was assumed to be represented by the larval biomass because larvae grow more or less exponentially. The larval biomass was calculated from areas on photographs, using the relation shown in figure 3.6. Figure 3.12 shows the relationship between the estimated larval biomass and the daily total of pollen entering the hive. This graph shows a rough correlation between pollen input and larval biomass, but it does not show larvae to be rejected at any particular state of pollen excess or deficit. The inconclusive result may be due to several complicating factors:

(1) The food demand of almost-mature larvae will be lower for their weight, than the food demand of rapidly growing larvae. This makes it very complicated to derive a reasonable relationship between larval biomass and food demand.

(2) Queen larvae grow faster than worker larvae (see figure 3.7) and therefore probably have a different food demand per unit biomass.

(3) Pollen is consumed by recently-emerged adult bees. Therefore, variable amounts of the daily pollen intake may reach the larvae, depending on the number of young adults present. This factor is probably quite significant. As

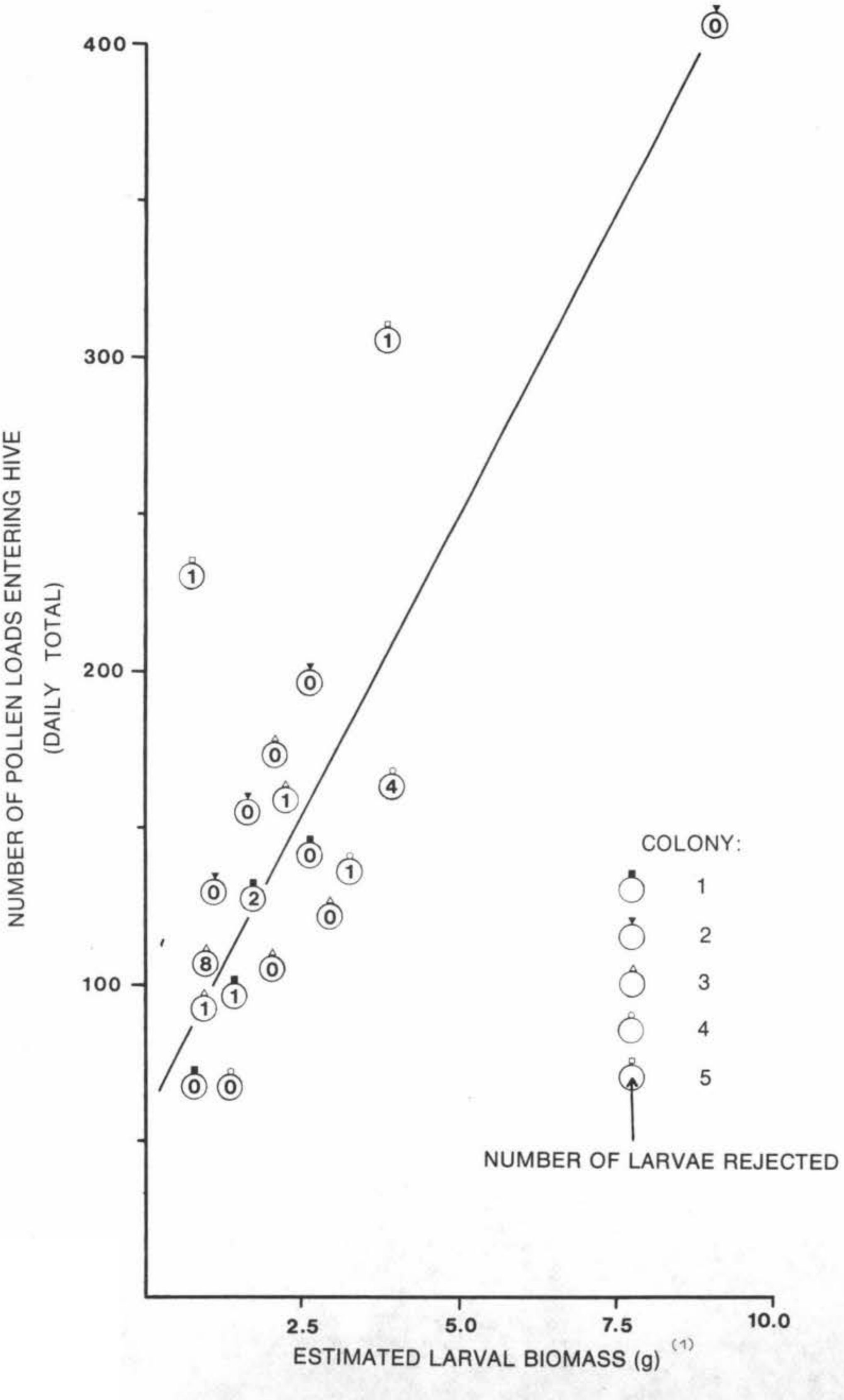

Figure 3.12 Daily pollen intake to colony versus the quantity of larvae present. The number of larvae rejected that day and following night also shown. Data were collected at various stages of the season, from five colonies.

(1) SUM OF INDIVIDUAL CLUMP BIOMASSES; CALCULATED FROM AREA ON PHOTOGRAPH, USING AREA : BIOMASS RELATION SHOWN IN FIG. 3.6.

mentioned in section 3.2.4, newly emerged queens and males (probably workers too) appear to consume almost one-third as much pollen as they do in the larval stage. Several of the peaks of larval rejection shown in figures 3.15.2 to 3.15.5 do in fact coincide with times when there was a sudden increase in the number of cocoons hatching.

The second approach to investigating the nutritional status of rejected and non-rejected larvae was a morphometric comparison of the two types. Figure 3.13 shows the relation between log. dry weight and head capsule diameter, for rejected and non-rejected larvae from the same nests. The three clusters of points represent the third, fourth, and fifth instars. There are about five rather light fourth-instar rejected larvae, but the rest show no tendency to be lighter for their head width, than non-rejected ones.

3.3.3 _ Post-spinning mortality

Fifth instar larvae which had spun silken envelopes around themselves were rarely rejected. However, a number died *in situ*, as did some prepupae, pupae, pharate adults and even partially emerged adults. The numbers of individuals dying at various stages are shown in table 3.4. Several causes could have been involved. Disease was suspected in colonies 1 and 2 due to their mortality being greater than I have usually seen in *B. ruderatus* nests. Most of the dead prepupae rattled loosely inside their cocoons, and did not seem to have decayed in the way often caused by bacterial or viral infections. An *Aspergillus*-like fungus was present on a very small number of dead specimens. Many of the dead individuals were on the underside of their clump. Some of

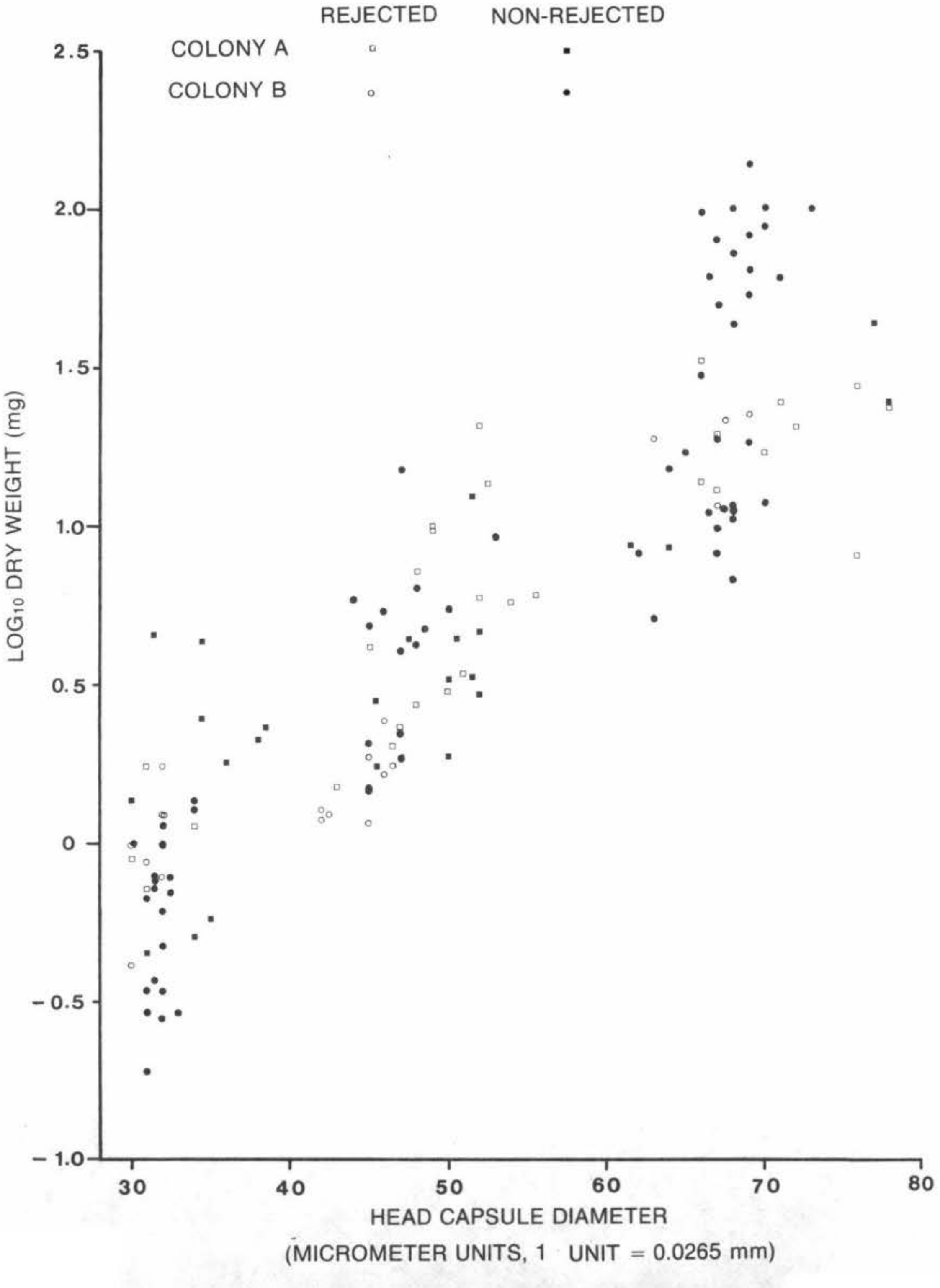

Figure 3.13 Relation between head capsule diameter and log. dry weight for rejected and non-rejected larvae. Rejected larvae collected during six days, prior to freezing the colonies on 18 and 19 Jan. 1975.

Table 3.4 Brood mortality occurring between late fifth instar (silk spinning) and adult stages

	Colony				
	1	2	3	4	5
Number of individuals surviving to late 5th instar	263	599	326	336	673
Number (and percentage[1] in brackets) dying as:					
larvae[2]	26 (10)	52 (9)	6 (2)	6 (2)	9 (1)
prepupae	36 (14)	55 (9)	7 (2)	15 (5)	1 (0)
pupae	2 (1)	4 (1)	1 (0)	0 –	3 (0)
pharate, and incompletely emerged, adults	12 (6)	22 (4)	12 (4)	5 (1)	4 (1)

[1] percentage to nearest 1%

[2] does not include rejected larvae, which were nearly all rejected before late 5th instar.

the dead adults thus positioned may have been physically locked in, and unable to escape from their cocoon, although many appeared to have died without even starting to bite through their cocoons. Nutritional causes were suspected in some cases, such as in clumps 81 and 85 of colony five, which occurred during queen production. Clump 81 had four normal-sized healthy queens on top, and two small females (sizes of a medium, and a large worker) underneath which died during their pupal stage. Clump 85 had eight healthy queens on top and a medium-worker sized female underneath which had shed its pupal skin but had apparently not attempted to escape from its cocoon. Why did these small females die? According to the idea that workers are undernourished queens, these small females should not necessarily have been so mortally affected by their poorer clump-positions and food quantity. An interesting possibility is that workers might control the size to which larvae are reared, partly with the use of growth hormones fed to the larvae. (Röseler and Röseler, 1974, found that juvenile hormone administered to *B. terrestris* and *B. hypnorum* larvae affected the size to which they grew.) It is possible that larvae in poor positions for receiving food, also sometimes receive inadequate quantities of developmental substances, and hence fail to metamorphase properly.

A number of males died apparently because they could not escape from their cocoons, although they had chewed an exit hole, and in some cases were partway out. Such males were probably not assisted by workers, who usually do assist emerging bees to open their cocoons. This worker neglect of males is consistent with Hamilton's (1964) theory that workers ought to be reluctant to rear their brothers.

3.4 COLONY DEVELOPMENT

In the study area B. ruderatus colonies are usually established during November (late spring) and die out in January or February (mid summer). The sizes, in terms of the number of individuals, of colonies under various conditions are shown in table 3.5. An additional colony, whose cocoons were not able to be counted, appeared to be somewhat larger than the biggest one listed in table 3.5. Colony sizes of 300 or more have been considered large by Sladen (1912) and Free and Butler (1959). Therefore, by British standards at least, B. ruderatus can be considered a prolific species.

The growth rate of any population equals its birth (and immigration) rate minus its death (and emigration) rate (Odum, 1959). In an annual colony cycle, such as bumblebees have, the birth rate exceeds the death rate while the colony is growing. But what makes a colony decline? Does the birth rate drop, does the death (and/or emigration) rate increase, or do both occur? In the following sections I describe some of the components of the birth and death rate at different parts of the season, in an attempt to trace the limiting factors of B. ruderatus colony growth.

3.4.1 Egg Production

Eggs are laid on fresh coooons (see section 3.2.1 and figure 3.1). "Growth trees" of the successive layers of brood clumps are shown in figures 3.14.1 to 3.14.5. A

Table 3.5 Number of cocoons found in completed *B. ruderatus* colonies

Season	Total cocoons	Queen cocoons	Site where[1] nest established	Site where[1] growth completed
1973-74	?	135	NU (7)[2]	NU
	382	116	NU (5)	OH
	ca 435	162	NU (6)	OH
1974-75	382	80	DU	OH
	377	20	DU	OH
	271	52	DU	OH
	554	186	DU	OH
	425	18	DS	OH
	277	7	DS	OH
	501	29	DU	OH
	254	11	NS (11)	NS
1975-76	237	0	DS	OH
	547	12	DU	OH
	320	22	DS	OH
	330	13	DS	OH
	662	228	DU	OH
	737	160	NU (12)	NU
Mean	418	74		

[1] NU = natural, underground; NS = natural, surface
DU = domicile, underground; DS = domicile, surface
OH = observation hive

[2] Identification number from table 3.1 where nest site details given.

○ COCOON CLUMP; AREA OF CIRCLE ∝ AREA OF CLUMP

◑ COCOON CLUMP CONTAINING SOME DEAD LARVAE (WHITE / BLACK) ∝ (COCOONS / DEAD LARVAE)

● NO COCOONS FORMED, DUE TO DEATH AND/OR REJECTION OF ALL LARVAE

· EGG CLUMP SHOWING NO SIGNS OF GROWTH

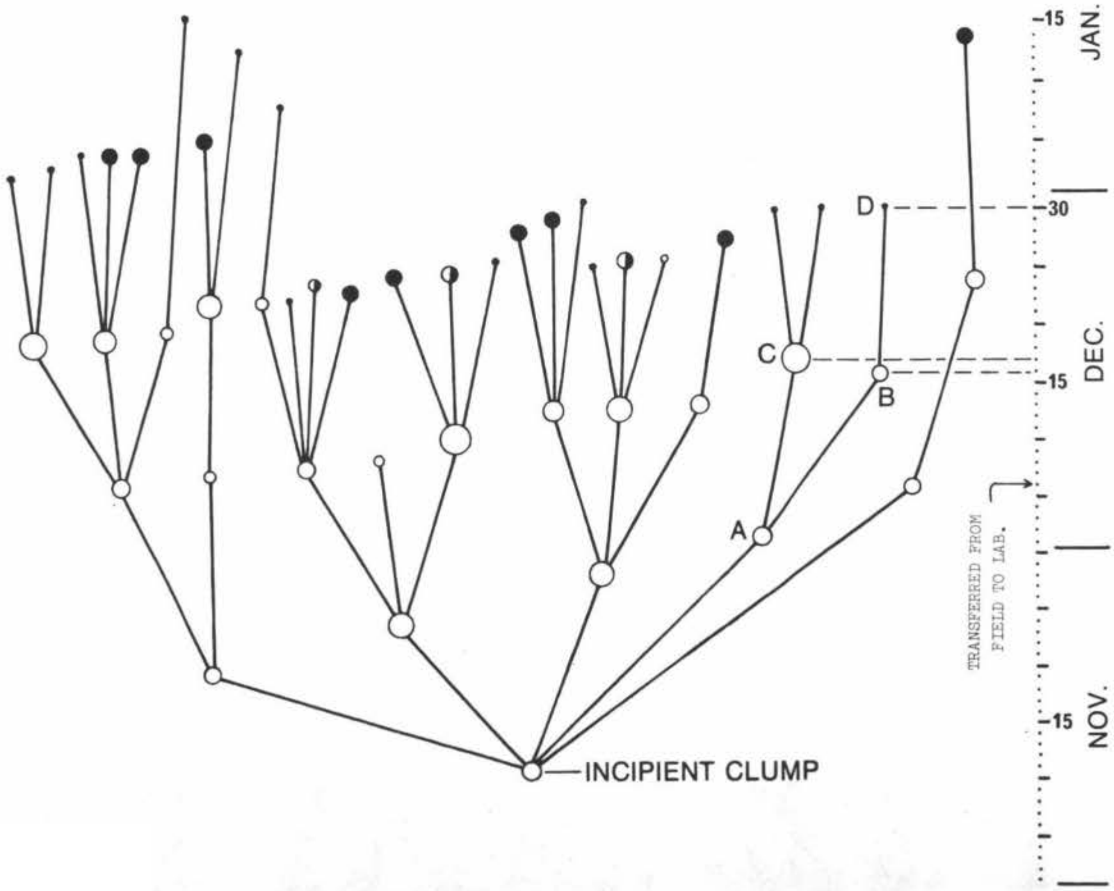

Figure 3.14.1 Spatio-temporal relations of the brood clumps of colony 1. Example: egg clump B was formed on the cocoons of A on 16 Dec. Clump C was formed on clump A on 17 Dec. Clump C grew to cocoon area 3.6 x the cocoon area of B. An egg clump (D) was made on B on 30 Dec. but it never grew.

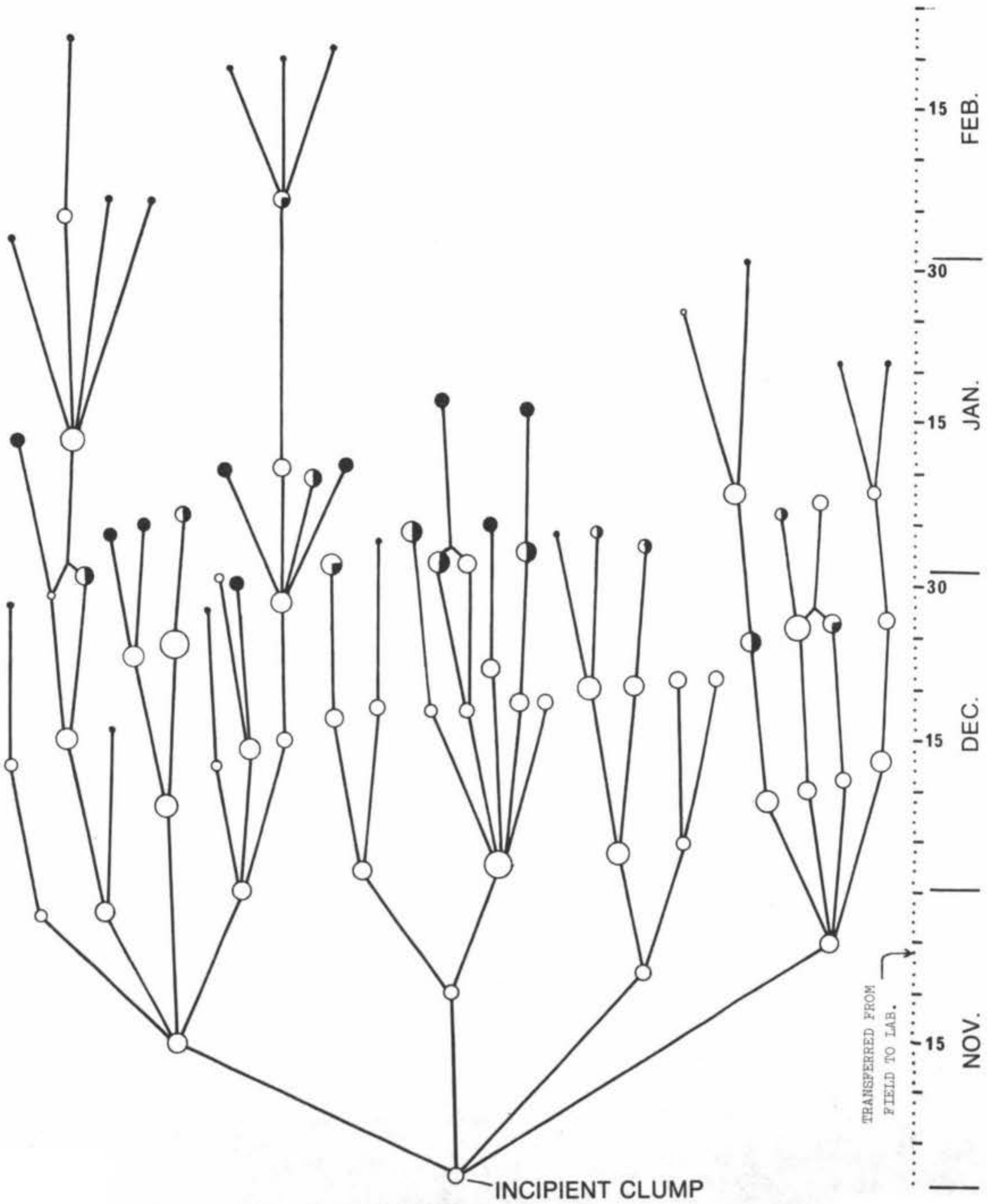

Figure 3.14.2 Spatio-temporal relations of the brood clumps in colony 2. See fig. 3.14.1 for legend and explanation.

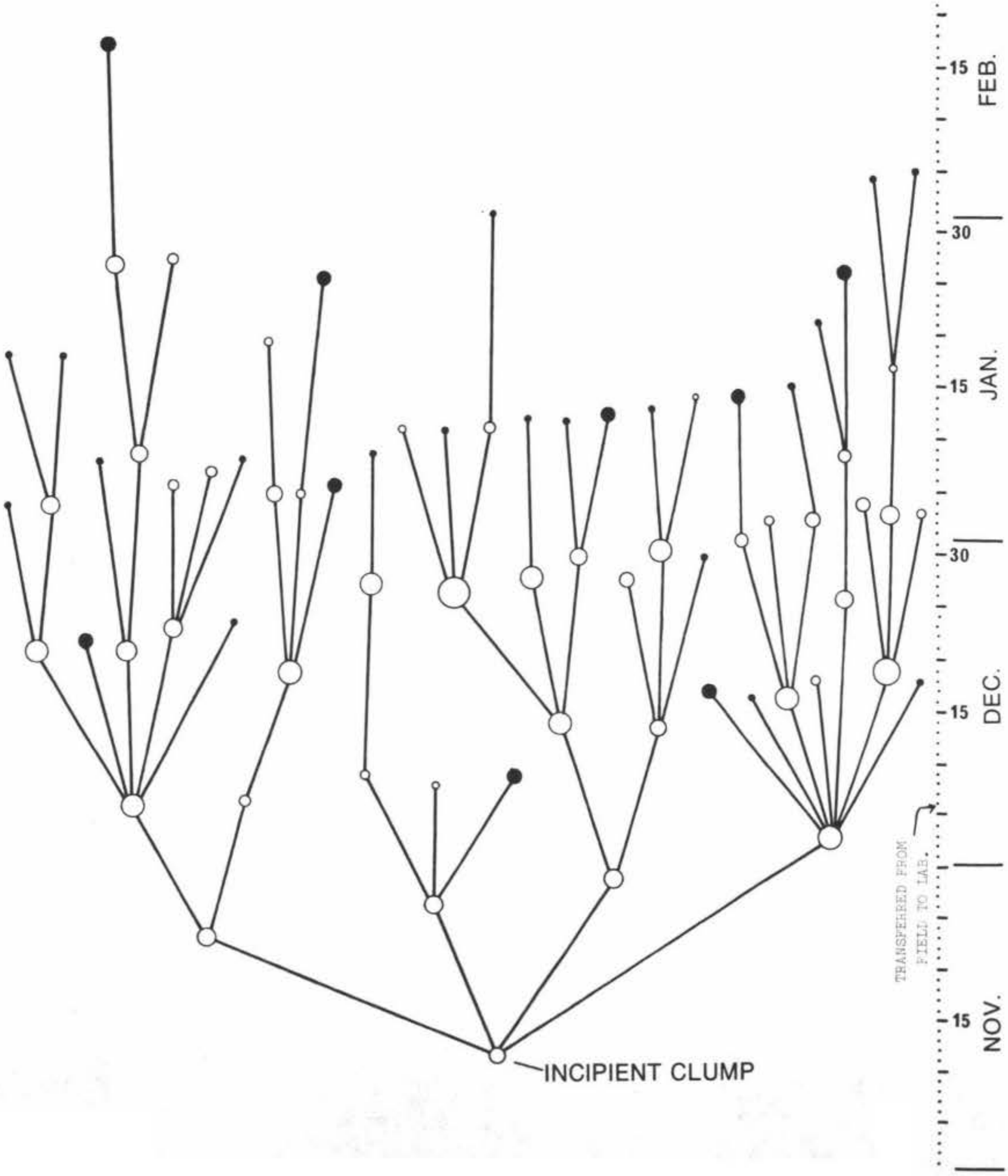

Figure 3.14.3 Spatio-temporal relations of the brood clumps in colony 3. See fig. 3.14.1 for legend and explanation.

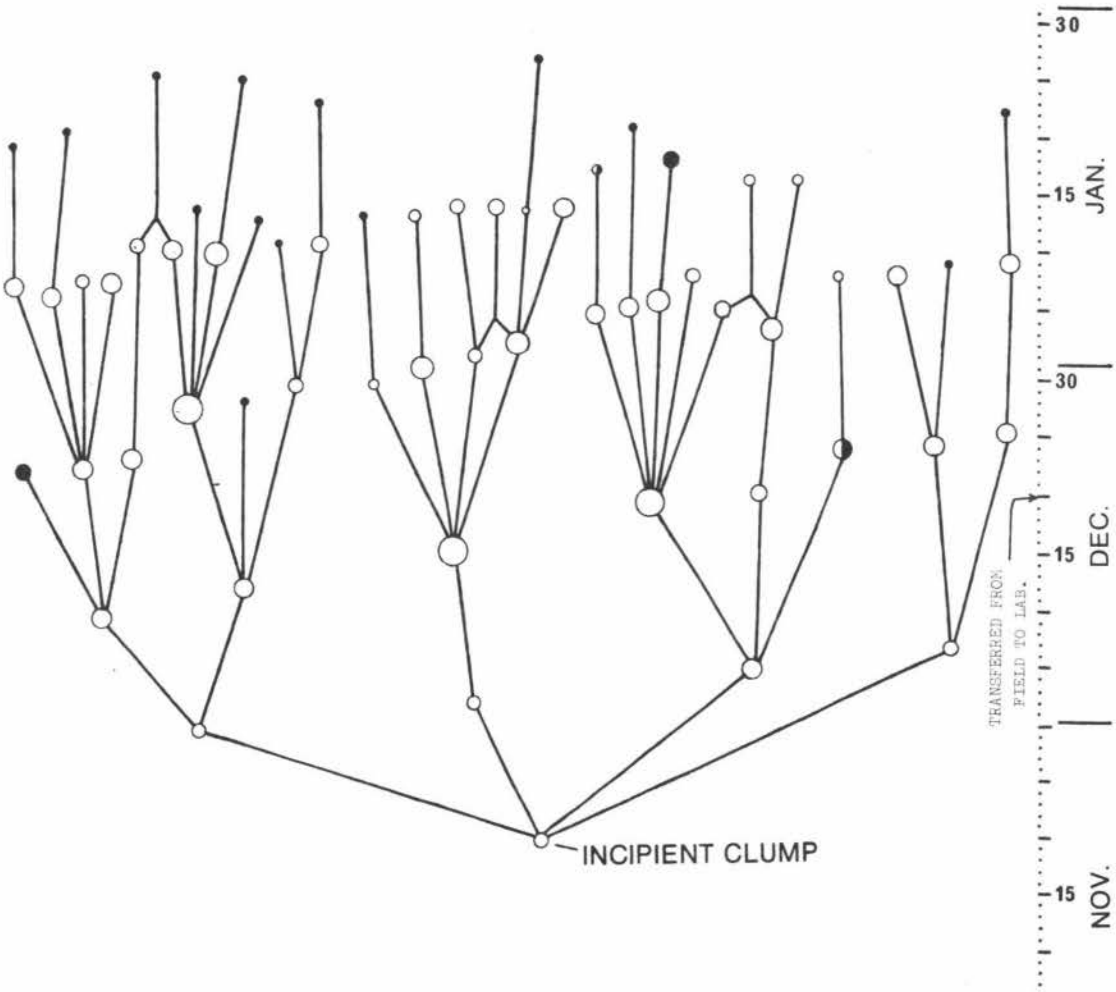

Figure 3.14.4 Spatio-tempoaral relations of the brood clumps in colony 4. See fig. 3.14.1 for legend and explanation.

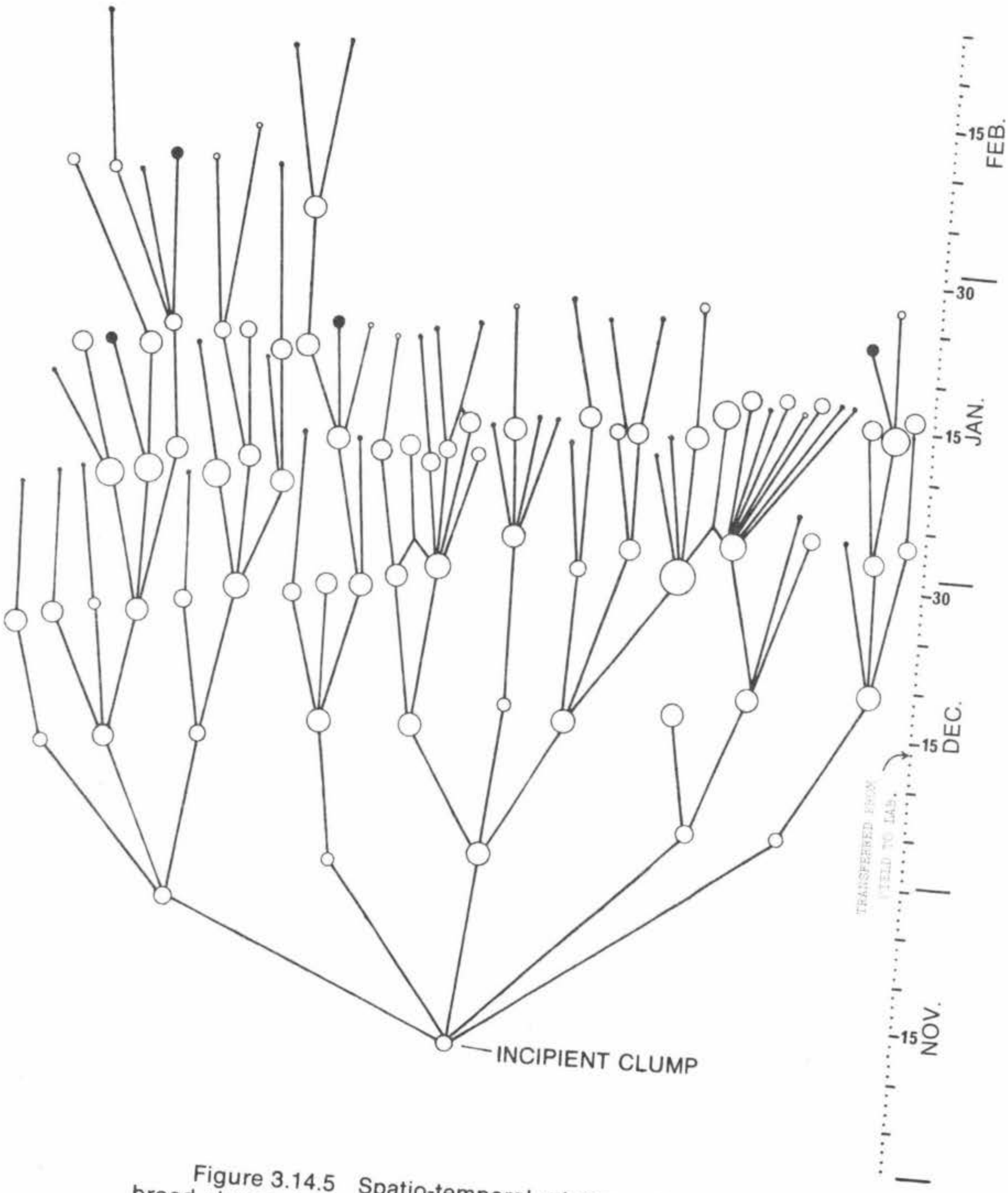

Figure 3.14.5 Spatio-temporal relations of the brood clumps in colony 5. See fig. 3.14.1 for legend and explanation.

tendency can be seen for larger[1] cocoon clumps to have more egg clumps built on them. Graph A on figures 3.15.1 to 3.15.5 shows the number of egg clumps and the number of substrate cocoons summed over five-day periods. There is an average of about one egg clump built on every five cocoons. At an average of 14 eggs per cell, and one cell per clump (although there are often more - figure 3.2), this represents 2.8 eggs per cocoon, which is very close to the figure Brian and Brian (1948) found for small colonies of B. agrorum. The Brians' work has been cited by Free and Butler (1959) and Michener (1974) as indicating that the number of eggs per cell varied in relation to the cocoon number. However, their paper does not state this, and I consider that their results, like mine, probably indicated that more cells of eggs were built on larger clumps of cocoons.

Brian and Brian's (1948) suggestion that the egg/cocoon ratio is such that the bees hatching from the substrate cocoons are the right number to assist rearing the new brood, has often been quoted (e.g. Free and Butler, 1959; Michener, 1974). However, my results show that the cocoon-mediated oviposition response continues virtually unchanged for male and queen cocoons, but the adults emerging from these cocoons do little or nothing to assist rearing the brood which was laid on them.

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[1] The cocoon-clump areas indicated on the diagrams are equivalent to the sum of the squares of the cocoon diameters in each clump. The actual area available for egg-cell construction would sometimes have been less, due to some cocoons being underneath others. Also, some cocoon clumps were covered up by the rapid growth of adjacent larval clumps, before eggs could be laid.

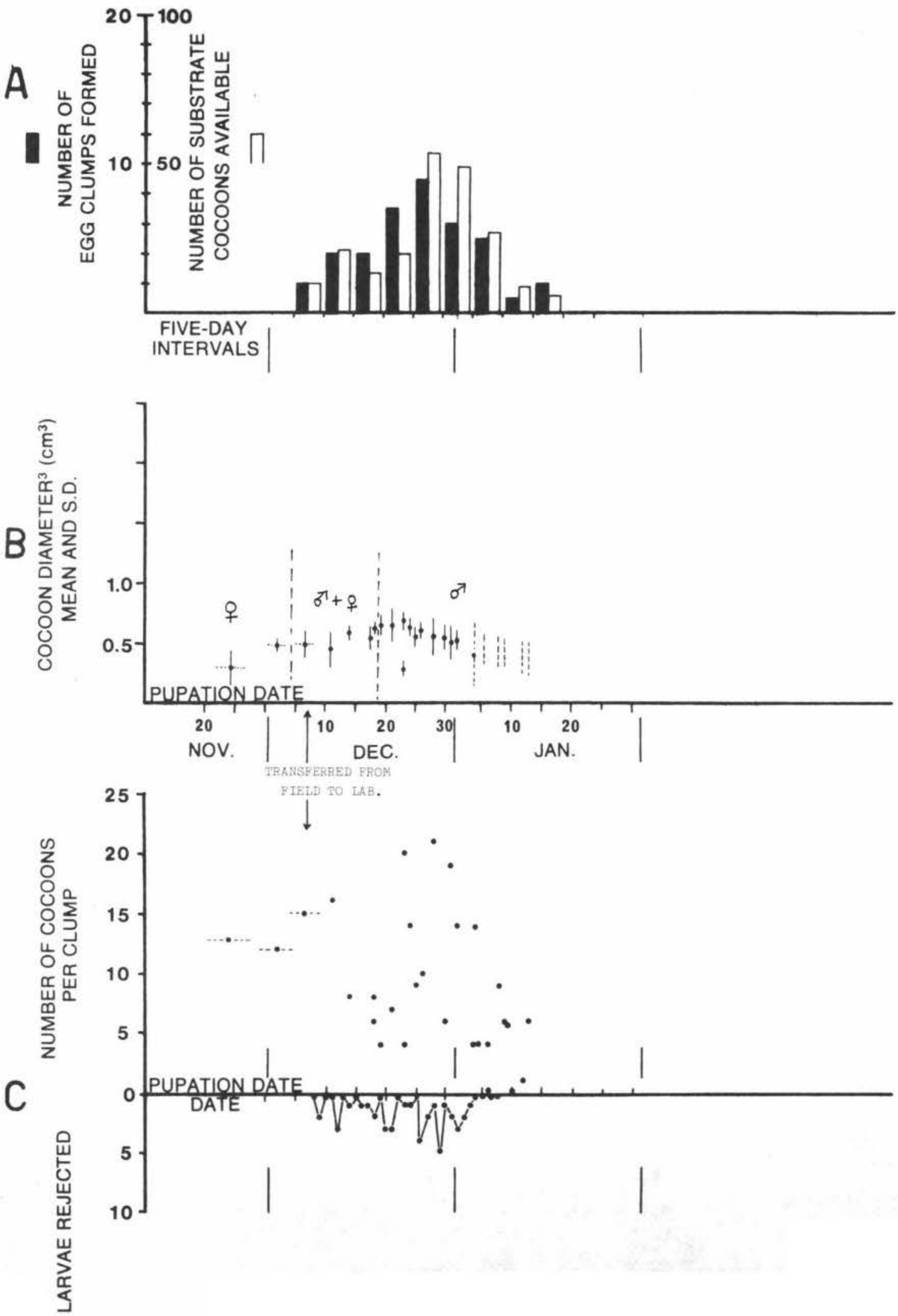


Figure 3.15.1 Seasonal changes in colony 1. (A) Egg clump formation in relation to the supply of substrate cocoons. (B) Cocoon "volume" (diameter³). (C) Numbers of cocoons per clump, and daily totals of rejected larvae.

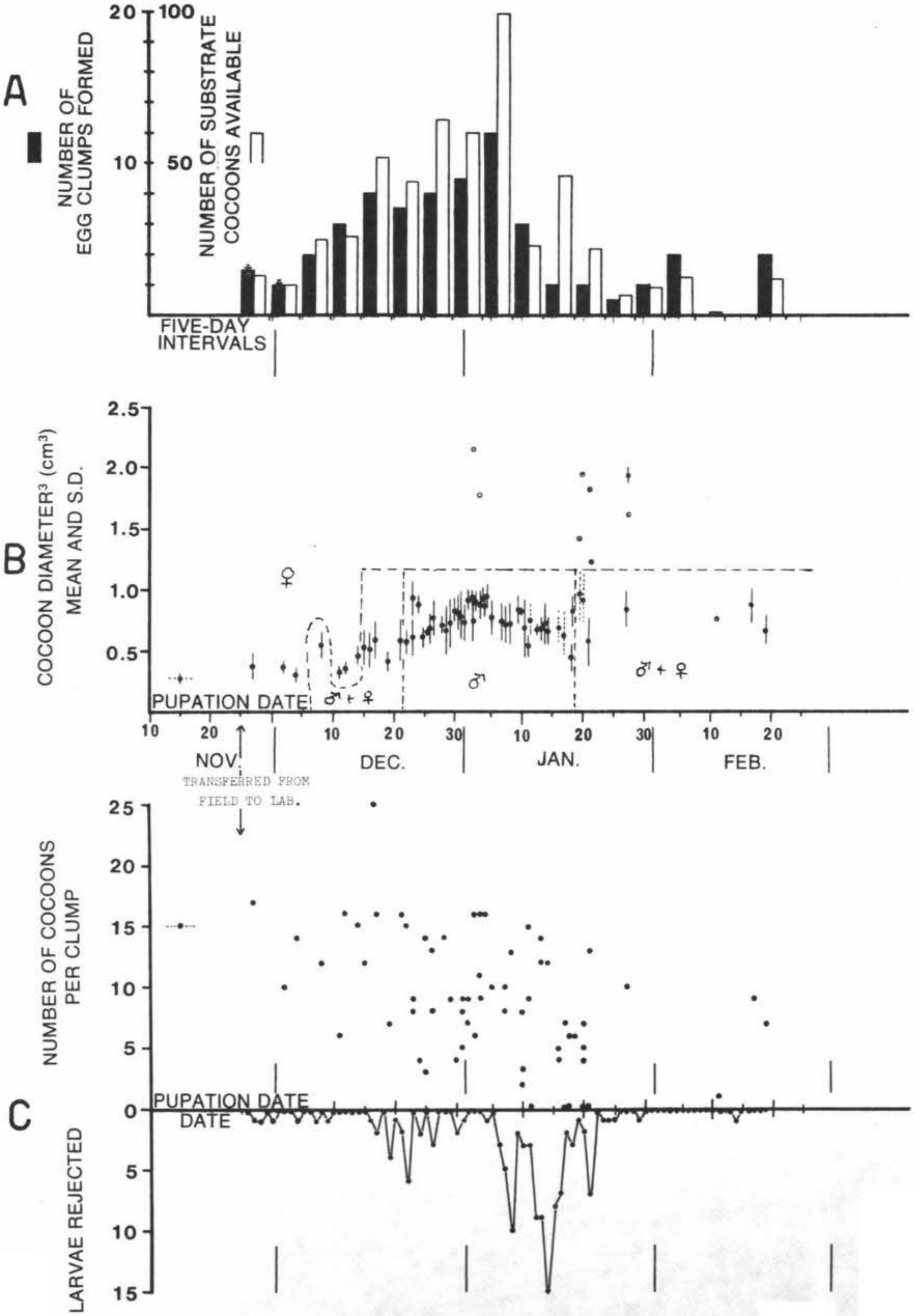


Figure 3.15.2 Seasonal changes in colony 2. (A) Egg clump formation in relation to the supply of substrate cocoons. (B) Cocoon "volume" (diameter³). (C) Numbers of cocoons per clump, and daily totals of rejected larvae.

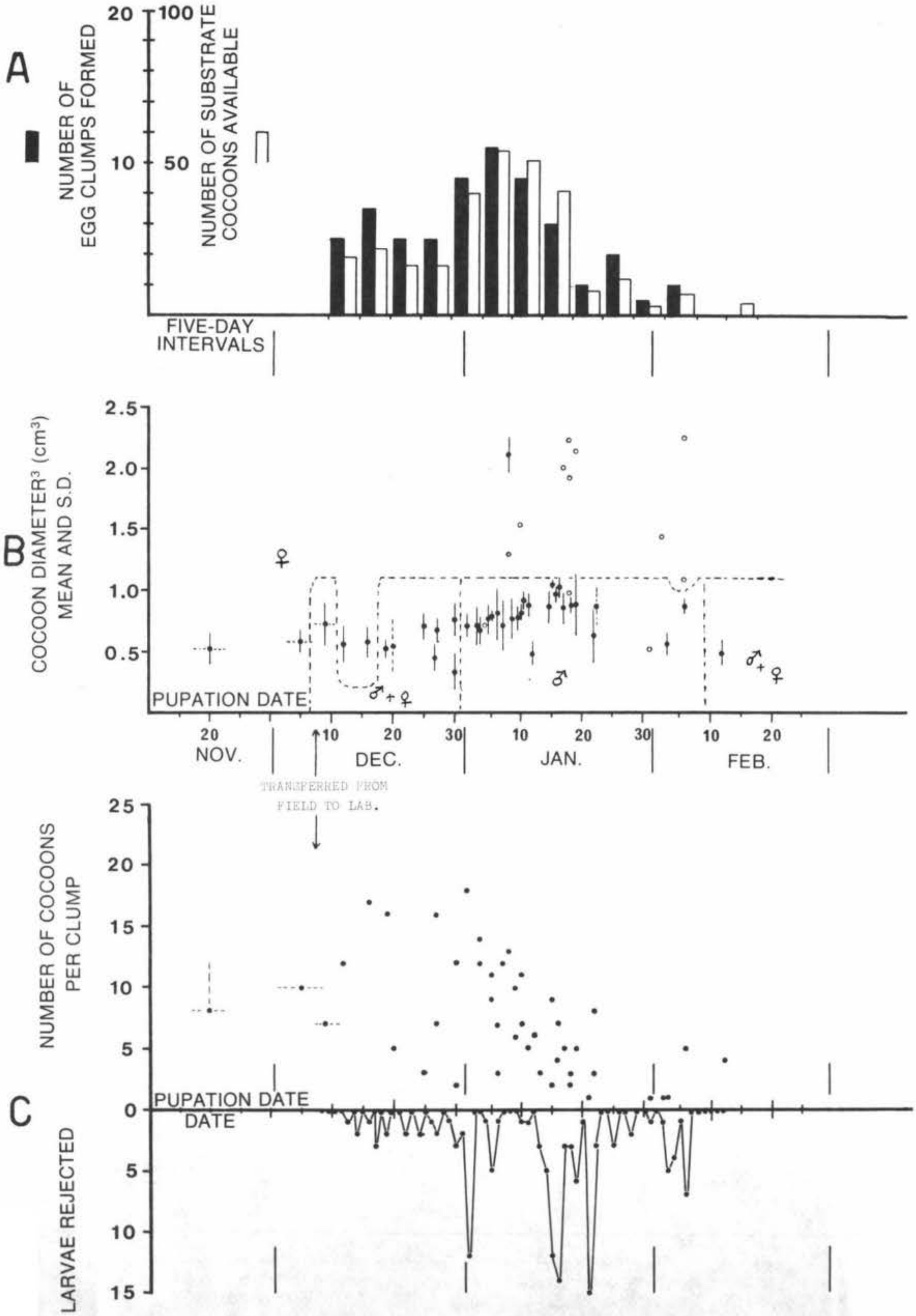


Figure 3.15.3 Seasonal changes in colony 3. (A) Egg clump formation in relation to the supply of substrate cocoons. (B) Cocoon "volume" (diameter³). (C) Numbers of cocoons per clump, and daily totals of rejected larvae.

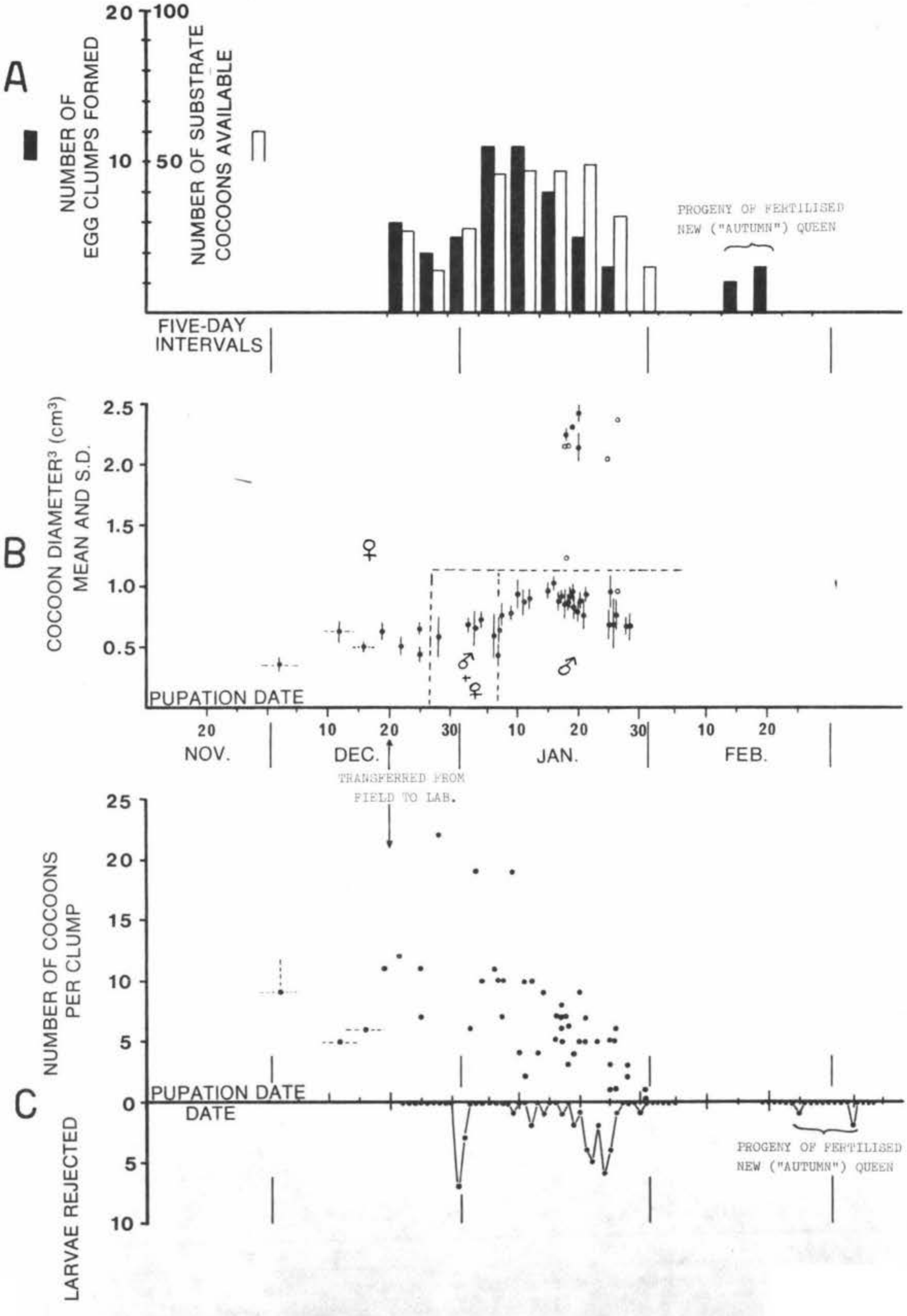


Figure 3.15.4 Seasonal changes in colony 4. (A) Egg clump formation in relation to the supply of substrate cocoons. (B) Cocoon "volume" (diameter³). (C) Number of cocoons per clump, and daily totals of rejected larvae.

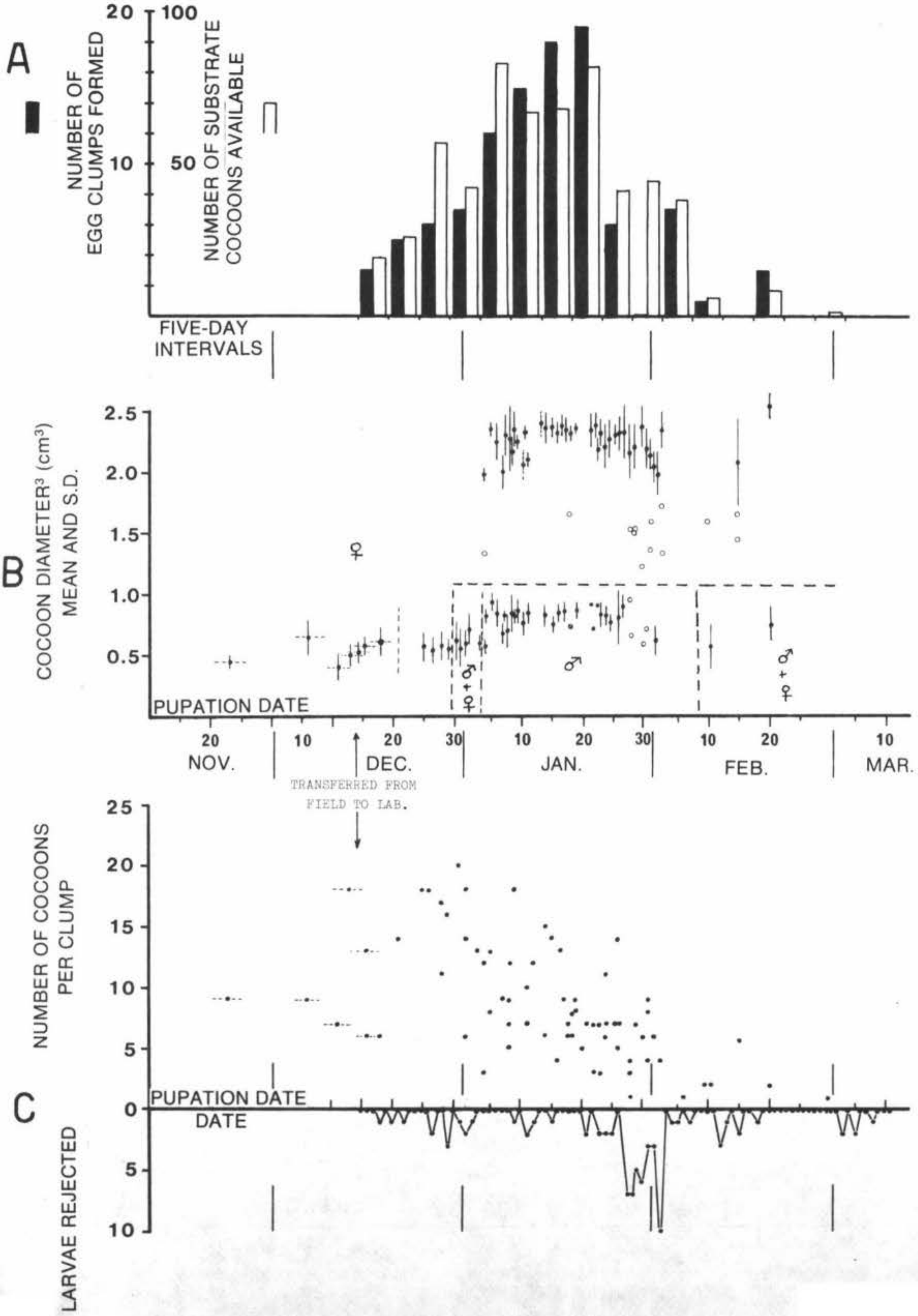


Figure 3.15.5 Seasonal changes in colony 5. (A) Egg clump formation in relation to the supply of substrate cocoons. (B) Cocoon "volume" (diameter^3). (C) Number of cocoons per clump, and daily totals of rejected larvae.

I suggest that the biological significance of the observed relation between egg production and substrate cocoon availability, is to ensure sufficient space for pocketed larval clumps to grow without undue distortion of the clump or its pocket. The larval clumps of pollen storers should not require precise spacing, because their mode of nutrition would be less disturbed by distortion (or even translocation) of their clumps, which might result from crowding. Several pollen storers are in fact known to lay their egg cells in large numbers without regard to spacing (e.g. B. terrestris - Sladen, 1912; B. terricola - Plowright, 1966; B. ignitus - Katayama, 1971).

Brian (1951) considered that the queen would be unable to maintain such an egg/cocoon ratio when the colony was larger, but I have found the oviposition rate to keep up with the cocoon supply fairly well in most colonies. The maximum egg laying rate for bumblebee queens has been suggested to be about one cell (i.e. 10 to 15 eggs) per day by Sladen (1912) and Free and Butler (1959). But in the present study, the foundress of colony five made four egg clumps per day on at least one occasion. I knew the eggs were laid by the queen and not by workers because the resulting brood contained queens. Therefore, the eggs were fertilised. A total of 25 adults were reared from these four clumps. However, in colonies 1, 2, 3 and 4, (figures 3.15.1A to 3.15.4A) the number of eggs per cocoon declined at the beginning of colony decline. There were peaks of larval rejection about these times too. Both the reduced oviposition and the larval rejection could have been due to large numbers of newly emerged males and/or queens consuming most of the pollen in the nest.

Is colony growth limited by the rate of egg production? For oviposition to have a limiting effect, we would expect most of the eggs to be raised to adulthood. I have little information on the egg mortality within individual cells, but I consider that the number of adults reared per clump, is often less than the average number of eggs per clump (see figures 3.15.1C to 3.15.5C). I do, however, have definite observations that whole clumps of eggs often fail to develop, especially during the decline of the colony (see figures 3.14.1 to 3.14.5). The failure of these clumps to develop, is usually associated with lack of food in the nest, and shortage of workers to attend to the larvae. Thus it would appear that colony decline results more from a lack of workers, rather than a lack of fresh brood.

3.4.2 Brood Growth

Possibly the most important aspect of colony development is the care and destruction of larvae. The size of adults, and thus to a large extent, their caste and role in the colony, is determined during the larval stage (Free and Butler, 1959). And conversely, the treatment given to larvae is the result of the numbers and behaviours of the existing adults.

I was able to assess the size attained by larvae, by examining the cocoons spun by them. As shown in figure 3.9, the diameter of a cocoon is closely correlated with the size of the adult emerging from it, and the cube of the diameter is closely related to the quantity of pollen invested in it. The frequency distribution of cocoon diameters for five colonies are shown in figure 3.8. Apart from colony 1 which produced no queens, each histogram is bimodal with few if any cocoons in the 10.5 to 12.0 mm size range. Workers and males emerged from cocoons which were less than 10.5 mm diam., and queens emerged from cocoons larger than 12 mm diam. Of the few intermediate-sized cocoons, some failed to hatch (described in section 3.3.3) while the others produced intermediate-sized females which behaved as workers i.e. they were regular foragers.

The generalisation has often been made that pocket makers have overlapping worker and queen size distributions (Free & Butler, 1959; Plowright & Jay, 1968; Wilson, 1971; Michener, 1974; Alford, 1975). B. ruderatus seems exceptional however, in having a more discontinuous size distribution which is usually considered typical only of some pollen storers. B. hortorum is one of the pocket makers often quoted as having some workers bigger than the smallest queens, and my field observations agree with this. When searching for specimens of B. hortorum queens in the spring, I have often been unable to decide whether some individuals are small queens or large workers, but the same confusion has never arisen with B. ruderatus queens. They are more uniform in size and clearly larger than workers.

The seasonal changes of cocoon diameter-cubed (and hence pollen investment per larva) are shown in figures 3.15.1B to 3.15.5B. The mean diameter-cubed for the cocoons within each male and/or worker clump is represented by a single dot. However, the queens of each clump are represented by another dot because they were usually easily recognizable and grossly different in size from the males (mixed clumps of workers and queens were seldom found). There were a few females of intermediate size (between 1.075 and 1.70 cm^3) and these have been plotted individually with open circles. Other open circles on the graphs represent clumps which are in the normal queen or worker/male size range but with only one cocoon in the clump.

The larvae of the incipient clumps received less food than larvae of most later batches. But between the second and final all-worker clumps little size increase was apparent.

After the last all-worker clump, five or six clumps containing males and workers were reared. Some of these clumps contained larger cocoons than previously, and there was often a slight net increase in mean size.

Colonies one to four changed to producing all-male clumps at the cessation of worker clump production. In colony one, male size increased slightly, then declined until the demise of the nest. No queens were produced. Colonies two, three and four produced progressively larger males until the maximum male size (about 10 mm diameter cocoons) was reached, whereupon male size declined. Near the time of peak male size, these colonies produced a few queens, and colonies two and three produced some more queens at subsequent intervals.

In colony five, which was the most prolific one (note the large number of queens produced - figure 3.8, table 3.5) there was an abrupt change from worker and male production to queen and male production, without an intervening period of all-male rearing. Photographs of colony five at 20-day intervals are shown in plates 11, 12, 13 and 14. The first clumps of queen larvae can be seen in plate 12. Queen larvae were fed at least twice as much pollen as male and most worker larvae. Only one intermediate-sized female was pro-

Plate 11 Colony five, two days after being transferred to the laboratory. About twenty days since its inception.

Plate 12 Colony five, about forty days old


1 cm
5

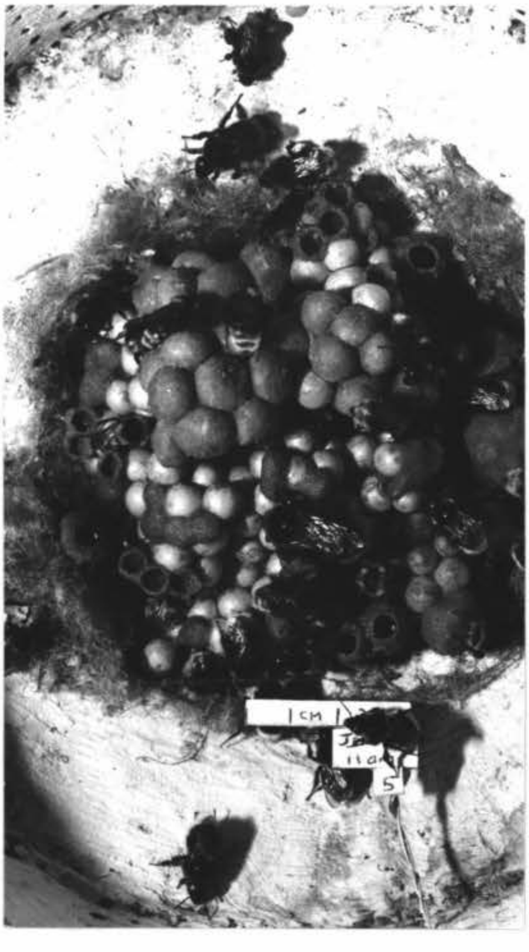
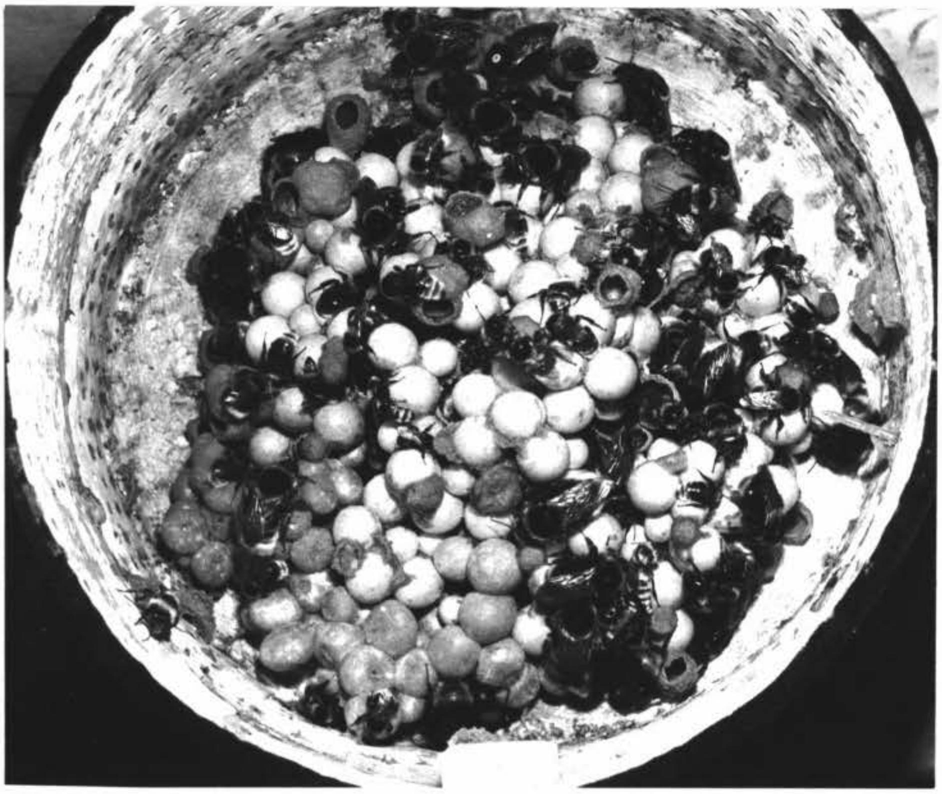
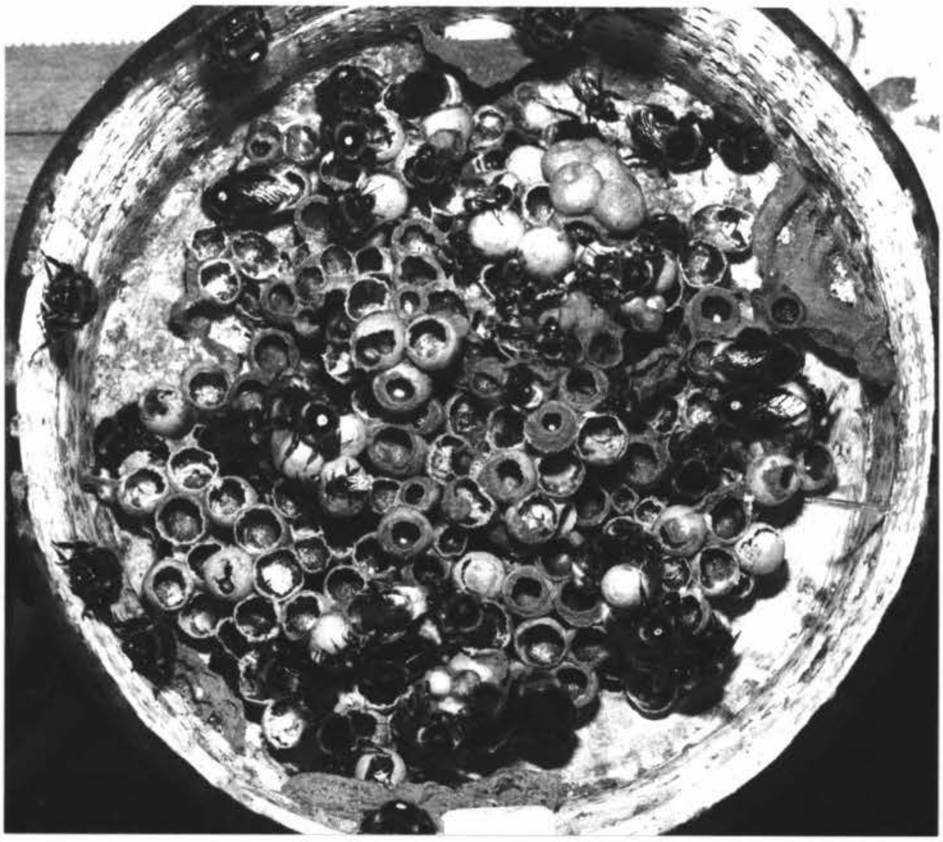

1 cm
5

Plate 13 Colony five, about sixty days old

Plate 14 Colony five, about eighty days old





duced during the transition from worker to queen production. There was a slight increase in the mean size of queens during the first week of queen production, but a more uniform size was achieved over the next two weeks. Finally queen size became more variable again and decreased. Most of the intermediate sized females were produced during the decline of queen production.

What controls the size to which larvae grow before pupating? Plowright (1976) has presented a theory which predicts that the size at pupation will be related to the rate of food intake during the silk-spinning stage of the fifth instar, which is when the greater part of larval growth takes place. He has shown that the rate of silk production may be inversely proportional to the feeding rate, and that infrequently-fed larvae sometimes spin so much silk around themselves that further food intake and growth is curtailed.

It has long been thought that the rate of food intake (and hence size attained) is associated with the worker:larva ratio (Cumber, 1949; Free & Butler, 1959; Plowright & Jay, 1968). The basic idea is simply that the more workers there are, the more pollen and nectar can be brought "home" and distributed to the larvae; and the fewer larvae are present, the more food they will receive each. Plowright & Jay (1968) have shown how the worker:larva ratio would normally rise through the season as a result of the pattern of worker survival and brood production rates. This would theoretically result in a gradual rise in female size until queens were produced. Such a gradual increase in the size of bees produced has in fact been found for some <u>Bombus</u> species by Knee & Medler (1965) and Plowright & Jay (1968). The cause of the sudden size increase at queen production of species like B. ruderatus however is not clearly understood.

3.4.3 The role of larval rejection

The daily numbers of larvae rejected from each colony are shown on graph C of figures 3.15.1 to 3.15.5. Some larval rejection occurred during the production of all three castes, but became most prevalent during later stages of colony development. As mentioned in section 3.3.2, 36% of the estimated larval population was rejected from one colony (No. 3) during its period in the laboratory. The percentage loss of invested food would have been much smaller however, due to the rejected larvae being only a fraction of their potential full size (i.e. they were rejected before the silk-spinning stage when most growth took place). Graph C of figures 3.15.1 to 3.15.5 also shows the number of larvae surviving to spin cocoons, in each clump. Not unexpectedly, there tend to be fewer larvae surviving per clump later in the season when more of them were being rejected. At this stage some colonies were still managing to produce a few queens however, i.e. although the total food investment in larvae was diminishing, the remaining larvae were still reaching almost maximum size.

Although the events during colony decline may seem a strange place to start, I think they give the clearest evidence for the function of larval rejection. As shown in section 3.4.1 and figures 3.14.1 to 3.14.5, there appears to be no egg shortage during colony decline. Rather, the cause of decline seems to be the dying off of the worker force, and the consequent loss of food and attention for the brood. If larval nutrition was merely a result of seasonal trends in the worker:larva ratio, female size would fall again as the trophic state of the colony deteriorated, and more workers would be produced at the end of the season. But it is well known that Bombus colonies rarely return to worker production after queens have been reared (Free & Butler, 1959; Alford, 1975). In B. ruderatus larval rejection is apparently the mechanism by which queen size is maintained.

But does larval rejection play a role in the initiation of queen production? My results are inconclusive on this point because larvae were being rejected through much of the season anyway. There was however a noticable peak of rejection from colony 3 on January 1st, which was an appropriate time to be influencing the first batch of larvae to become queens. Röseler (1967) (cited by Michener, 1974) suggests that larval rejection functions to increase the worker:larva ratio to an optimal level for queen production, and that larval rejection is stimulated by the presence of male brood. My observations suggest that male brood often do trigger rejection, but that such rejection serves mainly to increase the worker:larva ratio for male (not queen) production. Males, although only about half as big as queens, are more uniformly sized than workers. Note that in colonies 2 and 3 (figures 3.15.2B and 3.15.3B) an isolated male-containing clump during the worker-production period contained some larger cocoons than the contemporaneous worker clumps. The early male clump of colony 3 passed its larval stage while still in a field domicile and larval rejection was not being monitored. But some rejected larvae were in fact noticed in the domicile at that time. It would appear that some inherent property of male larvae stimulates the workers to reject enough larvae for the remaining ones to reach a sufficient size.

Evidence of larval rejection in aid of queen production comes from an observation of a small B. hortorum colony. I had anaesthetised the whole colony (about eight individuals) with CO_2, but the foundress queen failed to properly recover. When the workers and the ailing queen were reunited with their brood, one small worker rejected ten of the fifteen larvae present. The foundress died, and the remaining five larvae developed into new queens. Röseler (1970) discovered that B. terrestris foundress queens inhibited the rearing of new queens early in the season, probably via a pheromone. I have observed the workers of B. terrestris and B. ruderatus to behave in a distinctive manner when their queen is removed, possibly as a result of their "missing" the queen pheromone. Within a few hours or less of the queen disappearing, the workers perform exaggerated ventilating movements of the abdomen (such as when they are incubating chilled brood), and behave aggressively toward one another. I believe the hyperventilation may be associated with increased metabolic rate to speed up ovary development. The aggressive behaviour resembles that described by Free (1955b) for several British species. Admittedly the above observations relate to worker ovary-development rather than queen production, but the two features are possibly related. In Apis mellifera for instance, a queen pheromone inhibits both queen rearing and worker ovary development (Butler, 1960 - cited by Wilson, 1971). If indeed Bombus queens inhibit queen production via a pheromone, the initiation of queen production could result from either a reduction of pheromone output due to queen aging or loss, or a reduction in the effect of the pheromone on workers (or larvae) due to physical factors such as crowding.

3.4.4 Male production

Male production has much the same effect as queen production has, on colony growth, because neither males nor queens contribute much to the rearing of further brood. When the final worker larva has pupated and only male or presumptive-queen larvae are being reared, there will be about ten days before the last worker emerges and the worker population is maximal. This is the "beginning of the end" of the colony, the imminence of "the end" depending how long it takes for all the workers to die (workers often live for several weeks).

Male production differs from queen production in being irrevocably determined by the time an egg is laid, whereas female eggs are caste-plastic the determination into workers and queens being controlled trophogenicly during the larval stage. Thus queen production is under some degree of control by the workers, but male production is determined solely by the individual who lays the eggs (usually the foundress queen).

Male production can, and sometimes does, occur when colonies are quite small and thus cripple the chances of attaining a large size. The sizes to which my colonies grew (in terms of the total cocoon counts) were related to the length of the worker production period, which was in turn dictated by the earliness of male production.

Little seems to be known about the causes of male production in hymenoptera generally, except where it is a direct response to the nature of the oviposition site, such as ichneumons reacting to small hosts or Apis reacting to drone cells. Röseler (1967) (cited by Alford, 1975) suggested that Bombus male production resulted from a high density of bees in the nest. Alford does not say how "density" was defined in the original paper however. In view of the fact that males are sometimes produced in small or unprosperous nests, bee density would not seem to be very applicable as a general explanation. Since males arise from unfertilised eggs, one tends to think of exhaustion or viability-loss of the sperm in the queen's spermatheca. Sperm-exhaustion is unlikely, according to Röseler (1973), who found no significant reduction in quantity of sperm in queens sampled at the end of colony growth, compared to before colony founding. Further evidence against the loss of sperm viability is the fact that male production is often followed by a resumption of female production (queens).

CHAPTER 4 GENERAL DISCUSSION AND CONCLUSIONS

4.1 ECOLOGY AND MANAGEMENT OF B. ruderatus COLONIES

Natural colonies of B. ruderatus were found mainly in underground sites. This agrees with findings of Sladen (1912) and Postner (1952). Cumber (1953) implied that he considered B. ruderatus to have a greater tendency to nest above-ground than other species in New Zealand. However in my experience there have been no higher proportion of surface nests of B. ruderatus than surface nests of B. terrestris, a generally acknowledged underground nester (Sladen, 1912; Free & Butler, 1959).

The preference for underground nest sites was further demonstrated in the occupation rates of underground and surface domiciles. During the 1974-75 season, 93% of 45 underground domiciles were occupied (76% of the identified queens were B. ruderatus), compared with only 32% of 23 surface domiciles. Initially I thought that dampness could have made the surface type unattractive, but when a much drier version was tried the following season it failed to attract any more queens than the earlier type. During that second season however, the underground domiciles were reoccupied even though they contained damp nesting material (I had neglected them in my attempts to devise dry surface domiciles and the nest material had not been replenished). It appeared that damp underground domiciles were more attractive to queens of both B. ruderatus and B. terrestris than dry surface domiciles. This is of considerable interest because in the past, dampness has often been blamed for non-acceptance of domiciles by bumblebees (Sladen, 1912; Frison, 1926; Fye & Medler, 1954a). Since the work of Sladen and Frison, underground domiciles seem to have gone out of favour, probably on account of the extra labour required to instal them. However my results suggest that the high occupation rates might outweigh the extra cost per domicile because, although surface domiciles might be cheaper, more would be needed to get an equivalent number of colonies.

Two drawbacks with the design of my underground domiciles, were inaccessibility for the identification and removal of occupants, and impermeability of the plastic cavity roof causing condensation which seriously affected colony survival. At present I am trying to remedy both of these problems by replacing the buried plastic dome, with a partially buried porous-concrete domicile which is accessable via a lid. The same tunnel and entrance has been retained. Further details and occupation results are given in Appendix 5.

Underground domiciles have several practical advantages. For example if they were moulded in situ with concrete they would be relatively permanent, livestock proof, and maintenance free. A high concentration of such nest sites could be permanently provided in an area where B. ruderatus was naturally common (such as the Waverley study area in this project) and the colonies could be taken out and shifted to clover crops when needed. At the crop site a different domicile design could be used. This could be portable (even disposable) and would not need to be underground because this seems to be a behavioural requirement for nest-searching queens rather than an essential physical requirement for colony growth.

B. ruderatus has relatively large colonies (average cocoon count about 400) for a long-tongued species. Sladen's (1912) work would suggest that it is one of the most prolific of the British long-tongued species. Of such species in North America B. fervidus was found by Hobbs (1966) to have a maximum cocoon count of 287, and B. americanorum was considered by Frison (1930) to have an average colony size of 200 individuals. It has been suggested from time to time by various persons that these two species should be introduced to New Zealand (L. Gurr, personal communication). However, the success of domiciliation of B. ruderatus, and its apparently larger colony sizes would tend to indicate that we already have in New Zealand a suitable candidate for pollination management. A further argument against the introduction of B. fervidus and B. americanorum is their highly aggressive dispositions. Plath (1934) described how B. americanorum workers attacked anyone who ventured within 25 feet of their domicile. B. fervidus is reported to behave similarly. B. ruderatus however is quite docile, and there is little danger of being stung when manipulating colonies. Innocent passers by are certainly safe.

4.2 COLONY DEVELOPMENT

Special methods have been devised to house field-initiated B. ruderatus colonies in order to study their subsequent development. The conical observation hives were heated to 30C, and had a large porous wall-surface for draught-free ventilation. A window was removed from the laboratory, so that the workers could forage freely in the field. The observation colonies appeared to reach a similar size at a similar time to undisturbed ones in the field. The results obtained in my laboratory, can safely be considered therefore to reasonably represent natural colony development of B. ruderatus. Since there were many more aspects of colony development than could be covered in a project of this magnitude, I confined most of my effort to those aspects which caused the least disruption to the colonies.

The growth dynamics of social insect colonies are affected by a nexus of interacting factors, many of them species-specific behavioural phenomena. To assist me in providing a unidimensional narrative discussing B. ruderatus colony development, I have included a flow-chart, figure 4.1. It shows the twenty or so factors which seem relevant to colony development. Each numbered arrow is discussed in the numbered sections below.

1. Colony establishment

In the study area (hill-country margin, just South of Taranaki) colony establishment took place during November and early December, and coincided with the onset of foxglove (Digitalis purpurea) bloom. Prior to that time of year, flowers are very scarce in the area, and it is suspected that

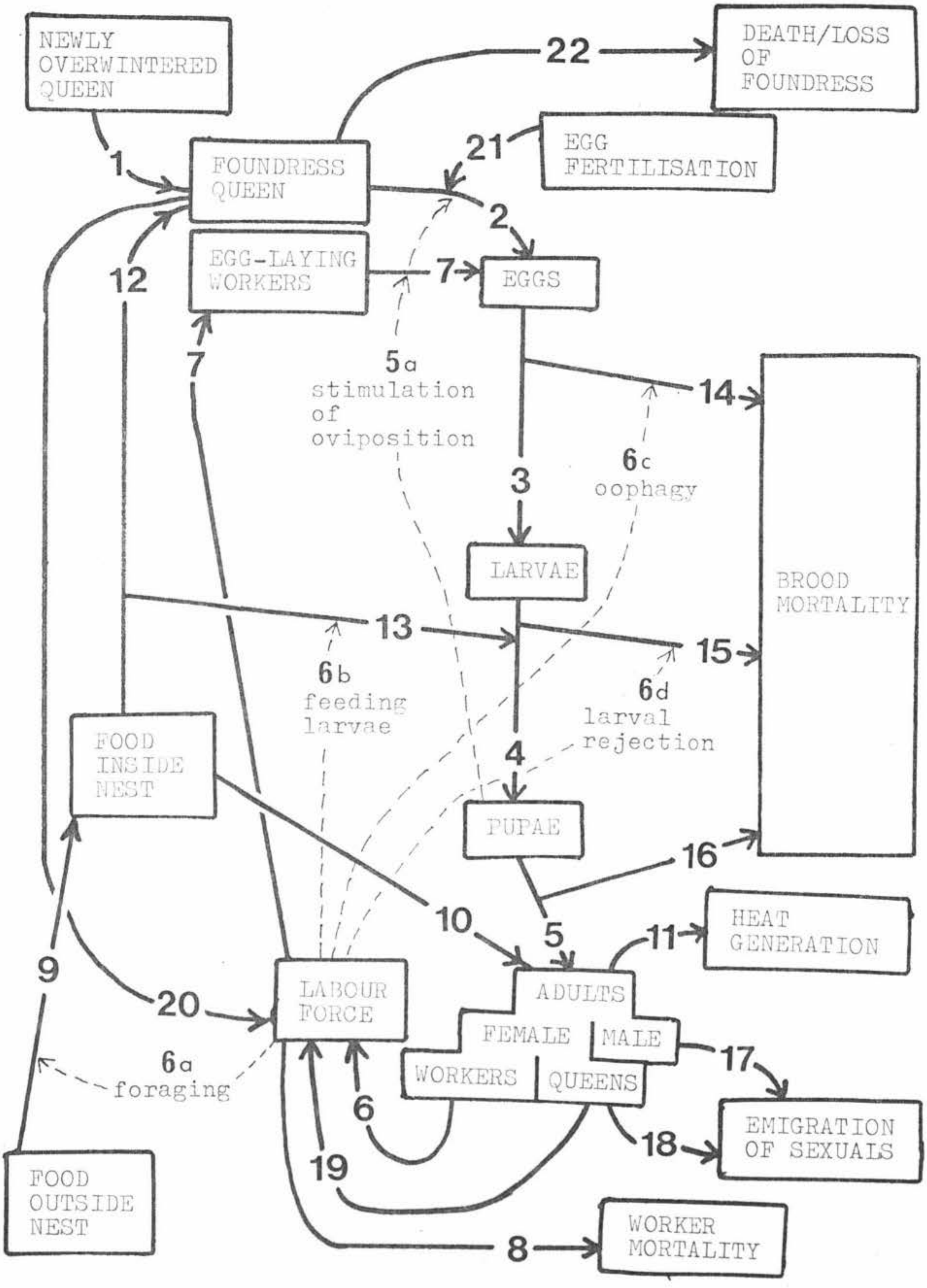


Figure 4.1. Flow diagram of the main factors affecting the development of Bombus ruderatus colonies. The solid arrows represent the transfer of materials or other resources. Dotted arrows represent behavioural interactions. Numbers refer to explanatory paragraphs in section 4.2.

this put the earlier emerging B. terrestris queens at a disadvantage. Where earlier forage is available B. terrestris competes more favourably with B. ruderatus and often outnumbers it (Gurr, 1957). Nest site preferences have been discussed in section 4.1. There is some evidence that overwintered queens may re-use their parental nest site. This is being investigated at the present time (see Appendix 5).

2. Egg laying

The queen lays an initial batch of about 16 eggs. Subsequent batches of 10 to 19 eggs are laid in wax cells on cocoons. (See 4a for details of the effect of cocoons on oviposition.)

3. Egg development

Eggs were concealed inside closed communal cells. Therefore neither their hatching time nor their survival rate could usually be determined. For one batch an oviposition to hatching duration of slightly less than four days was found. This agrees with the average of 3.6 days which Hasselrot (1960) found for several European species.

4. Larval development

B. ruderatus larvae receive food in two ways: (a) foragers deposit solid pollen in pockets on the larval clumps, so that the larvae come to lie on a bed of pollen which they eat; (b) workers (and queen(s)) ingest pollen from the pockets, and regurgitate it to the larvae in a mixture of about 1 mg pollen/µl nectar. Both modes of nutrition appear to operate throughout larval life and for all castes. Most North American pocket-making subgenera, except subterraneobombus, do not have pockets on queen and male clumps, or on 5th instar worker clumps (Hobbs, 1964). Hobbs considered the use of pockets for all stages and castes to be a primitive trait. However, B. ruderatus has a strong bimodality in female size (i.e. clear size distinction between workers and queens), and this is considered to be an advanced trait - unusual among pocket makers - by most authors (e.g. Wilson, 1971; Michener, 1974; Alford, 1975).

Plowright (1976) has presented a theory which could explain the discontinuity, where it occurs, in female size distributions. The theory is based on the observation that during the 5th instar, larval silk production rates are inversely proportional to the rate of food intake. By making certain assumptions about silk production rates, geometry and strength, Plowright showed the possibility that below a certain critical level of feeding, larvae might always spin so much silk around themselves that further feeding and growth would be restricted. Below this critical trophic level, larvae would thus pupate before they exceeded the upper size limit for workers. If, on the other hand, larval food intake exceeded the critical level the growth rate would exceed the restraining effect of the silk so that feeding could continue until the physiological limit of growth was reached (i.e. normal queen size). It would be very interesting to compare the silk production dynamics of B. ruderatus with that of B. hortorum, a very closely related species but which has a weak worker/queen size dichotomy.

Larvae take about six to ten days to reach the pupal stage. Very often the largest pupae are formed from larvae with the shortest development times; which agrees with Plowright's theory.

5. Pupal Development

The developing bee is enclosed in a cocoon for 9 to 14 days, the duration being proportional to the dimensions of the cocoon. Most of this time is spent in the pupal stage. However the first day or two (precise time not determined) is occupied by the prepupal stage, and an undetermined time prior to adult emergence is spent as a pharate adult.

5a. Effect of cocoons on oviposition

Freshly spun cocoons are the usual substrate for egg cell construction. Oviposition usually occurs when the underlying cocoons are one or two days old. The number of egg cells is limited by the size of the underlying clump. On average about one new egg clump is built per five substrate cocoons. This is similar to the situation B. agrorum, a pocket maker described by Brian & Brian (1948).

6. Workers entering labour force

Workers were not seen to perform "duties" (e.g. wax manipulation, feeding larvae) while less than 12 to 24 hours old. During this stage they are termed "callows" and are still developing coat colouration and wing hardness. The age distribution of "mature" working individuals was not studied. However, some individuals (especially large ones) were observed to take their first foraging flight on the third day after emergence. Very small workers were seldom seen foraging, an observation frequently made by other authors, e.g. Free & Butler (1959).

6a. Foraging

Foraging was not studied in detail in this project. The main food source for incipient colonies in the Waverley field area was foxglove (Digitalis purpurea). After the colonies had been shifted to Massey University they obtained most of their pollen from red clover (Trifolium pratense) and goats' rue (Galega officinalis). Other plants may have served as nectar sources.

Marked foragers (at Waverley) were seen up to 1 km away from their nest site.

6b. Feeding of larvae

As mentioned in (4) there were two methods of feeding larvae - direct regurgitation, and indirect provision of solid pollen.

The stimuli which cause workers to feed larvae, and which control the feeding rate, are not understood. When observing workers regurgitate food to larvae, I noticed that the degree of abdominal contraction varied. It appeared that large larvae stimulated workers to regurgitate more food per feeding than small larvae.

Solid pollen (pocket) feeding usually began five days after the eggs were laid, i.e. one day after their estimated hatching time. Sometimes however, pocket formation was delayed. This was considered to be more likely a result of food shortage rather than delayed egg-hatching.

6c. Egg-eating

Reciprocal oophagy between the foundress and workers was observed on several occasions. The only pocket-maker in which oophagy has been observed before is B. fervidus (Plath, 1934). In B. ruderatus colonies, the foundress generally appeared to be more successful at eating worker-laid eggs than workers were at eating her eggs. The proportion of eggs lost through oophagy was not determined, but it was not considered to be very high.

6d. Larval rejection

Larval rejection is a type of infanticide which prunes the larval population. Presumably the pruning is done in relation to the food supply to avoid the production of "undersized" individuals. However, the threshold for rejection (and hence the bees' definition of "undersized") must change through the season, because during worker production larval rejection is relatively infrequent and female size is small and variable, but during queen production more larvae are rejected and female size is large and uniform.

B. ruderatus foundress queens seem to produce an "optimistic" quantity of eggs. Under optimum conditions most of the larvae probably survive, and large colonies may develop with few larvae being rejected. However, a colony which is handicapped by high forager mortality or inclement weather may reject a considerable proportion of its larvae yet produce normal sized workers and a few queens. (One of my poorer colonies rejected 36% of its total larval population during a nine week period.)

Röseler (1970) suggested that larval rejection was stimulated by the presence of male brood, and that it served to increase the worker:larva ratio to an optimal level for queen production. My observations agree that larval rejection is often associated with the presence of male brood. However, in *B. ruderatus* such rejection seems mainly associated with ensuring the male larvae get enough food, males tending to be more uniform-sized than workers. Males are produced for variable periods before queen production is initiated.

There is much yet to be learnt about larval rejection. My project has provided descriptive information on its ocurrence in free-foraging B. ruderatus colonies. I hope that future investigations will be conducted under such conditions that the colonies' pollen budgets can be accurately monitored.

7. Egg laying by workers

This ocurred during the latter part of the season in most colonies, but the proportion of egg cells made by workers was not determined. Workers laid only 1 to 4 eggs per cell (compared to the queen's 10 to 19) and these were often eaten by the queen or rival workers. In the largest colony I observed, egg production by workers ocurred on a large scale after the death of the foundress and resulted in the rearing of nearly a hundred males.

8. Worker mortality

Due to my not engaging in an extensive marking program worker longevities were not determined in any detail. Average forager longevity was probably a small number of weeks. Maximum worker longevities exceeded ten weeks.

9. Colony food input

No measurement was made of nectar input. The average pollen "payload" was approximately 0.02 g. The maximum number of pollen loads recorded in one day was 400, which would give a total pollen input of about 8 g.

10. Food consumption by adults

Freshly emerged males and queens (kept in an incubator away from their nests) consumed considerable quantities of nectar and pollen. Queens each consumed an average of 0.4 g of 50% honey solution every day. Pollen consumption ocurred mainly during the first four days of adult life during which time each queen consumed about 0.3 g in total. Males were about half the weight of queens and consumed half as much food. The basic patterns of food intake of males and queens were otherwise very similar, both sexes eating about one third as much pollen during the young adult stage as during the larval stage. Thus, when large numbers of sexuals are emerging there may be considerable depletion of the colony's food stores. Hasselrot (1960) suggested this could hinder the rearing of further brood. I have found some of the biggest peaks of larval rejection to coincide with the emergence of large batches of queens and males.

11. Heat generation

Bumblebees raise the temperature inside their nest envelope as a result of brood incubation (a task performed by all castes) and incidental metabolic heat. A nest temperature of about 30°C seems optimal, and is probably achieved in most natural nests. The efficiency of nest heating depends on the thermal insulation of the nest envelope. Heinrich (1974) has shown that incubating queens consume twice as much nectar and generate twice as much heat at 20°C as at 30°C. Thus bumblebees need good home insulation if they are going to keep their heating bill down and have calories left over for other jobs such as brood rearing.

12. Food consumption by foundress

This was not measured. The queen must eat a certain amount of pollen to obtain proteins for oogenesis. It is probable that she can take advantage of her size and strength (at least until queen production) to obtain what she needs of the food stores.

13. Food consumption by larvae

The mode of feeding has been described in sections 4 and 6b. The total pollen intake during larval life was estimated to be 0.1 to 0.6 g for workers, and 0.9 to 1.1 g for queens. Males fell within the upper part of the worker size range. There was an approximately linear relationship between the cube of the cocoon diameter and the quantity of pollen grain walls in the meconium.

14. Egg mortality

Oophagy has been mentioned in section 6c, but as pointed out in section 3, total egg mortality could not be directly measured. However, destructive sampling revealed that there were sometimes more than forty eggs laid in a clump of three egg cells, yet such clumps rarely produced more than twenty pupae in live colonies. The difference was greater than could be accounted for by larval rejection. Close examination of dissected nests suggested that in brood clumps initiated as two or more contiguous egg cells, the larvae from the earliest hatching cell may crowd out the other eggs before they could hatch. Some dead eggs were also found intermixed with healthy ones in normal egg cells.

15. Larval mortality

Larval rejection has been discussed in section 6c. It appeared to be the main source of larval mortality. Some fifth instar larvae were apparently attacked by pathogenic microorganisms, but the ocurrence was only confined to certain colonies and was not investigated in detail.

16. "Cocoon" mortality, i.e. prepupae, pupae and pharate adults

The maximum cocoon mortality ocurred in colony 1, where it was 20%. This was a rather weak colony, but since the cocoon mortality ocurred mainly during male production it should not have affected the size of the worker population. More usual cocoon mortality rates were 1% to 6%, most of these deaths ocurring during the prepupal stage.

17. Emigration of males

Males usually left their parent nest on about the fourth day after emerging from their cocoons. No males were ever seen to make an orientation flight or return to a colony.

18. Emigration of queens

Young queens spent variable lengths of time in their parent nest. Some were seen to take their first flight when two or three days old. Unlike males they normally returned to their nest. Most queens apparently departed for hibernation quarters within about ten days of emergence, but this period seemed highly variable.

19. Queens entering the labour force

New queens were seen performing various household duties, but mostly they fed and rested. Some queen sized individuals became constant foragers, and some of these were known to die without departing to hibernate. Such individuals were functional workers. A recent (1976) paper by Plowright states that in B. perplexus, queen-sized females may become functional workers if subjected to high temperatures and low food intake during their first few days of adult life. This fits in with the observation that young queens are often seen resting outside their nest envelope, presumably a cooler place than inside. Perhaps they cannot develop proper queen-type metabolism in the warmer environment. In my observation hives young queens tended to rest around the periphery, perhaps seeking a cool place. But with the artificial heating they may have been too warm, hence many becoming functional workers.

20. Foundress queen entering labour force

Prior to the emergence of the first workers the foundress *is* the labour force. I have observed *B. ruderatus* foundresses foraging a week after their first workers have emerged. The length of time she continues foraging probably depends on the size and number of the first batch of workers. Household duties such as feeding larvae and manipulating wax are less hazardous than foraging, and the foundress assists with these throughout the season. During the period of most rapid colony growth however, much of her time is involved with egg cell construction and egg laying. The foundress contributes towards colony defence by buzzing menacingly when disturbed. The workers have a quieter buzz. However, if workers are present the foundress merely hides amongst the brood (still buzzing loudly!) while the workers attack the intruder. Young queens and males similarly "bark but don't bite".

21. Control of egg fertilisation

The spermathecae of mated *Bombus* queens usually contain so many sperm cells that only a tiny proportion of them are ever used (Röseler, 1973). The egress of sperm cells from the spermatheca for egg fertilisation is controlled by a muscular mechanism in most hymenoptera (Michener, 1974). Fertilised eggs become females (workers or queens), but if sperm is witheld the eggs become males. Röseler (1967)- (cited by Michener, 1974), suggested that crowding of the bees in the nest stimulated the queen to lay unfertilised eggs. My observations do not confirm Röseler's theory, as I have seen male production ocurring in colonies with low adult populations in relation to the available hive space. Much more information is needed on the causes of male production in Bombus, because the timing of the changeover from worker to male production is an important factor affecting colony size.

22. Mortality or loss of the foundress

Sometimes the foundress is killed and replaced by an invading queen, in which case the colony continues to develop more or less normally. Loss of the foundress without replacement during worker production curtails the production of female brood, and thus initiates the decline of the colony. Often however, she survives until depletion of the worker force prevents the rearing of further brood, and she is of no further use to the colony.

In summary we may describe four periods in colony development:

Establishment - depends on the foundress locating a suitable nest site, and on her success at rearing the first batch of workers.

Growth - is the worker production period. The rate of growth of the worker population depends mainly on: Egg production (which is limited by the area of cocoons); larval survival (which depends on there being enough food for them not to be rejected); and worker survival (which in conjunction with foraging conditions affects the amount of food available to larvae). Under optimum conditions the worker population could be expected to treble every sixteen days.

Maturity - is the production of sexuals. Worker production is usually terminated by a gradual change to male production, which is followed by a partial change to queen production. The number of queens and males raised depends on the size of the worker force.

Decline - results from the worker force dying off without any new workers being reared. Males and queens usually contribute little to colony maintainance, in fact their production speeds its decline.

APPENDIX 1

Nest Material Preferences

Aim: To compare the attractiveness to bumblebee queens, of mouse nests and some other materials.

Materials and Methods: Four nest materials were tested:

(1) Mouse nests: these were made of hay, plus variable amounts of upholsterer's cotton. A handful of the material was put into the cages of laboratory mice, which were kept in a 5°C cool-room, to stimulate them to build nests quickly. I tried giving them plain upholsterer's cotton, but they had difficulty in building nests with it. The nests were removed when built, which was every few days.

(2) Upholsterer's cotton.

(3) Fine dried moss.

(4) Hay and sisel fibres, chopped into lengths of 2 to 5 cm.

Fifteen samples of each material were put into polystyrene boxes (internal dimensions 16 x 16 x 10 cm), with a 2 cm wide hole in one side at floor level. One mouse nest was used per box. The other materials were used to half-fill boxes. The boxes were arranged at three levels (0, 1, and 2m, above floor) in a room (5.2 x 6.4 x 3.3m, dimensions) with large windows. Five boxes of each type of material were randomly ordered equidistantly along the walls at each level.

Flowering foxglove (Digitalis purpurea) and Aqualegia plants in pots were provided, as well as cut foxgloves in bottles of water, and honey solution in feeders.

Ten queens each of *B. ruderatus*, *B. hortorum* and *B. terrestris* were released in the room.

Results: Four *B. hortorum* queens started nests in the boxes of upholsterer's cotton. Their heights above the floor were 0m, 1m, 1m and 2m. The *B. ruderatus* and *B. terrestris* queens did not start nests.

APPENDIX 2

Nest Insulation

Aim: To determine how much warmer than ambient, various containers would become, with a heat input equivalent to that of a small colony of bumblebees.

Materials and Methods: Heinrich (1974) showed that incubating queens of *B. vosnosenskii* at 20°C generate 6.25 cal/g thorax/minute. Assuming the same heat output for queens and workers of *B. ruderatus*, I weighed the thoraces of a queen; small, medium and large worker. I then calculated the heat output of a small colony, arbitrarily choosing a population of 1 queen, 8 small workers, 10 medium workers, and 2 large workers. The heat output came to 11 cal/min, which equals 0.77 Watts. An electric circuit was constructed with resistors which each dissipated 0.77 Watts. A resistor was placed inside each of the following cavities:

(A) "Natural nest envelopes"; these were manufactured, one from upholsterer's cotton, and the other from carpet underfelt. A 2 cm layer of material was moulded over a 7.5 cm diameter light bulb, forming a cup, which was then inverted on to a layer of the same material.

(B) Artificial hive-like containers; (1) A box made of 12 mm thick particle board, with a glass top, and a hole of about 20 cm^2. This box was meant to resemble the observation hives recommended by Free and Butler (1959), and Alford (1975). Its dimensions were about 20 x 15 x 10 cm.

(2) A polystyrene box 16 x 16 x 10 cm, with a 2 cm wide entrance hole, and 50 cm^2 of ventilator area covered with glass wool.

(3) A slightly smaller, thicker walled polystyrene box, which was totally enclosed (i.e. it had no holes).

The temperature in each chamber was measured periodicly with a multi-channel thermistor themometer ("Telemax") until temperature constancy was obtained.

Results: The temperatures equilibrated within two hours. The ambient temperature was 28°C. The temperatures inside the "natural" domes of upholsterer's cotton and underfelt, were + 13°C and + 9.5°C above ambient, respectively. The Free and Butler hive was + 2.5°C above ambient, the ventilated polystyrene box + 3.75°C, and the totally closed polystyrene box + 5°C.

APPENDIX 3

Ventilation

Aim: To compare the rates of water vapour diffusion through various porous surfaces.

Materials and Method: Discs of the test materials were cut to a diameter of 5 cm. They were then fitted into a 2 cm length of 5 cm int. diam. PVC pipe, which was in turn fitted over the neck of a small jar containing 30 ml of water. Gaps were sealed with plasticine. The jars were left under room conditions, and weighed periodically to determine their relative rates of weight loss by water evaporation. Jars which were sealed shut, and jars with open necks, acted as controls. The test materials were:

(A) A 1 cm thick layer of carpet underfelt; to represent a "natural" nest envelope.

(B) Perforated zinc covered with polyurethane foam; as used for the walls of the most recent observation hive design.

(C) Wire gauze, covered with a 1 cm thick layer of glass wool; as used to cover the ventilation holes on an earlier type of hive.

Results: The rate of water vapour loss through the tops of open jars was 4.86 mg/hour/cm². Taking this value as 100%, the rates of loss through the test materials were:

Underfelt	- 42%
Perforated zinc and polyurethane	- 38%
Wire gauze and glass wool	- 47%

The following table shows the relative diffusion rates calculated for various areas of different materials.

	Surface Area (cm^2)	Total ventilation Index
Natural covering: 8 cm diam. sphere of underfelt.	201	84
Ventilator of Free & Butler hive	10	16
Ventilator of my recent hive	314	119
Ventilator of my earlier hive	50	24

It can be seen that the large area of perforated zinc and polyurethane, on the walls of my recent hive design (figure 2.4) permits more than the amount of ventilation (as measured by water vapour diffusion) provided by a small sphere of underfelt, while hives with smaller but more "open" ventilators would not receive as much ventilation as the natural covering.

Observations of ventilating behaviour

It has long been known that bumblbees fan their wings in response to high nest temperatures (Sladen, 1912; Plath, 1934; Free & Butler, 1959). However, I have sometimes observed bumblebees fanning in situations which suggest that high humidity and/or CO_2 concentration was the cause.

(a) A large B. terrestris colony which had been freshly excavated and divested of its nest envelope was anaesthetised with CO_2. The first workers to recover from the treatment climbed to the rim of their box and started fanning. Overheating was unlikely to have stimulated the fanning, because no bees were fanning prior to the application of CO_2, and the gas would have had a cooling rather than warming effect.

(b) I once placed a B. hortorum queen with her incipient nest in a polythene bag. She subsequently began to fan. It was in a reasonably cool place and I think overheating was unlikely, but the impermeable bag probably caused a buildup of water vapour and/or CO_2.

(c) The large B. terrestris colony mentioned in (a) was housed in a wooden box with no heating or ventilation. The workers tore up the newspaper from the floor of the box and dragged the pieces over their nest as if to insulate it from heat loss (I had removed their nest envelope), but meanwhile workers were often seen fanning near the exit hole. There were considerable accumulations of wet faeces, and these might have been causing excessive humidity.

APPENDIX 4

Essig's Aphid Fluid.

Lactic acid (reagent grade 85%)	20 parts
Phenol (saturated in distilled H_2O	2 parts
Glacial acetic acid	4 parts
Water (distilled)	1 part

Staining Solution

Essig's Aphid Fluid	15 ml.
Acid fuchsin (2% aqueous solution)	20 drops
Lignin pink (2% aqueous solution)	20 drops
Erythrosin (2% aqueous solution)	20 drops.

APPENDIX 5

Results from the 1976 - 77 season

Domiciles

I wanted to retain the basic stimulus features of the underground domiciles (described in section 2.1.2.1 and figure 2.1), but replace the plastic-dome roofed cavity with something more porous and accessable from above. I dug up thirteen underground domiciles and discarded the plastic domes, putting in their place partially buried lightweight concrete domiciles of the design described in section 2.1.2.2 and figure 2.3. Some of the concrete domiciles could be matched up to the original tunnels, but some tunnels had to be dug up and repositioned more shallowly. The actual depths to which the concrete parts were buried varied. Mostly the rims were flush with the ground surface but some domiciles were sunken into shallow pits while others projected a few centimeters above ground level. A layer of twigs was placed on the floor of each domicile and each was three-quarters filled with underfelt. Installation was done on 30 October, 1976.

By 26 December, 1976, 8 domiciles (62%) contained B. ruderatus colonies. None contained B. terrestris. The colonies had all successfully reared workers, and there was no sign of condensation.

Return of overwintered queens to parental nest sites

The entrance-hole decorations, tunnels, etc. in my laboratory were left intact after the 1975 - 76 season to see whether any queens would return after hibernation. During early October 1976 I placed a piece of smoked paper in the distal end of each exit tunnel. The nest chambers were set at 20°C and a handful of underfelt placed in each.

Queens did return to the entrance which had belonged to colony 5 during the previous season. Colony 5 had produced over 200 queens, of which I had marked 85.

The first footprints on the smoked paper were found on 4 November. More footprints were found on 17 November. In order to see whether the queens were going all the way to the nest chamber (a 2.5 m walk) I put a second piece of smoked paper at the nest-chamber entrance. On 20 November there were more tracks over the outer paper but not at the nest chamber. I suspected the tunnel might be too transparent (made of coarse nylon mesh) to satisfy the queens' nest site requirements, so I shortened the tunnel to 1 m, covered it with a tent of black paper, and connected it to a closer nest chamber (no. 6) without vestibules. From 21 November onwards queen tracks into the nest chamber were discovered almost daily. But no bees were "caught in the act" until 28 November. On that day a marked B. ruderatus queen was seen walking about in the nest chamber, but she left and was not sighted again. She was "37-yellow", and had hatched in colony 5 on 31 January 1976. Thus she had retained her label for eleven months. On 5 December an unmarked B. ruderatus queen started nesting there. She had made her egg clump by 9 December, but then she disappeared and was not seen again.

Meantime (24 November) another unmarked B. ruderatus queen started a nest in chamber no. 5, from which the tunnel had been disconnected. Her presence was somewhat puzzling because her entrance hole was not one through which any queens had departed the previous summer. It was just an aperture in the wall of the outer vestibule. Whatever her origin, she did successfully start a colony, and the very early stages are described below.

Colony founding by a B. ruderatus queen

The queen voluntarily nesting in one of my observation provided an unusual opportunity for me to observe the the incipient stages of a B. ruderatus colony.

She was first seen on 24 November. There was a tunnel about 6 cm long leading to the centre of the underfelt in the observation hive. I could hear rustling noises in the underfelt as if the queen was shuffling about in a confined space. I guessed that she was forming her nest cavity, and refrained from investigating further for fear of causing her to abandon the site.

At 11 am the following day the queen was out foraging, so I examined her nest cavity. There was a pyramidal lump of dark brown material (about 2mm wide and 4mm high) in the centre of the cavity floor. Later in the day the lump was larger and had about four vertical ridges which converged at the apex. The ridges appeared to be made of wax on a central pyramid of pollen. On one side they were curved, forming the rim of a shallow, oval cup, which looked like a narrow egg cell. When the queen came home again I watched her through an opening in the roof of the nest cavity. Her body almost filled the cavity, so the pollen lump was out of sight beneath her. She very actively rotated around the lump with her "back" arched and her head down. She appeared to be shaping the lump with her mandibles. At one stage she stopped moving, and pointed her abdomen down towards the place where I had seen wax cup. After a few abdominal contractions she turned round and continued manipulating movements. I think she laid an egg. It then became apparent to me why the eggs in incipient clumps are orientated more or less vertically, in contrast to the horizontally laid eggs of all post incipient batches. This feature has often received comment (Alford, 1975) but no explanation seems to have been offered. When my B. ruderatus queen appeared to deposit an egg she stood "on tiptoes" with her thorax held high and her abdomen and head pointing downwards. In such a posture her abdomen would pass a vertically orientated egg. She would have been forced to adopt such a posture because of the smallness of the cavity. There was simply not enough room for the queen to lie out flat and be able to reach the egg cell with the tip of her abdoman. (Compare with plate 5, which shows a post-incipient egg batch being laid.) The construction of such a cramped cavity presumably aids heat retention. On the subject of heat, I noticed that whenever the queen returned from foraging she immediately straddled the brood clump and sat on it for several minutes, before unloading her nectar or pollen. This was probably to warm the brood, because it would have cooled during her absence.

Daily photographs were taken of the nest from 26 November until cocoon formation twelve days after the nest's inception. Some of the photographs are shown in plate 15.

Two rejected larvae were found amongst the nesting material on 3 December, following two days of wet and windy weather. Hobbs (1967) and Alford (1975) have stated that brood mortality is unusual among the incipient batch, and that adverse weather results in smaller rather than fewer bees being reared during the colony-founding period.

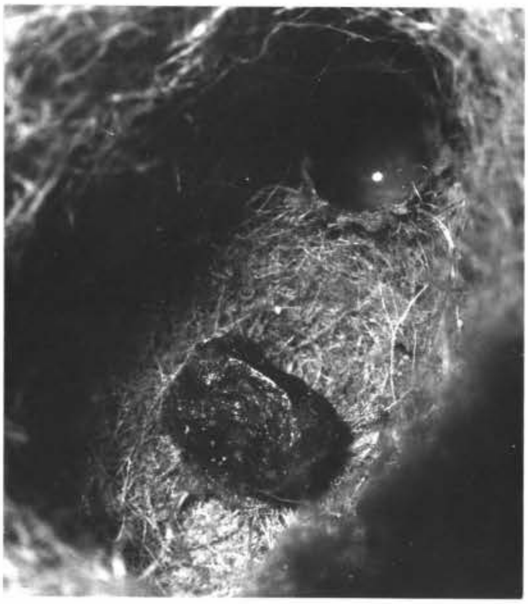
Plate 15 Incipient brood clump of *B. ruderatus* queen

A
Four days old:
completed egg clump

B
Six days old:
young larvae

C
Eight days old:
medium larvae

D
Ten days old:
almost-mature larvae.
The asymmetrical shape
is a result of some
larvae having been
rejected.



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