SOME STUDIES ON THE BIOLOGY OF BUMBLEBEES
(HYMENOPTERA: BOMBIDAE)

DAVID VICTOR ALFORD B.Sc. (London)

Thesis submitted for the degree of
Doctor of Philosophy
in the Faculty of Science in the
University of London
1967

ABSTRACT

The ecological conditions under which bumblebees hibernate have been investigated and differences noted in the type of site chosen for overwintering by certain species. Queens were most often found 8 cm. below the surface, but the depths at which hibernacula are constructed vary according to soil conditions. Spring soil temperatures in hibernacular positions typical of early-emerging species such as _Bombus lucorum_ (Linn.) show higher daily maxima than temperatures in positions typical of later-emerging species such as _B. lapidarius_ (Linn.).

The characteristics of the adult fat body of bumblebees are described, with particular reference to queens. Both fat and glycogen, but not protein, are stored in the trophocytes. In queens about to enter hibernation, fat forms 34% of their total dry weight. The utilization of both fat body reserves and honey stomach contents during hibernation, particularly in the autumn, has been demonstrated.

Investigations of natural incipient colonies of bumblebees have shown that 1st brood adults produced may be divided into two groups according to size. Typically, the larger individuals develop in the centre of the brood clumps and are the first adults to emerge. The number of eggs laid in incipient colonies varies according to the species. In _B. agrorum_ (Fabr.), for example, 8 eggs and in _B. hortorum_ (Linn.) 12 to 14 eggs are

generally laid.

The immature stages of Syntretus splendidus (Marshall), a gregarious endoparasite of adult bumblebees, are described and an account given of its life history. Five larval instars have been recognized. In the late spring and early summer of 1967, the incidence of parasitism among foraging bumblebee queens exceeded 17 %, and in foraging workers 14 %. The brood rearing ability of parasitized queens is impared and parasitic castration occurs. The host dies shortly after the emergence of the full-grown parasite larvae from its body.

CONTENTS

ACKNOWLEDGEMENTS

This work was carried out during the tenure of a Scientific Research Council Studentship. The receipt of a Royal Holloway College Postgraduate Studentship is also acknowledged. It is a pleasure to thank Dr. A.J. Pontin for his interest and advice throughout this study. I would also like to thank Professor P.M. Butler for providing facilities at the Department of Zoology, Royal Holloway College. Thanks are also due to other members of the Department, particularly Dr. E.J. Binyon and Dr. J.G. Thomas, for useful discussion and advice. My thanks also go to Mr. G.E.J. Nixon for identifying the adult Braconids reared in this study. Finally, I wish to express my gratitude to my wife for her encouragement, and invaluable help with the German literature.

INTRODUCTION

The foundations of the knowledge of British bumblebee biology were laid at the turn of the present century by Sladen, and his notable monograph "The Humble-bee", published in 1912, forms a basis for much of the biological work on bumblebees conducted since that time. Sladen's (1896) division of Bombus species into either "pouch-makers" (later changed to "pocket-makers") or "pollen-storers", based upon their method of storing pollen within the colony, is an important one, since each group has a characteristic method of larval nutrition. In the "pocket-makers" the larvae feed directly on the pollen stores of the colony which are housed in wax pockets adjoining the larval clumps. The larvae may also, at times, be fed by the nurse bees on a regurgitated mixture of pollen and honey. "Pollen-storers" keep their pollen in wax cylinders which are separated from the brood, and the larvae are fed only by regurgitation, although in the initial egg clump of a colony some pollen is provided. This classification of Sladen's is relevant to certain problems outlined in the present work.

Publications on various aspects of bumblebee biology date back for more than two hundred years and references to many of the more important papers are given by Plath (1934) and Free & Butler (1959). Several, more recent, papers are included in the review by Holm (1966). Although considerable advances have been made in studies on bumblebees over the past fifty years, and many of the imaginary concepts of earlier writers have now been discredited, there are many topics which still require critical study.

In recent years attempts at the more efficient utilization of bumblebees as agents of pollination, have increased interest in their domestication. Both the artificial hibernation of bumblebees and the initiation of colonies in the laboratory by captive queens are important aspects of this subject, as is a knowledge of bumblebee diseases and parasites (see Holm, 1966). The natural hibernation of bumblebees and the initial stages of colony development in the wild, however, have not received the attention they deserve and few papers dealing with either of these topics in any detail, exist. Tkalcu (1960) refers to the fragmentary information so far accumulated on bumblebee hibernation and points out that field work by "apidologists" often ends shortly before autumn and does not start again until hibernated queens appear in the following spring. Certainly a study of both hibernation and natural incipient colonies of bumblebees involves much intensive field work in which the development of techniques for locating material is of considerable importance. Since most of the data so far published on these topics are the result of casual observations a more complete study was considered desirable.

In Part I the work on natural hibernation is presented, and that on the incipient colonies of wild queens is reported in Part III. Part II on the adult fat body of bumblebees, has been included as an adjunct to the brief physiological studies made on hibernating queens, and lastly, in Part IV, new developments in the study of a parasite of adult bumblebees are reported.

Unfortunately, in the past many observations on bumblebees have been rendered almost useless as the various species concerned are not stated. No great purpose would be served by quoting a series of such cases, but a notable example is the scarcity of specific examples in the literature to illustrate the number of eggs laid by queens in their first brood batch (see Part III).

The Bombidae include various species or groups of species with widely different habits and physiology. It is essential, therefore, that each species under investigation is correctly identified and the name of the species to which a particular observation or series of observations refer, is quoted. Descriptions and excellent colour plates of all the British bumblebee species are given by Sladen (1912) and keys to the British Bombidae have been published by Richards (1927) and Yarrow (in Free & Butler, 1959). The nomenclature adopted in the present work follows that given by Kloet & Hinkes (1945).

Most of the present studies have been conducted within the grounds of Royal Holloway College, Englefield Green, Surrey, in particular at the Department of Zoology ("Alderhurst") and the adjacent area covered by Appendix Map 'A'. Where observations were carried out elsewhere, mention of this is made in the text. A list of all bumblebee nests obtained in the field during the course of the present work is given in Appendix Table I. Most of these were obtained from within the area covered by Appendix Map 'A'. With the exception of four colonies, all others were obtained from adjacent areas in the Englefield Green - Egham district (see Appendix Table I).

Nine Bombus Latrielle species were recorded from within the main study area. Of these, B. lapidarius (Linn.), B. terrestris (Linn.), B. pratorum (Linn.), B. hortorum (Linn.), and B. agrorum (Fabr.) were more or less common, while B. jonnellus (Kirby), B. ruderarius (Müller) and B. humilis (Illiger) were infrequent. Five species of Psithyrus Lepeletier, P. vestalis (Geoffrey in Fourcroy), P. bohemicus (Seidl), P. barbutellus (Kirby), P. campestris (Panzer) and P. sylvestris (Lepeletier) were also recorded. None, however, was common and only a single specimen of P. bohemicus, a spring queen, was found.

PART I : THE HIBERNATION OF BUMBLEBEES.

1. Introduction and review of the literature.

In the temperate parts of the world such as the British Isles, bumblebee colonies are annual, since only the young queens or gynes are able to survive the winter, while the old queens, workers and males, all die. The young, fertilized queens produced during the summer months enter hibernation and it is these bumblebees which reappear in the following spring and later form colonies of their own. In this country, the period of hibernation may last anything from six to eight, or even nine months, depending on the species and to some extent on the spring temperatures. Hibernation is therefore a vital, and important, yet neglected aspect of bumblebee biology.

To refer to the whole length of this period of inactivity as 'hibernation' is strictly incorrect, since some queens enter their winter quarters at the height of summer. The term 'diapause' is therefore more accurate, but since the period of quiescence is continuous and has evolved as a means of maintaining the species through the winter months, the term 'hibernation' is here retained and used to refer to the whole length of diapause, and not just to that part of it which coincides with the winter.

Some earlier writers on the hibernation of bumblebees (Huber, 1802; Hoffer, 1882-3; Wagner, 1907) considered that the queens overwintered in subterranean cavities at considerable depths from the surface. These early opinions, which are now known to be incorrect, were no doubt based on the assumption that queens would not be able to survive at low temperatures and would therefore need to burrow down deeply into the ground below the frost line, in order to survive the winter. Sladen (1912), considerably modifying his earlier (Sladen, 1900) estimates, states that bumblebees hibernate in the ground at depths of from one to three inches (2.5 to 7.5 cm.). Plath (1927, 1934) also found queens hibernating at these depths, while Bols (1937) found queens as deep as four or five inches (10 to 13 cm.).

According to Sladen (1912) and Bols (1937, 1939) bumblebees normally hibernate in small, spherical cavities which they excavate in the soil. Sladen records the cavity diameter as being about one and one eighth inches (29 mm.), while Bols describes them as being about the size of walnuts, and states that in them the queens lie on their backs with their wings in repose. Each of these authors has reported that well drained banks or slopes with a north or north-west exposure are favourite sites for hibernating bumblebees, and Sladen (loc. cit.) has pointed out that such situations will tend not to be warmed by the winter sunshine and thus the queens will remain undisturbed until the warm days of spring. Both Wagner (1907) and Sladen (1912) have stated that bumblebees hibernate under trees, but do not give precise details. According to the latter author both

Bombus terrestris and B. lucorum show a preference for such sites. Bumblebees have also been recorded hibernating in rotten tree stumps (Frison, 1926; Tkalcu, 1960, 1961), in cavities in stone walls (Skovgaard, cited by Free & Butler, 1959), in the interstices of walls of wooden buildings (Tkalcu, 1960), and under moss, leaves and piles of rubbish (Sladen, 1912; Verrill in Putnam, 1864; and others).

There are a few reports of the finding of many hibernating queens in very close proximity to one another (Plath, 1927, 1934; Frison, 1929; Townsend, 1951). Presumably in all these cases, which refer solely to the New World species B. impatiens Cresson the queens were hibernating about the entrance to their maternal nest.

To date, only a few accounts of the behaviour of bumblebee queens at hibernation sites have been published. The most important contributions are those of Bols (1937, 1939). The area in which Bols carried out his work near Lubbeek in Belgium, is evidently ecologically unusual in that queens for many miles apparently migrate to a particular hibernation site measuring little more than 50 x 10 metres. This is apparently due to the unfavourable ground in other areas, and the result is that many queens occur within this one small area. The unusual circumstances of these observations may account for some of the differences between Bols' results and those of the present writer.

Much of the information on the hibernation of bumblebees so far published, has accumulated from casual observations which have not been followed up by more critical data. In the present studies an attempt has been made to obtain data on various aspects of natural hibernation and where possible differences between certain species or groups of species have been investigated and the significance of those found are discussed.

2. Methods of locating hibernation sites and hibernating queens.

Spring queens of both Psithyrus and Bombus species that are parasitized by the nematode Sphaerularia bombi Dufour, do not succeed in forming colonies but instead return to their old or similar hibernation sites where they eventually die (Bols, 1939; Cumber, 1949a). Such queens often occur in considerable numbers (Bols, loc. cit.) and therefore the localities where greater or lesser concentrations of parasitized bumblebees were found in the late spring or early summer were examined later in the year for hibernating queens, since such areas were almost certainly suitable hibernation sites and likely to be occupied.

Well drained banks, tree-covered slopes and a wide variety of terrestrial undulations were searched in the late summer, autumn and winter, in order to locate hibernating queens. In many instances the actual hibernation burrows of hibernating queens were located, while in other cases queens were obtained by selective digging in banks, under moss, under leaf litter and so on. A few queens were seen actually searching for hibernation quarters and occasionally queens were observed in the act of excavating their hibernacula.

Most observations were carried out within the area covered by Appendix Map 'A', where much of the area consists of deciduous woodland comprised principally of oak and birch trees. Areas of beech and Scots pine also occur. The soil is basically Bagshot sand with occasional outcrops of gravel. Several queens were obtained from beech-covered chalk slopes on the Chiltern Hills at Tring, Hertfordshire, but few observations were made elsewhere.

3. Ecological and behavioural studies.

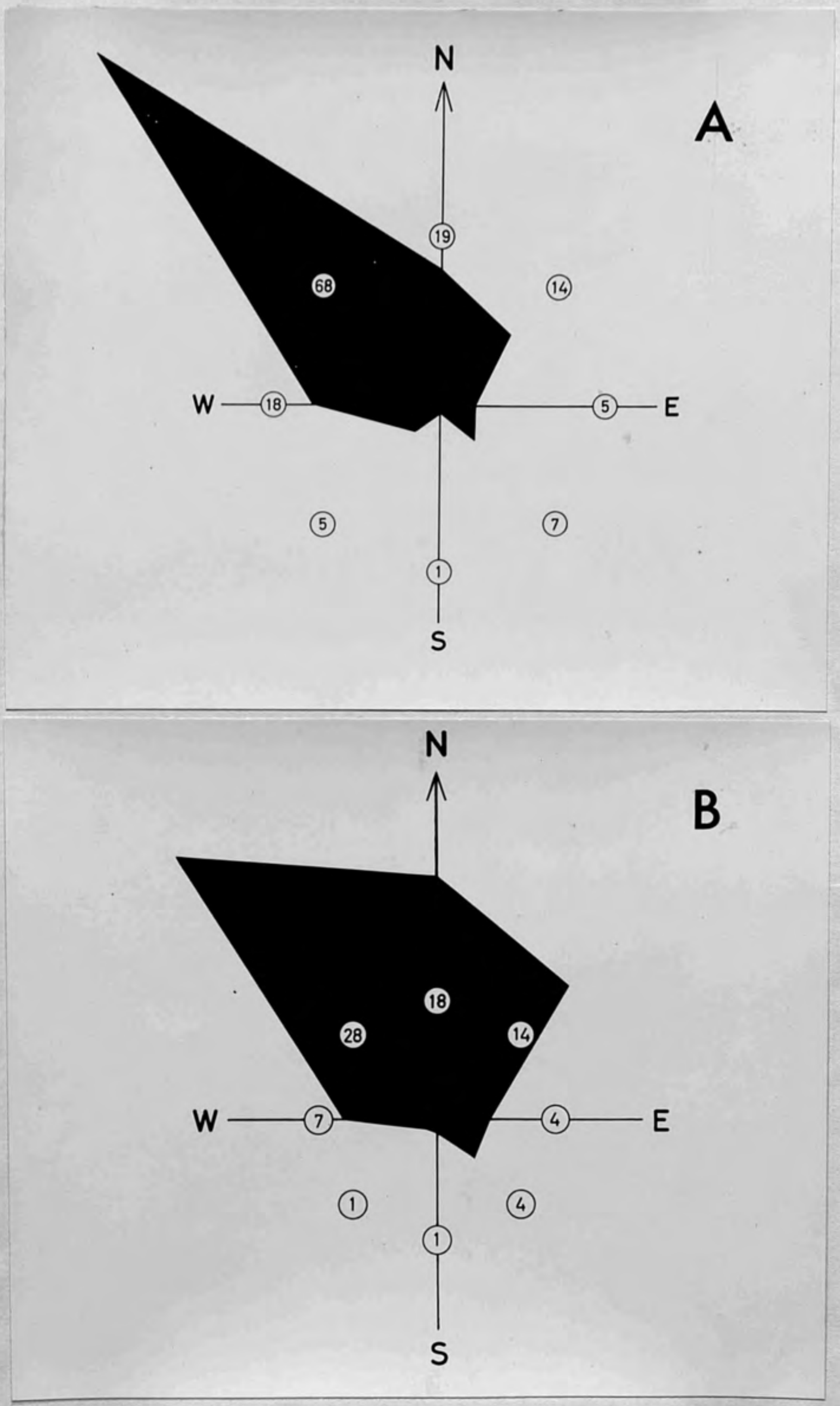
a) The hibernation sites of bumblebees.

The various sites chosen for hibernation quarters by 142 queens representing 10 different species are summarized in Table 1. Most queens were found in ground which was well drained and shaded from direct sunlight. Bees found hibernating in the soil tended to avoid overgrown ground, although a covering of litter or moss was no barrier. The number of individuals of each species found under various ground covers is given in Table 2.

i. Exposure.

The approximate exposure of the banks, slopes and other undulations in which hibernating queens were found was determined in each case. Results for all queens are given in Fig. 1.1(A), while in Fig. 1.1(B), results for queens present within the area covered by Appendix Map 'A' are given.

Fig. 1.1. Exposure of ground in which bumblebees were found hibernating.



A ..... All queens recorded.

B ..... Queens recorded within area of Appendix Map 'A' only.

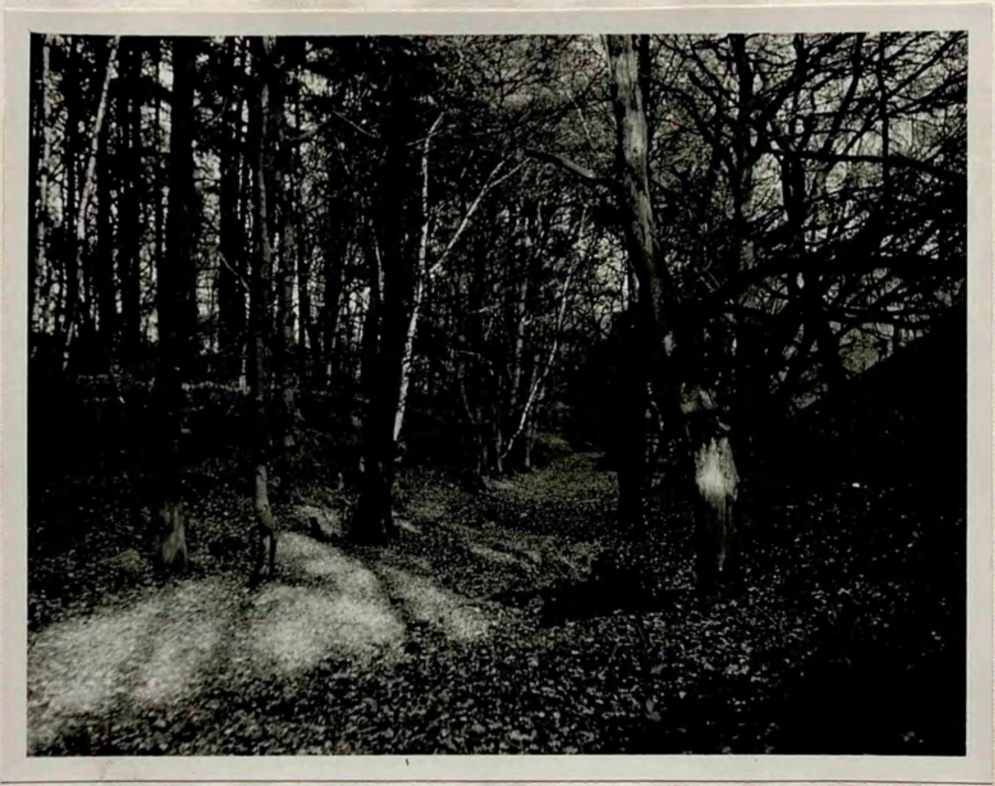
Number of queens found in ground of each exposure given within circles.

Half of all queens found, occurred in ground with a north-west exposure. The remainder were found mainly in sites with a west, north, or north-east exposure. Although some queens were found in ground with a more southerly aspect, such sites were always well shaded. It is probably worthy of mention that queens occurred in southerly facing banks even when suitable, and occupied, north or north-west exposed sites were available nearby, as in Fig. 1.2.

Table 1 — Sites chosen by queens for hibernation quarters.

Species	In banks etc.	Under trees	Under herbage (away from trees)	Others
B. lapidarius	76	2	-	-
B. terrestris	1	5	-	—
B. lucorum	3	13	1	2
B. pratorum	-	2	-	-
B. hortorum	17	5	-	-
B. agrorum	-	-	3	-
P. vestalis	2	-	-	-
P. campestris	3	1	-	-
P. barbutellus	3	-	-	-
P. sylvestris	1	2	-	-

Fig. 1.2. Sites for species hibernating in banks or slopes.



Queens were found hibernating in the mossy bank to the left (north-west side) of the path and in the steep slope on the right. The area is shaded by beech, birch, oak and pine trees.

Table 2 — Number of hibernating queens found below various ground covers.

Species	Condition of ground surface Clear	Moss	Litter or herbage
B. lapidarius	57	19	2
B. terrestris	-	2	4
B. lucorum	2	1	14
B. pratorum	-	-	2
B. hortorum	9	10	3
B. agrorum	-	-	3
P. vestalis	2	-	-
P. campestris	4	-	-
P. barbutellus	3	-	-
P. sylvestris	1	2	-

---

ii. Hibernation in banks, slopes and other terrestrial undulations.

Queens were found hibernating in banks, in the walls of ditches, in small undulations at the sides of paths and tracks, in more general slopes such as those under a mature beech canopy, and occasionally in isolated mounds of soil. In all instances the ground into which the queens had burrowed was shaded, usually by nearby trees. Three typical sites located within the area covered by Appendix Map 'A' are shown in Figs. 1.2, 1.3, and 1.4. Most queens found in terrestrial undulations had burrowed into clear ground or through moss. Very few were found beneath litter or herbage.

B. lapidarius was by far the most common species present in the situations included in this section, and was found in all manner of places from the smallest undulation of only a few centimetres, to banks and slopes many metres in height. There appeared, in this species, to be little or no selection so far as the degree of slope was concerned. Some queens were found in steep banks and others in more gently sloping ground, even when this was inclined at less than 10 degrees from the horizontal.

Several Psithyrus species (P. vestalis, P. campestris, P. barbutellus and P. sylvestris) were found in similar situations to those selected by B. lapidarius, but only relatively few queens of this genus were encountered (Table 1). However, Psithyrus queens were not common at any time of the year in the areas under investigation and surprisingly, in view of the commonness of its host B. lapidarius, no specimens of P. rupestris were seen.

Several queens of B. hortorum were found hibernating in banks, etc., but few specimens of other Bombus species. Most queens of B. hortorum were found in steep banks with or without a covering of moss.

The virtual absence of very common species such as B. terrestris and B. lucorum, and the complete absence of the abundant species B. agrorum from these sites was considered to be of significance. Of all habitats examined, those covered in this section provide the easiest conditions for finding queens because, especially in clear soil, the entrances to hibernacula are often clearly visible (see later).

Fig. 1.3. Further sites for species hibernating in banks or slopes.

B. lapidarius queens were found hibernating in slight undulations adjacent to the path, and *B. hortorum* queens in the steep mossy bank on the right.

Fig. 1.4. Bank at edge of wood in which *B. lapidarius* queens were found hibernating.

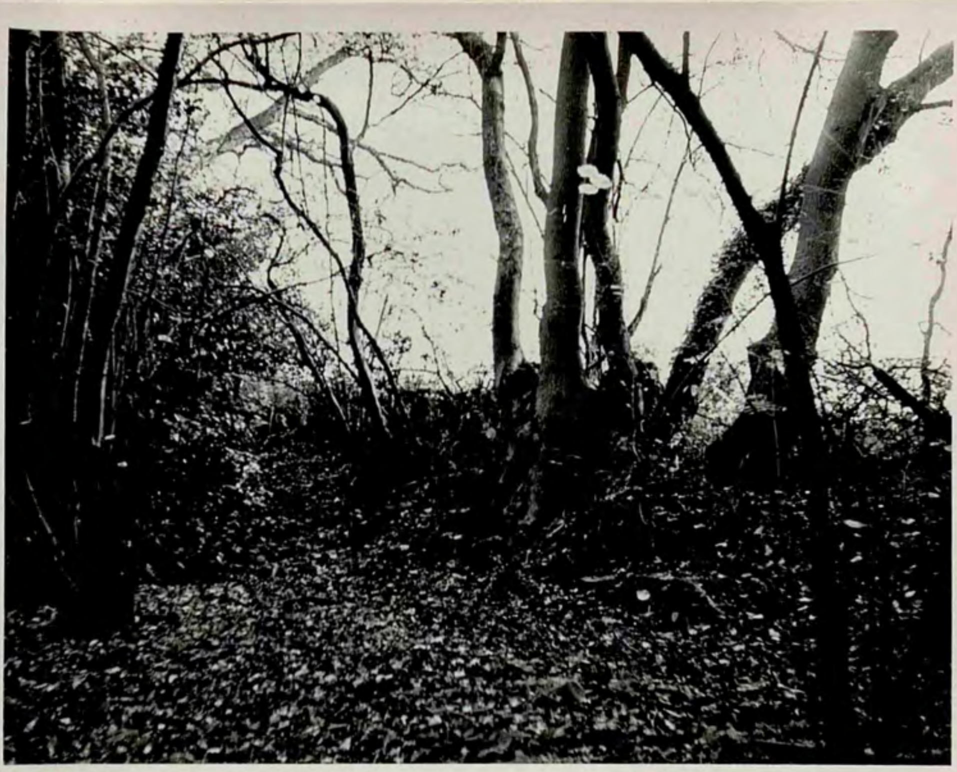


Fig. 1.5. Site at edge of plantation where queens were found hibernating under trees.

iii. Hibernation under trees.

The ground surrounding trees growing in a wide variety of places was searched during the autumn and winter months for hibernating queens. Little or no outward sign of the presence of queens in such places is present since the ground around the trees is usually covered by leaf litter, which makes the location of bumblebees in such places more difficult. In all, a total of 30 queens, belonging to 7 different species were found (Table 1).

Hibernating queens were found close to trees that were growing at the edge of woods or plantations (Fig. 1.5), or adjacent to clearings, or in lightly wooded areas (Fig. 1.6), but always where the ground was sloping and usually where the slope exposure was more or less northwards or westwards. Such sites were always well drained, and there was always reasonable shade provided by the trees. In most cases the ground was covered by a layer of litter and was, at least near to the tree trunks, more or less free of living ground vegetation.

In order to confirm that queens were actually hibernating in relation to the trees, searches were also made in ground identical to that near the trees (at least as far as surface conditions were concerned), at some distance from the actual tree trunks, but under the tree canopy and therefore similarly illuminated. All such searches proved negative. No queens were found under trees that were growing in thickly wooded sites, and none was found under isolated trees in open country.

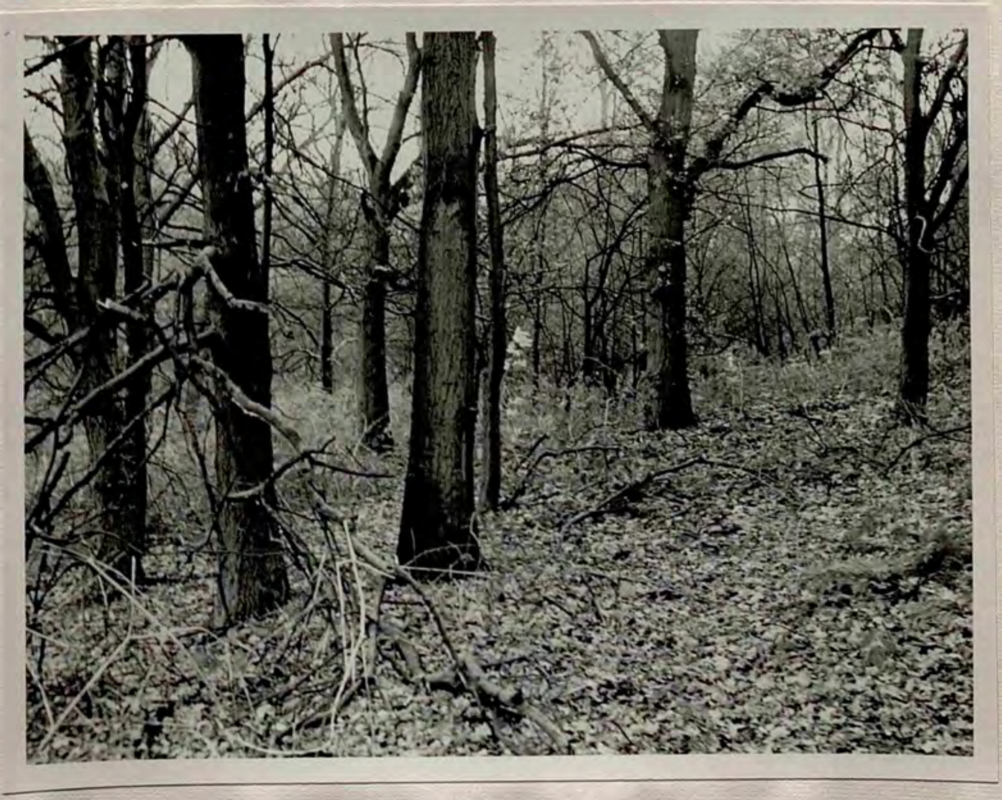
Several queens were found in association with trees with diameters of 40 cm. or more, but very dry soil which often occurs close to the boles of such trees, was never frequented. Smaller trees were also selected by queens. The smallest "occupied" tree had a diameter of only 15 cm.. In one instance 2 queens, one *B. terrestris* and the other *B. lucorum*, were found beneath the same tree.

Individuals of 4 species, *B. lucorum*, *B. pratorum*, *B. hortorum* and *P. campestris*, were found to have constructed their hibernaculum so close to the bole of the tree that on one side, little or no soil separated the chamber from the bark of the tree. More usually, bees were found a short distance from the bases of the trees (mean distance 15.4 cm.). The frequency of individuals at various distances from trees is given in Fig. 1.7.

Queens were found on all sides of tree trunks, being most usually located in the ground on the downward side of the slope. However, some queens were found up the slope from the trees, even when this meant that they were on the south or south-east side of the trunk.

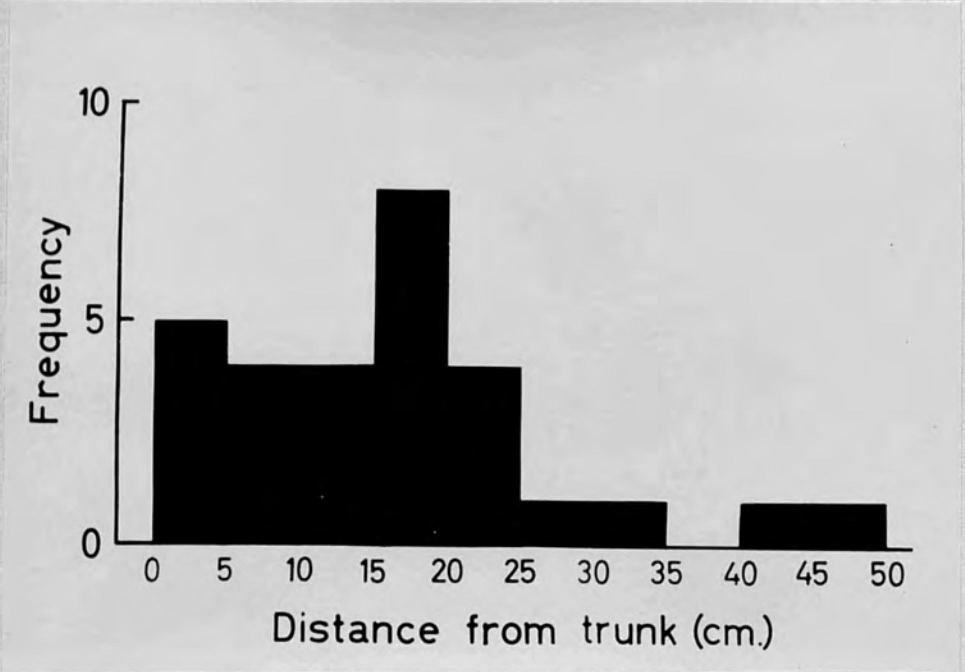
Fig. 1.6. Typical sites for species hibernating under trees.

a



b

Fig. 1.7. Frequency of hibernating queens at various distances from tree trunks.



Distances are measured to the nearest cm. from base of tree trunk to centre of hibernation chamber.

iv. Hibernation under herbage or litter not in relation to trees.

The absence of such a common species as B. agrorum from all of the previously examined sites, led to the searching of other kinds of habitat. It was noticed that in June, many B. agrorum queens were flying low over the ground at the periphery of clearings in woods or at the edges of woodland, in areas where there was a definite open, but generally shaded area of ground covered with grass clumps or low herbage such as bracken and brambles. Dissection of some of the queens revealed that they were, as expected, parasitized by S. bombi. In the late autumn a search of these areas was made by clearing away the ground cover and revealing the underlying soil. Three hibernating queens of B. agrorum were found in this way, two under grass and one in the soil immediately below a layer of bracken litter. In two cases the ground was more or less flat, although well drained.

Quite by chance a queen of B. lucorum was found hibernating under a grass sod in a shaded grass-covered glade of sloping ground in a lightly wooded area.

v. Hibernation within or near to the maternal nest.

Appendix Map 'A' shows the distribution of both nests and hibernating queens found in the main study area from 1965 to 1967. In order to emphasize the spatial relationship of the two distributions, results have not been separated into different years. From the map it is clear that there is a negative correlation between the sites chosen by queens for hibernation and those chosen for nesting.

When a queen-producing colony was located in the field, a search was also made in that area for hibernating queens and similarly in areas where hibernating queens were known to occur, particular attention was paid throughout the spring and summer to searching for any nests that might be present. In only one instance were queens found hibernating near to a nest. This particular case was of three B. lapidarius queens which were found hibernating in a chalk slope on the Chilterns, at the edge of a beech wood. The entrance to a nest of the same species was found within 2 metres of these queens. That the colony was producing queens was confirmed by the finding of a crippled specimen at the nest entrance, but unfortunately no queens which could be marked were seen to fly from the colony and it was not possible to dig up the colony to determine how many queens were being or had been reared. This particular nest was on a slope which was previously known to be a suitable site for hibernating queens and therefore it is doubtful if any significance can be attached to the spatial relationship of nest and hibernating queens in this instance, especially since queens of B. lapidarius and other species were found hibernating elsewhere on the slope, where, as far as was known, there were no colonies.

In October 1964 a torpid gyne of B. pratorum was found amongst the nest material of a colony which had been placed in a nest box several months earlier. Since this colony was not taken by the present writer and only examined after it had died out no details of its past history were known, except that to judge by the old comb, several young queens had been reared. Probably the queen in question had been prevented from leaving the colony and had settled down in the nest debris as best it could.

vi. Miscellaneous sites.

A wide range of miscellaneous sites in the field was examined for hibernating bumblebees but without success. However, by chance, two queens of B. lucorum were found hibernating in the folds of a curtain in an unheated, north-facing 1st floor bedroom. A queen of this species was recorded in 1965, hibernating in the soil under a stone (Pontin, personal communication).

vii. Analysis of site selection data summarized in Table 1.

The accuracy of the null hypothesis that the relative number of queens of two given species in one kind of hibernation site does not differ from the relative number of queens of the same two species in another kind of site is tested in each case, using 2 x 2 contingency tables.

It is clearly shown (Table 3) that the null hypothesis is disputed in each analysis. It may therefore be assumed that bumblebee species are selective in the type of site chosen for overwintering.

Table 3 — Examination of data summarized in Table 1 to determine whether or not site selection by queens occurs.

Compared species	No. of individuals in each compared site		χ^2	Probability that null hypothesis is correct
	In banks	Under trees		
B. lucorum	3	13	10.486	$p < 0.01$
B. hortorum	17	5		
B. lapidarius	76	2	55.567	$p < 0.001$
B. lucorum	3	13		
B. lapidarius	76	2	37.594	$p < 0.001$
B. terrestris	1	5		
	In banks	Under herbage §		
B. lapidarius	76	0	53.998	$p < 0.001$
B. agrorum	0	3		
	Under trees	Under herbage §		
B. lucorum	13	1	7.241	$p < 0.01$
B. agrorum	0	3		

χ^2 with one degree of freedom.

§ away from trees.

---

b) The hibernation quarters of bumblebees.

i. Outward signs of the presence of queens.

Normally queens hibernating under litter, as previously mentioned, leave no outward sign of their presence, although in the case of the single B. lapidarius queen found under litter, some particles of soil were present on the surface. Queens hibernating directly in the soil, on the other hand, leave very clear signs on the surface which until washed away by the rain, enable queens to be located. Typically the signs take the form of a pile of excavated soil displaced to a greater or lesser extent down the slope of the ground, surmounted by the entrance to the burrow which leads down to the hibernation chamber. The entrance holes of occupied burrows (Fig. 1.8) are usually less obvious than those of deserted burrows (Figs. 1.9 and 1.10), since in the latter, little or no soil remains in the burrow, because it is forced out as the queen backs out of the ground. The hole tends, therefore, to be more obvious and the pile of soil often larger. Where a layer of moss covers the surface of the ground, freshly excavated soil may be particularly obvious, as in Fig. 1.10.

ii. Hibernacula.

Although some of the hibernation chambers discovered were spherical in form, others were distinctly oval. The size of each chamber naturally depends upon that of the queen. Those of B. lapidarius had maximum diameters of about 26 to 30 mm., and those of B. hortorum a maximum of about 25 mm.. Bumblebees never hibernate in waterlogged soil, and excessively dry soil is also avoided, possibly because of the dangers of desiccation and also as it is unlikely that a suitable hibernation chamber could be hollowed out if the soil is too dry and loose. An excavation revealing the hibernation chamber of a B. lapidarius queen, made in the soil of a moss-covered slope, is shown in Fig. 1.11, and in Fig. 1.12, that of a B. hortorum queen, in the soil close to the base of a tree, is illustrated.

Fig. 1.8. Entrance holes to hibernacula of *B. lapidarius*.

(x 1)

(x 0.5)

Fig. 1.9. Entrance hole of deserted burrow dug in chalky soil.

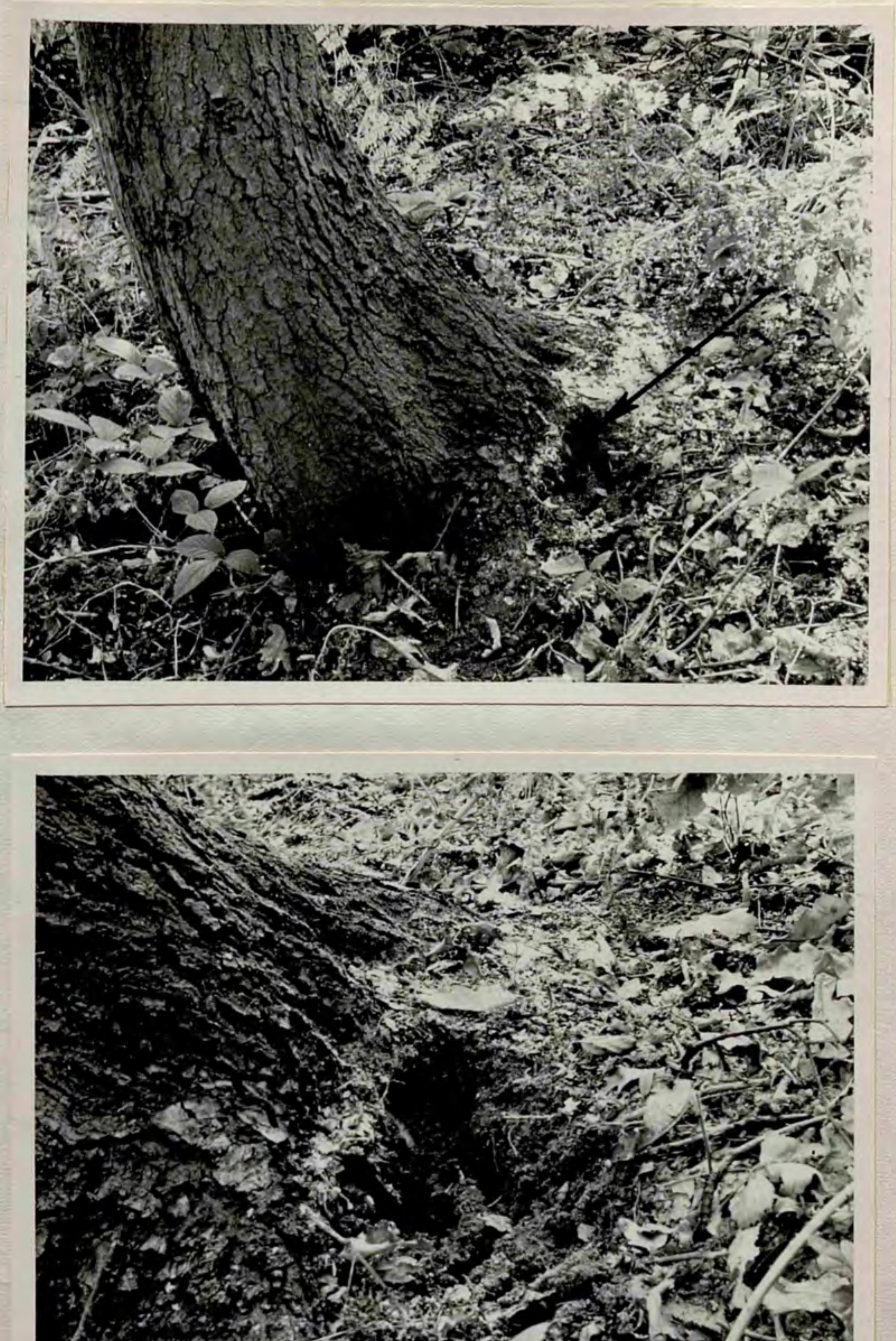
(x 0.5)

Fig. 1.10. Entrance hole of deserted burrow dug in a sandy, moss-covered bank.

(x 0.25)

Fig. 1.11. Excavation revealing hibernation chamber of *B. lapidarius* in moss-covered slope at depth of 7 cm..

Fig. 1.12. Excavation revealing hibernation chamber of B. hortorum in soil beneath shallow litter layer close to tree.



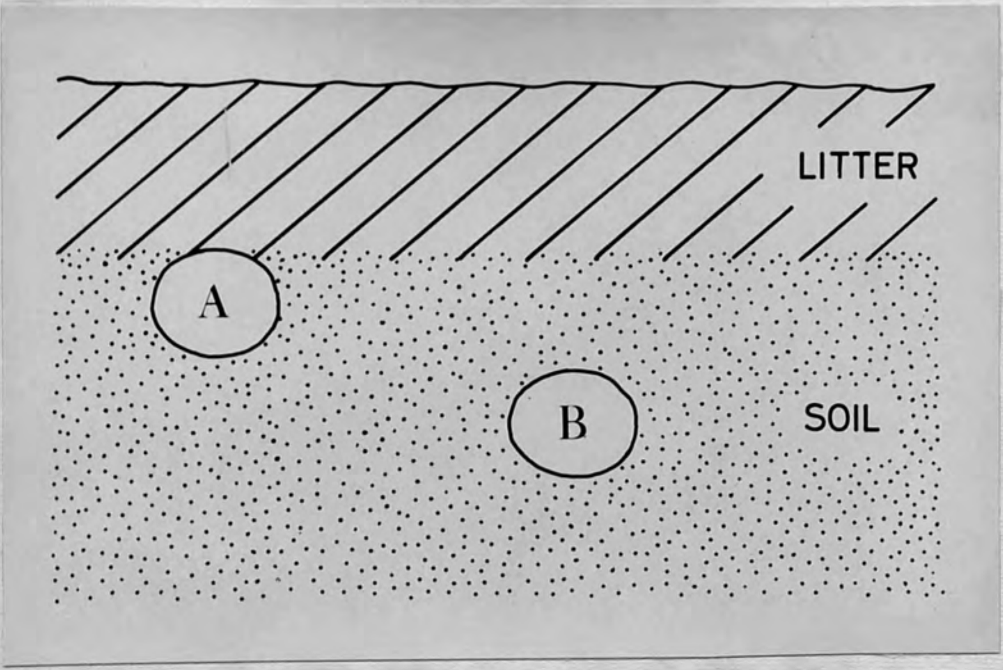
Where bumblebees hibernate in banks and directly in the soil in other situations, the hibernation chamber is connected to the surface by a straight burrow which, during the presence of the queen, is blocked by soil. This soil was pushed up from below during the hollowing out of the inner chamber. Each burrow lies more or less at right angles to the surface, but may be inclined somewhat horizontally. The chamber was usually found at the extreme inner end of the burrow, but sometimes it was at the end but to one side. Burrows of *B. lapidarius* were about 11 mm. in diameter.

Bumblebees hibernating beneath a layer of litter fell into two distinct groups in so far as the position of the chamber was concerned. Some species (*B. terrestris*, *B. lucorum*, *B. pratorum* and *B. agrorum*) constructed their chambers immediately below the soil-litter interface (Fig. 1.13), whereas others (*B. lapidarius* and *B. hortorum*) always burrowed down further into the soil before hollowing out their chambers. The latter two species would consequently hibernate at a greater depth than the others mentioned if in the same site. The positioning of both types of hibernation chamber is shown diagrammatically in Fig. 1.14.

Fig. 1.13. *B. lucorum* in hibernaculum immediately below soil-litter interface close to tree.

The litter layer has been removed to display the queen which is lying on its back in the hibernation chamber. The trunk of the tree is visible at the top left hand corner of the picture.

Fig. 1.14. Position of hibernation chambers of certain species in relation to soil-litter interface.



A - Chamber constructed in soil immediately beneath the soil-litter interface (B. terrestris, B. lucorum, B. pratorum, B. agrorum).

B - Chamber constructed deeper in the soil (B. lapidarius, B. hortorum).

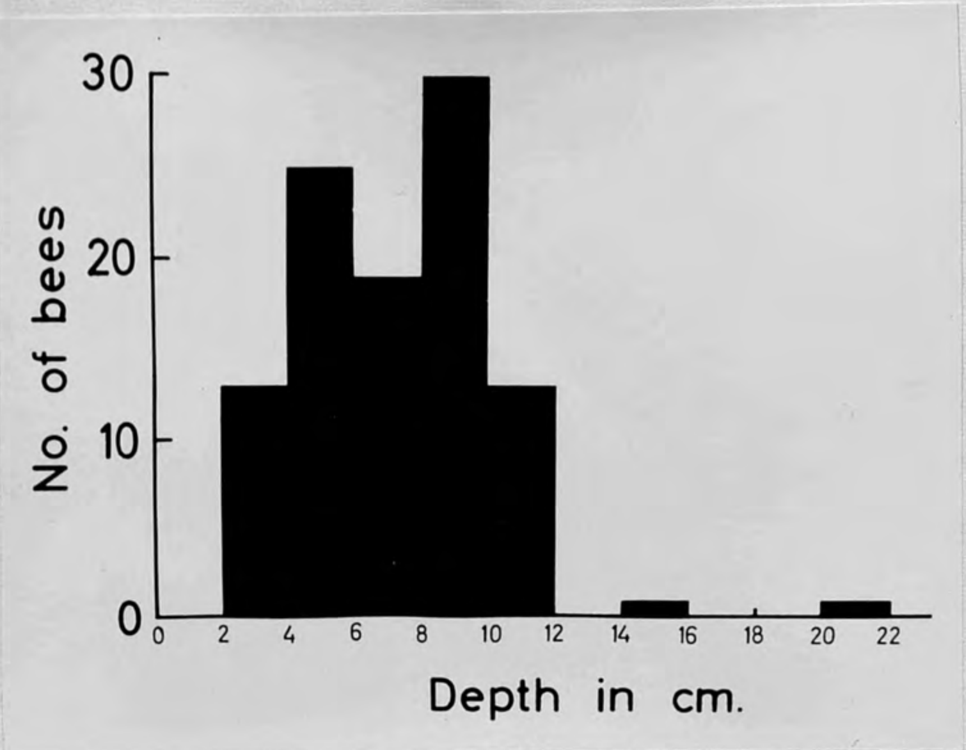
---

iii. The depths at which queens hibernate.

The depths at which individuals of various species were found are given in Appendix Table II, and results are summarized as a frequency distribution for all species in Fig. 1.15. Measurements were made to the nearest centimetre from the surface of the ground to the centre of the hibernation chamber along a line at right angles to the ground surface. Some queens were found as shallowly as 2 cm. but only one queen was found deeper than 15 cm.. The latter queen (B. lapidarius) had burrowed through 8 cm. of pine needle litter to a depth of 20 cm.. Queens were most frequently found at a depth of 8 cm.. The depths at which many queens were hibernating could not be determined exactly, either due to the bees being brought to the surface whilst being dug up and the hibernaculum destroyed, or due to collapse of the soil containing the queen and hibernaculum, such as when digging in banks. However, it is possible to state that none of these queens was hibernating at a depth greater than 11 cm..

Although from Table 4 it would appear that some species may hibernate at greater depths than others in similar situations, the small sizes of samples, B. lapidarius being an exception, coupled with the wide range in the depths at which individuals of each species were found, prevents any significance being shown between different species or groups of species.

Fig. 1.15. Depths at which queens were found hibernating.



Distances were measured from the surface of the ground to the centre of the hibernation chamber along a line at right-angles to the ground surface.

Table 4 — Mean depths (cm.) of queens hibernating beneath various ground covers and in different soils.

Species	CLEAR SOIL			MOSS COVER			Under	All bees
	Chalk	Intermediate^x	Sand	Chalk	Intermediate^x	Sand	Litter	
B. lapidarius	5.6(18)	7.8(9)	9.5(2)	3.8(4)	7.6(10)	8.2(5)	14.0(2)	7.0 ± 2.8
B. terrestris	-	-	-	2.0(1)	8.0(1)	-	4.8(4)	4.8 ± 2.0
B. lucorum	-	10.0(1)	5.0(1)	-	4.0(1)	-	6.5(13)	6.5 ± 2.3
B. pratorum	-	-	-	-	-	-	4.5(2)	4.5 ± 1.5
B. hortorum	3.5(2)	7.1(7)	-	2.0(1)	7.2(6)	10.0(1)	7.0(1)	6.2 ± 2.7
B. agrorum	-	-	-	-	-	-	8.0(1)	8.0
P. vestalis	-	-	7.0(1)	-	-	-	-	7.0
P. campestris	4.0(2)	4.0(1)	15.0(1)	-	-	-	-	6.8 ± 5.0
P. barbutellus	-	-	-	-	8.0(1)	-	-	8.0
P. sylvestris	10.0(1)	-	-	4.0(1)	11.0(1)	-	-	8.3 ± 3.1

x :- Soil heavier than sand but lighter than chalk.

(The number of observations contributing to each mean is given in parenthesis).

Some of the figures given in Table 4 are not strictly comparable since the depth of a queen hibernating under litter, and possibly other ground covers such as moss, may be affected by the thickness of such a layer. No difference was found, however, between the depths of queens burrowing through moss or into clear soil for either the B. lapidarius or B. hortorum samples.

One factor with an effect upon the depth at which queens hibernate is the texture of the soil into which they must burrow. Queens of B. lapidarius were found at a mean depth of 5 cm. in chalky soil (22 queens sampled from Tring, Herts.), and at a depth of 8 cm. in more or less sandy soil (26 queens sampled from Englefield Green, Surrey). The difference between the depths of these samples was significant ($p < 0.001$). Data for other species (Table 4) also suggest that queens will burrow deeper in a light soil than in a heavier one.

c) Observations on the behaviour of queens at hibernation sites.

i. Before hibernation.

Queens of B. lapidarius searching for hibernation quarters were observed from the time that they arrived at hibernation sites. Once in a suitable area, queens flew over the ground at a height of a few centimetres, tacking from side to side, settling at intervals and then crawling over, and occasionally scratching at, the surface with their forelegs. On finding a suitable place to dig, a queen would start to burrow into the soil. All queens observed, were found digging into slopes of about 45 degrees, and they began to burrow while facing up the slope of the particular site selected. They continued to face in this direction during the time that they were observed to dig, and the result of this behaviour, coupled with the effect of gravity, was that the pile of soil thrown up occurred only on the downward side of the slope (see Figs. 1.8, 1.9 and 1.10). In his description of the burrowing of a P. vestalis queen, Bols (1937) states that the bee frequently turned round whilst burrowing so that soil was thrown out on all sides of the burrow. It is possible that this method may be adopted where the ground is less sloping but all excavated burrows of both Bombus and Psithyrus examined by the present writer on a variety of degrees of slope, had piles of soil only on the downward side from the burrow. Certainly it would not be of much advantage for a queen to attempt to force soil up a slope since it would tend to drop back into the hole and impede digging. Probably the spot chosen by the queen observed by Bols was not particularly steep.

Queens were so engrossed in their digging that they could be covered with a glass tube or pill box without being disturbed. A queen of B. lapidarius burrowing into semi-chalky soil took 32 minutes from commencing to dig until disappearing from sight. This time agrees with the estimates of both Wagner (1907) and Bols (1937), but no doubt the speed at which a queen will burrow depends upon the hardness of the ground.

Queens use their mandibles and legs whilst digging, and occasionally back out of the burrow to force loosened soil out of the way (Wagner, 1907). This soil forms a mound at the entrance to the hibernaculum, while soil from the chamber serves to block up the burrow. Occasionally it was noticed that a queen would back out of the burrow she was excavating and start to dig another one a short distance away. Examination of these deserted burrows always showed that they ran up against a stone or root at a depth of only a few centimetres. No evidence was found to suggest that a queen ever attempts to bypass underground obstructions. In several cases where deserted burrows were found, an occupied hibernaculum was found nearby. One B. lapidarius queen, however, which experienced considerable difficulty in forming a burrow due to shallow chalky soil, gave up after three abortive attempts in one small area of ground, and flew away. Each burrow this queen had started ran up against a flint or solid piece of chalk a short distance from the surface.

ii. During hibernation.

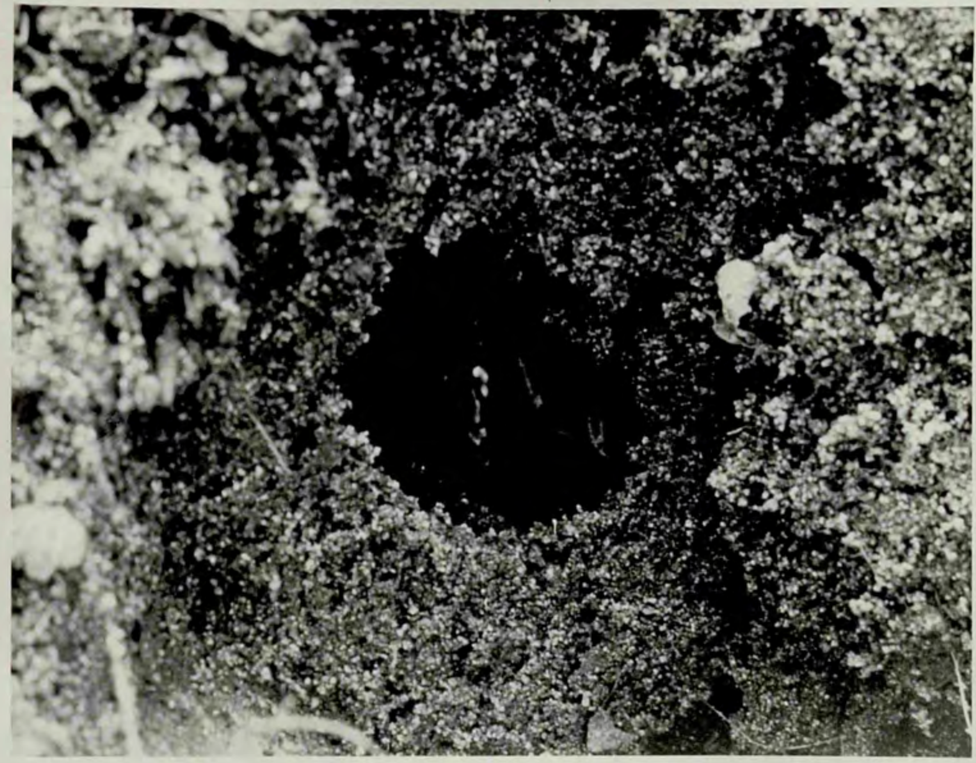
When hibernating queens are disturbed in the late summer or early autumn, they quickly become active, and vibrate their wings, producing an audible, low pitched buzz. Such behaviour was exploited to locate queens when digging in the soil during dull weather or in dimly lit sites, since on many occasions queens could be heard long before they were actually sighted. The vibration of the wing muscles rapidly raises their body temperature and in a short time they are able to fly. When dug up in cold weather later in the year, queens are normally in a torpid state and remain more or less motionless, although they will become active if held in the palm of the hand. This observation has also been made by Plath (1927, 1934).

Although some queens were on their backs when found, as was the B. lucorum queen in Fig. 1.13, others were in a crouched position (Fig. 1.16). Due to the tendency of queens to alter their position whilst being dug up, especially during the early stages of hibernation, it was often difficult to say whether or not a queen was in its original position when first seen. However, observations of 6 torpid queens in mid-winter showed them to be in a crouched position within the hibernation chamber. Most queens confined in tubes or boxes during periods of hibernation under artificial conditions, remained crouched on the floor of their container. Occasionally queens were found on the vertical walls with their heads upper most. Only rarely were individuals found on their backs.

iii. After hibernation.

Known hibernation sites were visited frequently at various times during the spring, but no queens were observed to emerge from their winter quarters. Following their emergence, however, queens were commonly found foraging on sallow catkins and various other flowers. The principal sites where spring queens were found foraging in the vicinity

Fig. 1.16. *B. hortorum* queen inside chamber of hibernaculum (x 3).



of the Zoology Department of Royal Holloway College, are indicated in Appendix Map 'A'.

Cumber (1953) states that in cool weather, before colonies have been founded, spring queens often crawl beneath vegetation in the vicinity of their food plants and reappear when conditions become more favourable. The present writer observed several queens creeping into matted vegetation towards evening in places which were not considered to be suitable for hibernation quarters, presumably to spend the night. It should be emphasized, however, that hibernation sites were never found to be used as night refuges for bumblebees and no tendency was found for healthy spring queens to assemble in such areas.

Queens parasitized by the nematode S. bombi, however, were found to inhabit known hibernation sites both in the spring and early summer. Some of these queens were seen to attempt to burrow directly into the ground while others flew slowly over the surface and occasionally forced their way under leaf litter and other dead vegetation. Such queens in the earlier part of the spring were reasonably active and easily disturbed, but those present at a later date were more laboured in their movements and less easily distracted. These parasitized queens were excellent indicators of suitable hibernation sites and as mentioned earlier, this fact was exploited when searching for hibernating queens later in the year.

In the Englefield Green district, *Sphaerularia* infected queens of all bumblebee species known to occur in the area, with the exception of *B. jonellus* and *P. bohemicus*. In March and April 1965, 6.8% of foraging spring queens and for the same period in 1966, 10.9% of foraging spring queens were infected. The most affected species was *B. lucorum*.

Observations on the various species of parasitized queens present at the different hibernation sites, revealed that there was a distinct preference shown for certain types of site by particular species. The following species were recorded flying over and along banks, including those illustrated in Figs. 1.2 and 1.3 :- *B. lapidarius*, *B. hortorum*, *P. campestris* and *P. barbutellus*. Queens of *B. terrestris* and *B. lucorum*, and one queen of *B. lapidarius* were found flying over tree-covered slopes including those shown in Fig. 1.6. In addition, several *B. agrorum* queens and a single queen of *B. ruderarius* were found flying over more open ground with a rough grass or low bracken cover, but within the shadow of adjacent trees. One queen of *B. humilis* which forced its way under a grass tussock on an open east facing slope was found to be a parasitized individual. Reference to the recorded distribution of hibernating queens in different kinds of site (Table 1), will show that the specific differences between the distribution of parasitized queens, are consistant with that for hibernating queens. This is further evidence to show that hibernation site selectivity by different bumblebee species does exist.

4. Some physiological studies on hibernating queens.

a) Changes in weight and water content.

The relative amount of water in gynes, prior to their entry into hibernation, shows considerable variation, but generally forms between 60 and 70% of their total live weight. Water forms about 57% of the live weight of queens at the commencement of hibernation, and this figure remains relatively constant throughout the whole period of hibernation, during which time there is a reduction of approximately one half in both the live and dry weights of queens (Fig. 1.17). Immediately after hibernation, however, there is a marked increase in the actual and relative amounts of water in the body, following post-emergence feeding (Tables 5 and 6).

b) Utilization of fat reserves.

Considerable reserves of fat are present in queens at the commencement of hibernation (Table 5). They form from 11 to 16% of the total live weight (mean, 13%). Most of this fat is utilized during the first half of the hibernation period (Fig. 1.17) and queens sampled shortly after their emergence from hibernation generally contain relatively little fat in their fat bodies (see Part II).

c) Utilization of honey stomach contents.

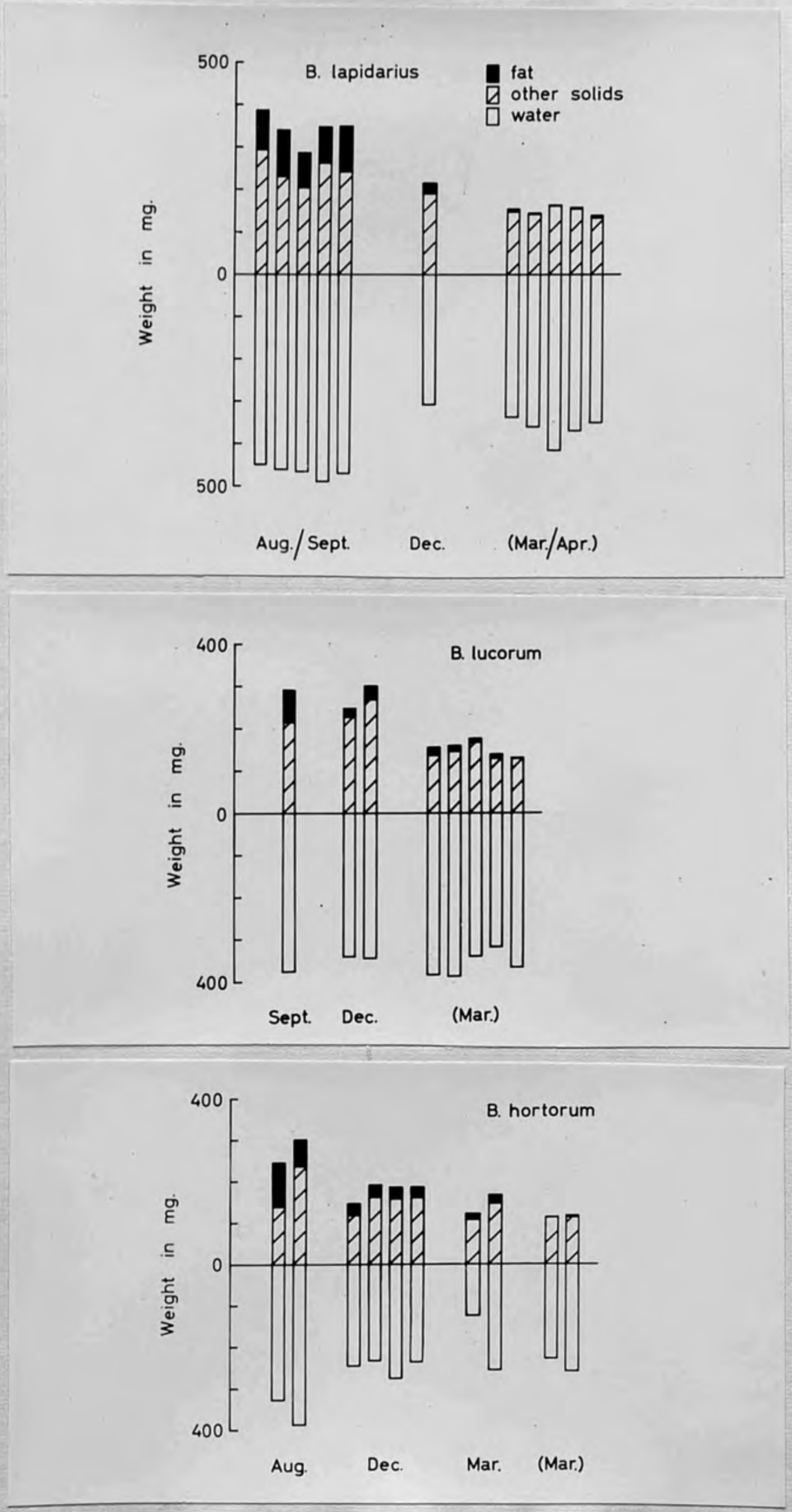
At the commencement of hibernation the weight of the honey in the crop of queens often exceeds 200 mg., and on average represents 23.3% of the live weight of Bombus queens. The mean for Psithyrus is lower (12.7%) in view of the relatively higher body weight in this genus attributable to the thicker cuticle. The honey in the crop of hibernating queens contains 20% water. During the autumn, the content of the crop is reduced considerably and apparently, as with fat, more honey is utilized at this time than in the second half of the hibernation period (Fig. 1.18). Although the relative amount of honey in the crop of queens at the commencement of hibernation was reasonably constant, the quantities present in individuals sampled in mid-winter showed considerable variation, ranging from 8 to 127 mg. (Appendix Table III).

Table 5 — Mean water and fat content of queens sampled during or immediately after hibernation.

Species	(n)	Live weight (mg.)	Dry weight (mg.)	Water (mg.)	Fat (mg.)
In hibernation: autumn					
B. lapidarius	(5)	810.4	342.5	467.9	96.7
B. lucorum	(1)	663.8	288.4	375.4	76.1
B. hortorum	(2)	634.5	277.2	257.3	99.4
In hibernation: winter					
B. lapidarius	(1)	524.3	215.5	308.8	28.4
B. lucorum	(2)	619.0	276.0	343.0	30.4
B. hortorum	(4)	426.2	177.0	249.2	31.0
In hibernation: spring					
B. hortorum	(2)	336.4	148.2	188.2	21.9
Immediately after hibernation					
B. lapidarius	(5)	517.6	151.4	366.2	6.1
B. lucorum	(5)	510.0	149.6	360.4	12.5
B. hortorum	(2)	361.2	118.0	243.2	6.4

(n) = number of observations.

Fig. 1.17. Amounts of fat, other solids and water in queens of various species during and (months in parenthesis) shortly after their emergence from hibernation.



Each column represents one individual.

Fig. 1.18. Reduction in honey stomach contents during hibernation.

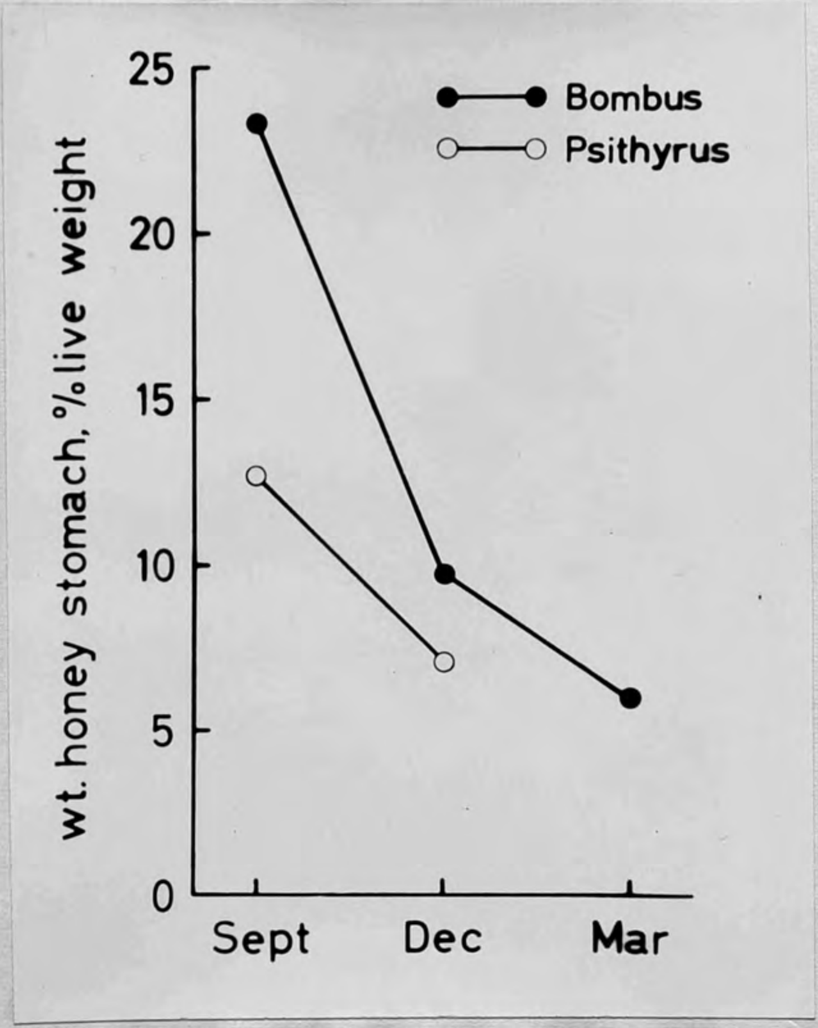


Table 6 — Mean water content of queens expressed as a percentage of their live weight.

Species	In hibernation			Immediately after hibernation
	Autumn	Winter	Spring	
B. lapidarius	58	59	-	71
B. lucorum	57	56	-	71
B. hortorum	56	59	55	67

5. The spring emergence of bumblebees.

a) Introduction.

It is generally agreed that the emergence of bumblebees from hibernation is governed by temperature and it is known that some bumblebee species appear earlier in the spring than others (Hoffer, 1882-3; Sladen, 1912; Plath, 1934; and others). Both Siivonen (1942) and Stein (1956a) have produced data which relate the emergence of bumblebees to spring temperatures. Stein (loc. cit.) also investigated ground temperatures at a depth of 20 cm. and found that maxima were about 5 to 6°C. at the time of the appearance of B. terrestris and 9°C. when B. lapidarius appeared. No details are given of the site chosen for the measurements and why a depth of 20 cm. was chosen is not stated. The assumption usually drawn from observations made on the appearance of hibernated queens in the spring is that the various species require different temperatures to arouse them. However, such a view does not take into account the ecological conditions under which different species hibernate and any effect these may have on the influence of spring temperatures on the queens.

The present ecological studies have shown that the early-emerging species <u>B. terrestris</u>, <u>B. lucorum</u> and <u>B. pratorum</u> typically hibernate immediately below the soil-litter interface close to trees, while later-emerging species such as <u>B. lapidarius</u>, <u>B. hortorum</u> and <u>Psithyrus</u> species, usually hibernate in banks or slopes. In order to investigate the possible effect that these site differences may have on the emergence of spring queens, a study was made of the relationship of spring ambient temperatures and subterranean temperatures in hibernation sites typical of early- or late- emerging species.

b) Methods.

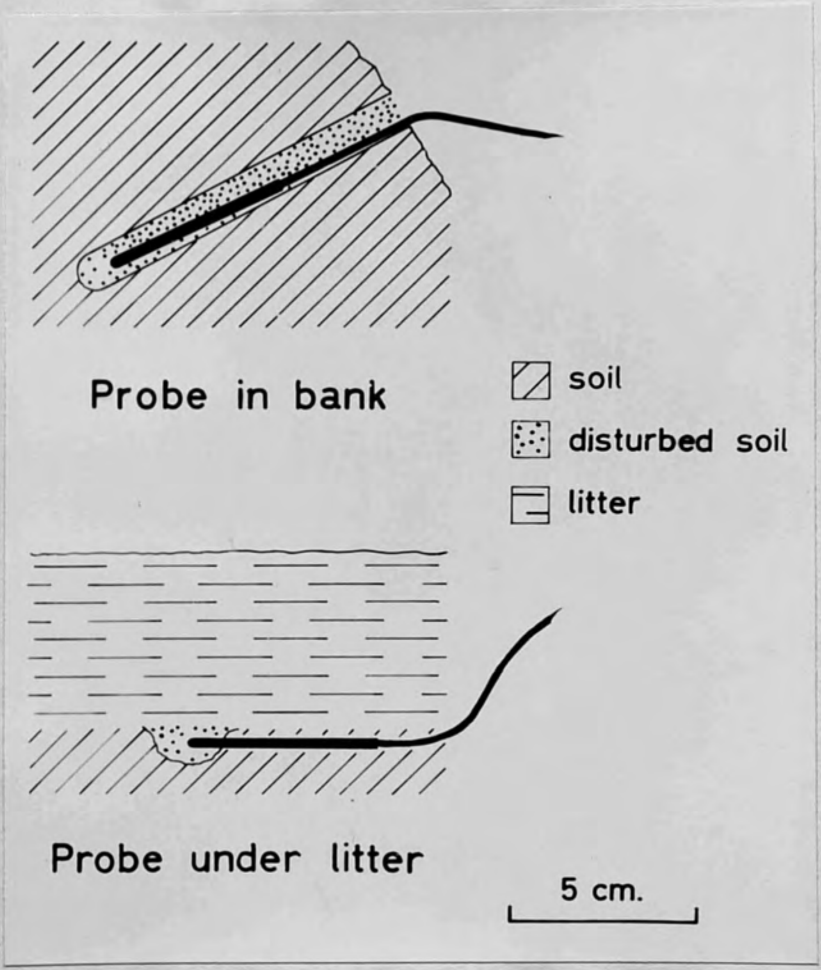
Subterranean temperature measurements at hibernation sites were obtained using matched Grant thermistor thermometer probes (Type 'C'). Each probe consisted of a thermistor bulb enclosed at the tip of a stainless steel tube measuring 51 x 3 mm. and was attached to a lead which led to where readings could conveniently be taken. The reaction speed of each probe was approximately 20 to 30 seconds. Spot readings were made with a Grant thermistor thermometer (Model 'S'), and continuous recordings from two probes were obtained by Grant miniature temperature recorders. Under field conditions the power source supplied with the continuous recorders (mercury cells) proved wholly inadequate, even after modifications, due both to failure at ambient temperatures below +4°C. and to rapid loss of power during continuous operation at higher temperatures. Four Nife cells wired in series were substituted for the mercury cells on each recorder and these operated satisfactorily during continuous running under a wide range of ambient conditions. Recharged Nife cells were fitted every two to three weeks.

In March 1966, provisional data were obtained using two probes and recording temperatures from these with the Model 'S' thermometer. One of these probes was located in a bank and the other under litter near a tree. The position of each probe tip was intended to approximate to the centre of a natural hibernacular chamber. Both probes were located in known hibernation sites.

In January 1967, four probes were set out in the sites indicated in Appendix Map 'A'. Two probes (A and B) were dug into the soil of banks in which B. lapidarius queens were known to hibernate, and two (C and D) were placed in the soil below the soil-litter interface under trees at a site where B. lucorum and other early appearing species had previously been found hibernating. The relative positions of the probes in both kinds of site are shown diagrammatically in Fig. 1.19.

Spring ambient (air) temperatures were obtained at "Alderhurst" using thermometers located inside a Stevenson screen.

Fig. 1.19. Positions of thermistor probes in the two types of situation from which temperature records were obtained.



Probes A and B were sited as shown in upper diagram.
Probes C and D were sited as shown in lower diagram.

c) Results.

The daily maximum and minimum air temperatures for February, March and April, for 1965, 1966, and 1967, are given in Fig. 1.20, and the date from which spring queens were recorded in the study area in each year is superimposed above the relevant temperature graph. In Table 7 details of the emergence dates for individual species are given.

Table 7 — Date of first appearance of spring queens of various species.

Species	1965	1966	1967
B. terrestris	25th March	7th March	7th March
B. lucorum	25th "	8th "	7th "
B. lapidarius	30th "	8th "	22nd "
B. pratorum	26th "	6th "	20th "
B. jonellus	29th "	9th "	14th "
B. hortorum	29th "	9th "	22nd "
B. ruderarius	29th "	10th April	22nd "
B. agrorum	29th "	7th March	20th "
P. sylvestris	31st "	18th "	—

B. lucorum and *B. terrestris* were always the first species recorded, and both became common in advance of other species with the exception of *B. pratorum* and *B. jonellus*.[++] Individuals of other species appeared more spasmodically, *B. agrorum* and *B. hortorum* becoming 'common' slightly

---

[++] Although *B. jonellus* was not common in the main study area, a few miles away on Chobham Common spring queens of this species generally made their appearance at about the same time as queens of *B. pratorum*.

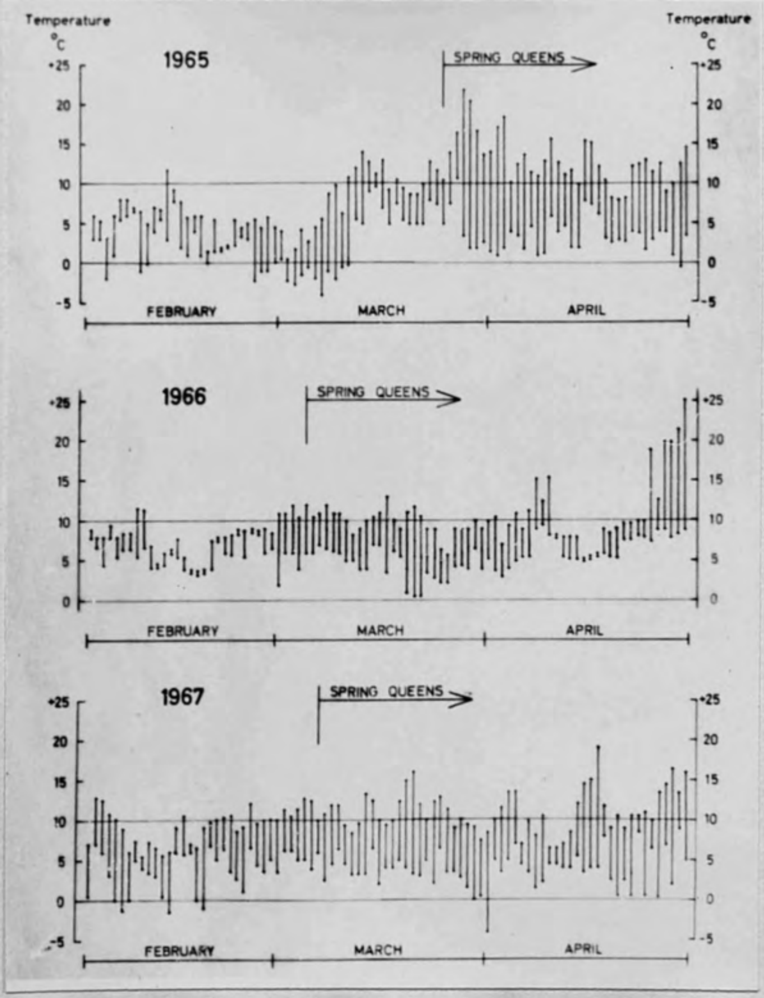
earlier than B. lapidarius. Data for other species, including Psithyrus queens, are unreliable because relatively few individuals were recorded at any time. In all three springs queens of B. lapidarius were well out by the end of April, and in view of this no temperature measurements were taken after this time.

Fig. 1.21 shows the highest recorded daily temperatures obtained from a thermistor probe in a bank and a probe under litter near a tree for the period 8th March to the 18th March, 1966 inclusive. The daily maximum air temperatures are also shown. The probe results are subject to error since without continuous recordings the true daily maxima may not be recorded. However, they do suggest that temperature differences between the two types of site exist.

Spot readings from four probes in 1967 are given in Table 8. Although these readings are subject to the same errors as those taken in 1966, they do suggest that the temperature in banks is lower than that under litter.

The continuous recordings obtained from probes A and C, enable the actual daily maxima to be obtained and these are given in Fig. 1.22, along with the daily maximum air temperatures. Readings from probe C were ended on the 25th March because the litter cover was disturbed during the night, probably by a small mammal. The gap in the trace from probe C at the beginning of February was due to the jamming of the chart winding mechanism and that in the bank trace at the end of March, to battery failure. When readings were ended, it was confirmed that each probe was still in its original position.

Fig. 1.20. Daily air temperature range in experimental area during period of emergence of hibernated queens.



The appearance of bumblebees in each year is superimposed above the relevant temperature graph.

Fig. 1.21. Maximum recorded temperatures for experiment in 1966.

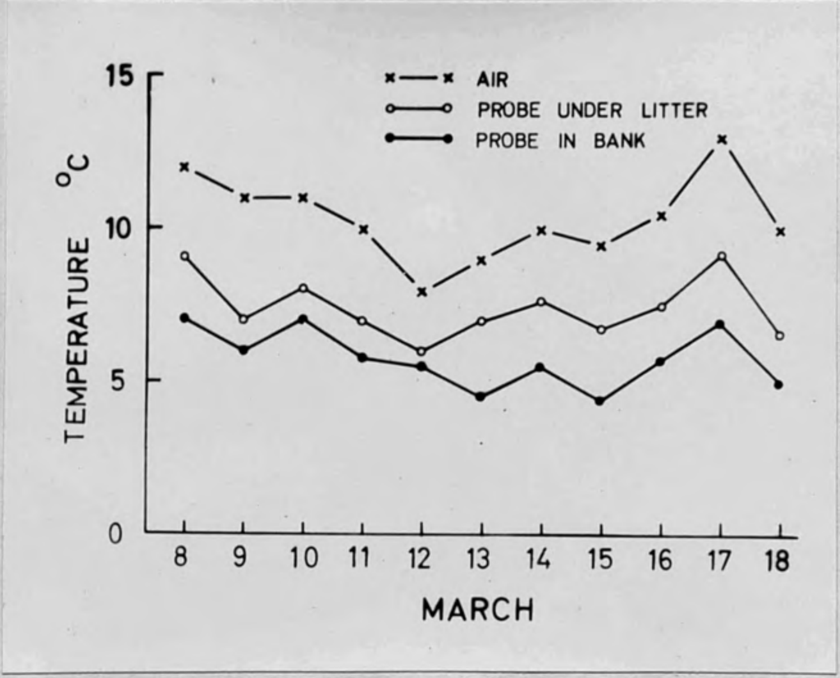
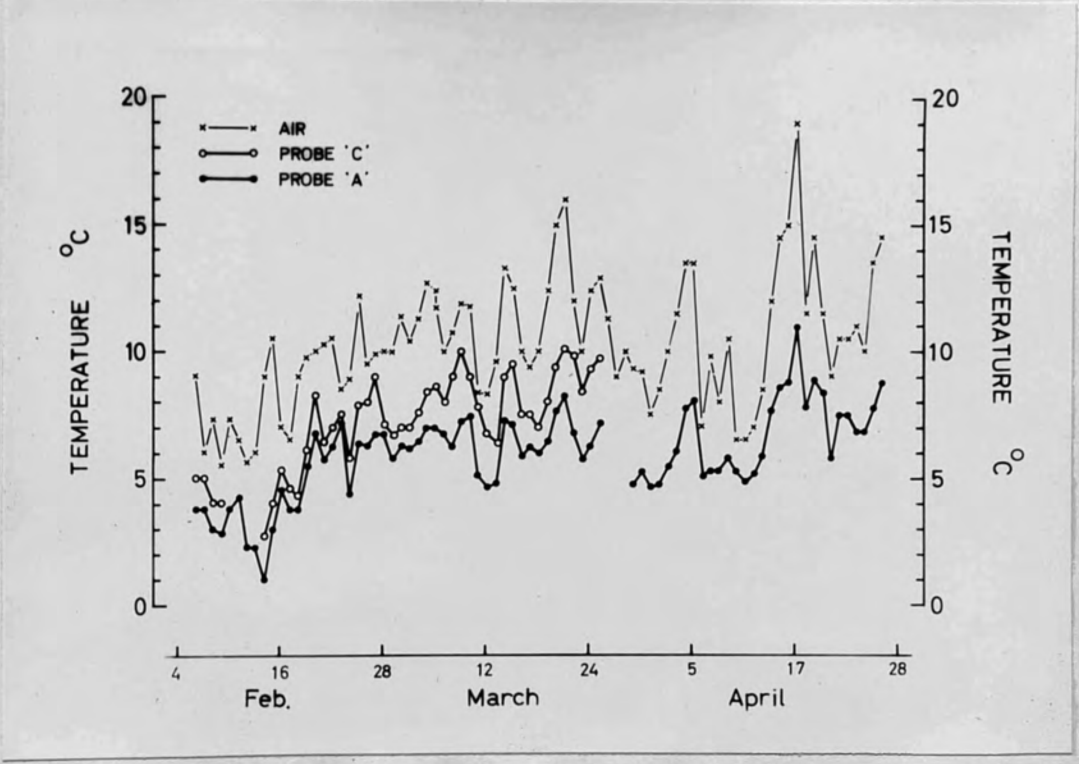


Fig. 1.22. Daily maximum temperatures for experiment in 1967.



Probe 'A' in bank.
Probe 'C' under litter close to tree.

Table 8 — Spot readings of thermistor probe temperatures (1967).

Date	Approximate time of readings (G.M.T.)	Ambient (air) temp.	Probe temperatures			
			(Bank)		(Litter)	
			A	B	C	D
6th February	0830	3.0	2.75	1.5	3.5	3.5
	1500	7.5	3.8	3.3	4.9	4.9
7th "	1400	6.0	4.0	4.0	5.0	5.2
8th "	1400	6.0	2.75	2.0	3.75	3.5
9th "	1345	6.0	2.5	2.5	4.0	4.0
13th "	0830	1.0	1.5	0.25	2.0	1.7
	1445	4.0	2.25	0.6	2.5	2.3
14th "	1100	1.0	0.5	-0.3	1.25	1.25
15th "	1400	7.0	2.7	2.0	4.0	3.75
20th "	1500	7.0	5.0	5.0	5.7	5.5
21st "	1030	9.0	5.0	5.0	5.9	5.9
23rd "	1115	6.5	5.2	5.0	5.2	5.4
24th "	1020	6.5	3.0	2.0	4.0	4.0
	1445	7.0	4.4	4.3	5.8	4.8
27th "	0930	-	4.1	3.4	4.6	4.2
1st March	1530	8.5	5.5	4.6	6.0	5.6
2nd "	1415	-	5.5	5.5	7.0	6.0
6th "	0845	8.5	6.2	5.5	6.2	5.75
	1315	9.0	7.7	7.7	8.2	7.0
8th "	1045	7.0	5.9	6.0	7.5	6.25
14th "	1200	-	6.3	6.25	7.1	7.3
21st "	1240	-	6.6	7.0	9.0	8.75

(All temperatures in °C.)

Throughout the 1967 experimental period the daily maximum temperatures from probe C were above those from probe A (Fig. 1.22). The mean daily difference for February was 1.05 ± 0.50°C. and for March, 1.80 ± 0.70°C.. The trace obtained from probe A was gently undulating showing that temperature changes were gradual, and the daily maxima were generally reached in the late afternoon or early evening. The trace from probe C was similar, except that, especially in response to rising temperatures, it was more uneven and the maximum point was reached an hour or more ahead of that of probe A.

6. Discussion.

Wagner (1907) assumed that queens of the various bumblebee species entered hibernation at about the same time of year, in response to the onset of autumn weather. In this connection he thought that queens reared in sheltered nests took up their winter quarters later than those from more exposed nests. More recently, Stein (1956b), in contrast to the observations outlined in another paper (Stein, 1956a), suggested that queens were stimulated to enter hibernation, instead of initiating a second generation of colonies, by the effects of the wide range in day and night temperatures which occur in the late summer and autumn. Stein neglects to mention, however, that in the spring when colony initiation does take place, there are also wide differences between night and day temperatures, and since queens do not at this time have nests in which to shelter, they may be even more subjected to this range than would autumn queens.

That the time of entry into hibernation of both European and American bumblebees depends upon the species and not nest situation or temperature is correctly stated by Plath (1934). B. pratorum is one of the first species to enter hibernation (Hoffer, 1882-3), since colonies of this species are normally at an end by mid-July or even earlier. This is often long before some species such as B. agrorum normally produce queens (see Cumber, 1949b). In the present studies, queens of B. lapidarius were found to enter hibernation from July onwards, showing that initiation of hibernation is not governed by the onset of autumn weather.

Details of the stimuli which are responsible for initiating a queen's entry into hibernation are unknown. According to Detinova (cited by Lees, 1955) there appears to be in Anopheles maculipennis an association between the inactivity of the corpus allatum and hibernation, and a similar association has been demonstrated in the Colorado beetle (de Wilde, 1953). Since in bumblebees the corpora allata are inactive until the final stages of hibernation (Palm, 1948) there may be a connection between their inactivity and hibernation. The corpora allata of spring queens parasitized by Sphaerularia are also apparently inactive (Palm, loc. cit.), which is indicative of similarities in the physiology of these queens and those before hibernation. This probably explains why the behaviour of parasitized spring queens is similar to that of autumn queens about to enter hibernation.

Schmiedeknecht (1878) was of the opinion that both queens and workers could survive the winter and found colonies in the following spring. This belief was no doubt based on the fact that small queens are sometimes produced in colonies and these may be able to overwinter and initiate colonies. Wagner (1907) reports seeing workers, as well as queens, attempting to enter hibernation, adding that the former do not survive. This observation is difficult to understand unless here too small queens were mistaken for workers.

Queens entering hibernation have considerable reserves of fat in their fat bodies, and are normally fertilized. Cumber (1954) found that all the queens he dissected in the spring were fertilized, and all hibernating and spring queens dissected in the present studies were mated. The absence of unfertilized queens in the spring may be explained in several ways. Either unmated queens are usually unable to survive the winter or do not normally enter hibernation, or the chances of a queen mating are very high so that few, if any, queens remain unfertilized.

The development of fat bodies in queens is not dependent upon previous fertilization (Free, in Free & Butler, 1959) and as shown in Part II of this thesis, the reserves mainly accumulate in the first few days of adult life, that is, before the normal time of mating. This strongly suggests that there is no reason why unmated queens should not survive the winter, and Cumber (1953) found unfertilized queens surviving among the bumblebees he was overwintering under artificial conditions. It is possible that an unmated queen is reluctant to enter hibernation but there is no evidence to show that such a queen will not do so. As pointed out by Free & Butler (1959) the sex ratio — that is of queens and males — is probably adjusted so that the greatest possible number of queens becomes fertilized. Sladen (1912) has estimated that twice as many males as queens are usually produced and a preponderance of males in Psithyrus has been noted by Frison (1926) and others. If, as seems likely from the evidence of dissections, most queens do become fertilized, then this is sufficient to explain the absence of unfertilized queens in and following hibernation.

Before leaving the maternal colony in order to enter hibernation, a queen fills her crop with honey (Sladen, 1912). Sladen considered that this honey was especially needed as a source of food "during September, when the ground if often very dry and warm." It is at this time that a queen's metabolic rate will probably be somewhat higher than during most of the hibernation period and results have shown that most of the honey in the crop is used up during the autumn, as suspected by Sladen. However, it is unlikely that the water content of the honey is of importance in offsetting desiccation since sufficient metabolic water is probably available from the breakdown of the fat body reserves. The remaining honey in the crop is probably of importance at the end of hibernation to bridge the period of increased metabolic activity from the end of diapause until the queen can supplement its food reserves by feeding.

The situations in which queens hibernate are varied but are typically well drained and usually shaded from direct sunlight. Hence sites with a north-west exposure predominate. The present work establishes that queens do not normally hibernate close to their maternal nests, and shows that Plath (1934) is not justified in concluding from Sladen's (1912) observations on B. lapidarius that this species usually does so. There would appear to be no reason why queens should hibernate next to their old nest, unless the colony were situated on or in a suitable hibernation site.

Site selectivity by certain species is confirmed by the present work. B. terrestris, B. lucorum and B. pratorum, which typically hibernate immediately below the soil-litter interface close to trees, all emerge earlier in the spring than B. lapidarius, B. hortorum and Psithyrus species, which hibernate in banks and slopes. Bols (1937, 1939) records these latter species hibernating in such sites and also found many B. ruderatus (Fabr.) queens. This species, however, was not resident in the areas covered by the present work. It is possible that species such as B. ruderarius, B. humilis, B. sylvarum (Linn.) and B. muscorum (Linn.), may choose to hibernate in similar situations to those in which the three examples of B. agrorum were found. However, this may be hard to demonstrate, since searching for queens in such ill-defined sites is difficult and rarely successful.

Since the appearance of queens in the spring is governed by temperature, it is clear that the conditions under which individuals hibernate will have an effect upon their emergence. Results from temperature measurements in hibernation sites have indicated that hibernation chambers of species hibernating immediately under the soil-litter interface close to trees, are subjected to higher daily maximum temperatures in the spring than chambers in banks or slopes.

It is significant that the warmer sites are typical of early-emerging species, while the later-emerging species generally hibernate in banks or slopes. Later-emerging species may tend to hibernate at greater depths than early-emerging species, but this could not be positively demonstrated. It may be that both an effect due to hibernaculum position and to specific physiological differences is involved in governing when a queen of a particular species will emerge from hibernation. However, although the suggestion that different bumblebee species have different arousal threshold temperatures cannot be ruled out, ecological conditions alone could explain this phenomenon.

It is unlikely that minimum or mean daily temperatures influence the time of emergence of bumblebees since queens will appear on warm days whether nights are cold or not, so long as their hibernating quarters warm up sufficiently. Also, as previously mentioned, hibernating queens may become active, even in mid-winter, if warmed artificially. Further, it has been shown by Soulié (1957) that the hibernation of ants (Crematogaster scutellaris Ol.) may be disrupted by frequently repeated temperature maxima and that neither mean nor minimum temperatures are involved.

Inevitably local topographical features will affect the time of emergence of spring queens, by influencing ambient temperatures. **Also**, queens hibernating in sandy soil will probably be aroused earlier than queens in similar situations, but in damper, heavier soil. A tendency for queens to burrow deeper in sandy conditions, because of the ease of burrowing, would close the gap between emergences in such cases, and it is interesting to find that queens in sand were deeper than those in chalky sites.

Observations by Latter (in Fox-Wilson, 1946) suggest that light penetrating into the hibernaculum may be effective in arousing hibernating wasps. Most bumblebees hibernate in the dark and so will not be affected. However, occasionally queens do hibernate in places where light may penetrate, as did the B. lucorum queens found in the folds of a curtain, and such bees may be influenced by light intensity. A temperature-aroused queen in a natural hibernaculum will be subjected to light and other factors just prior to its emergence from the ground, but whether such a queen will remain in situ under dull and/or wet, although warm, conditions, is not known.

Little precise information is available concerning the habits of spring queens in relation to the places in which they spend the night or periods of inclement weather, before they have founded colonies. As described earlier, the habits of spring queens parasitized by Sphaerularia are distinctive, but unfortunately in the past, the observers of spring queens have usually neglected to determine whether healthy or parasitized queens were being studied. The result is that many of the habits of parasitized individuals have been credited to healthy queens as well. Réaumur (1742) found queens digging into the soil of a slope during the spring and Plath (1927) suggested that these observations were actually made on queens that were about to enter hibernation and that Réaumur had "placed them in the spring by mistake." In his later account of hibernation, Plath (1934) alternatively suggests that Réaumur was observing queens that had overwintered and were digging into the ground to spend the night. It is more likely, however, that the queens Réaumur observed were parasitized by S. bombi and had returned to a hibernation site.

Bols (1937) observed many queens in late June spending the night under leaves in the area of his "summer camp" but at that time he did not suspect the occurrence of nematodes in the bees. Later, however, Bols (1939) confirmed that bumblebees frequenting his "summer camp" were parasitized. Unfortunately this original observation has been perpetuated as applying to normal (healthy) spring queens, as in Free & Butler (1959). Bols (1939) also refers to observations, presumably made on healthy queens, which suggest that bumblebees remain at the hibernation site for a time before departing, often in groups, in order to found colonies elsewhere. According to Bols this departure occurs from mid-June onwards. These observations were presumably conducted on B. lapidarius and various Psithyrus species. During the present work no evidence was found of healthy queens using hibernation sites as night refuges, and certainly no lingering at such sites after emergence or mass exodus of queens as described by Bols, was ever seen. Further, by mid-June colonies of even B. lapidarius were past the incipient stage and some nests (B. pratorum) had already produced queens. The unusual behaviour of queens described by Bols may be attributed to the peculiar ecological circumstances of the area under investigation.

Between the time of their emergence from hibernation and the founding of colonies, queens probably pass the night in any convenient sheltered situation, especially to avoid the dangers of a night frost, and they will seek out any suitable place nearby. They do not attempt to find or habitually return to special sites each night. Burrowing into the soil would appear quite unnecessary and since the duration of their stay may be measured in hours rather than months, there would appear to be no great ecological requirement of such a site other than that it should offer a certain degree of protection.

PART II : THE FAT BODY OF ADULT BUMBLEBEES.

1. Introduction.

Schmieder (1928), and others, have described the characteristics of the fat body of the Hymenoptera, and much has been published concerning the fat body of honey bees (see Snodgrass, 1956). However, little reference has been made in the literature to that of bumblebees, although Hüsing (1954, 1955) has described the larval fat body of Bombus and compared it with that of some related Aculeates.

Reference has been made in Part I to the utilization of fat by hibernating queens, and in the present section the fat body of adult bumblebees of various categories is briefly described, and the nature of the metabolic reserves and the extent to which they are accumulated in different categories of bumblebee, with particular emphasis on queens, is examined.

2. Methods.

a) Histological.

Observations on the gross histology of the fat body were made on fresh tissue dissected in 0.8% saline solution. More detailed histological observations were made on paraffin (m.p. 56°C.) sections cut at 8 μ following fixation in alcoholic Bouin (Duboscq-Brasil), Carnoy, or Aqueous Bouin. Most sections were stained in Ehrlich's Haematoxylin, although Chlorozol Black, Delafield's Haematoxylin and eosin were also used. Mayer's Haemalum was used to demonstrate the nuclei in fresh or formalin-fixed tissue which was then mounted in glycerine jelly mounting medium.

b) Histochemical.

i. Lipids.

For the demonstration of lipids, fresh or formalin-fixed tissue was stained in a saturated solution of Sudan Black B in 70% ethanol or ethylene glycol. Lipids were demonstrated in whole mounts and also in frozen sections cut on a Cryostat at thicknesses of 12 and 15 µ. Neutral fat was demonstrated by staining with Nile blue sulphate, Oil Red O, or Fettrot 7B. The Fettrot 7B method given by Pearse (1960) was found to be most convenient and gave particularly good results.

ii. Glycogen.

Alcoholic Bouin was used as a fixative for all fat body tissue on which tests for glycogen were made. Paraffin sections were cut at thicknesses of 8 and 10 µ, and tested for glycogen by the Best's Carmine method (Casselman, 1959) or the periodic acid Schiff reaction (McManus & Mowry, 1960). Control sections were incubated for 30 minutes at 37°C. while covered with saliva, which successfully removed glycogen from sections while distilled water did not. Glycogen was also demonstrated in whole mounts by the iodine method (Wigglesworth, 1942).

iii. Protein.

Protein was demonstrated in Carnoy and Bouin-fixed tissue using the ninhydrin Schiff test after Yasuma and Ichikawa (Casselman, 1959), and the Mercury-Bromophenol Blue method after Bonhag (Pearse, 1960).

iv. Uric acid.

The hexamine silver method given by Pearse (1960) was used to demonstrate the presence of uric acid in alcohol-fixed tissue.

3. Histological observations.

a) Gross structure.

The fat body of adult bumblebees consists of two cell elements, the actual fat cells or trophocytes, and the oenocytes. The trophocytes are large, spherical to oval cells, which form the bulk of the fat body tissue. The oenocytes are smaller, spherical cells which are scattered throughout the fat body tissue, usually abutting the trophocytes. Both types of cell were observed to be associated with the fine, ultimate capillaries of the tracheal system.

The colour of the fat body in general terms, depends upon the amount of pigment present in the oenocytes. Thus the fat body tissue appears white when no pigment is present in the oenocytes, and yellowish to golden or dark brown when the oenocytes contain pigments. However, in even the darkest tissue, the unpigmented trophocytes are clearly visible amongst the oenocytes when viewed under the low power dissecting microscope.

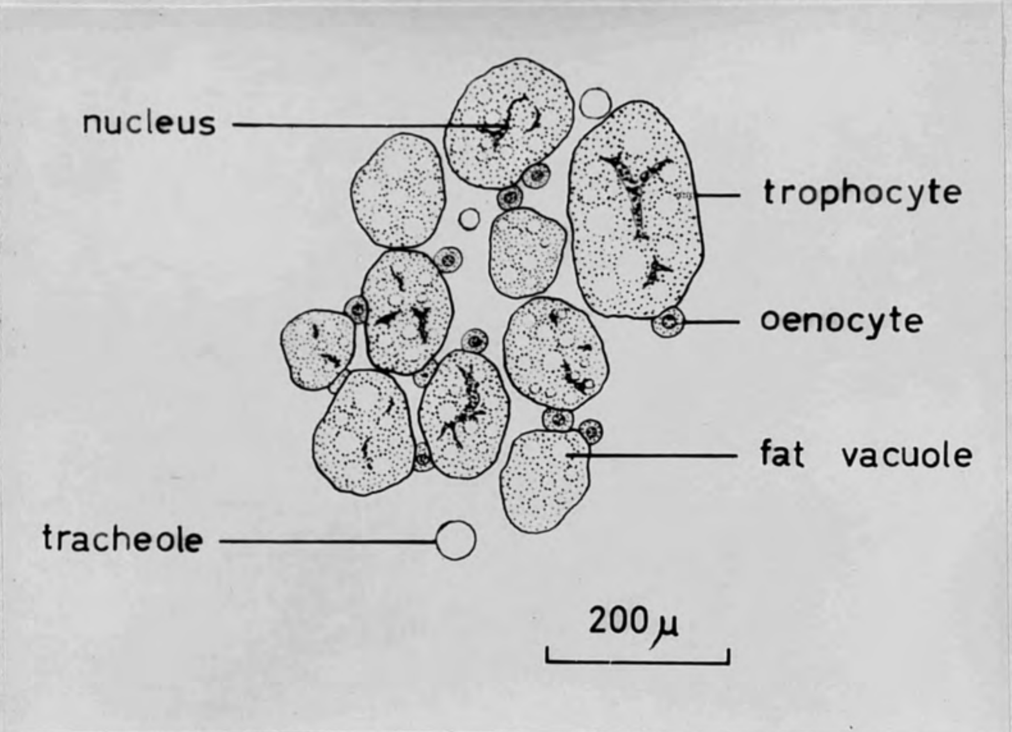
In fresh, unstained tissue, the trophocytes have a glistening, beady appearance due to the numerous fat globules present in the cytoplasm. The trophocyte nucleus is usually invisible in unstained tissue. The oenocytes have a more or less homogenous, dense, appearance and a central, spherical nucleus which is visible without staining.

In profuse tissue, the oenocytes tend to be distorted since they become compressed by adjacent trophocytes, a condition described by Lotmar (1939) as occurring in the fat body of overwintering honey bees. Unlike the condition in the honey bee, however, the trophocytes of bumblebees retain a more or less rounded outline and do not become flattened against each other to any great extent, so that the tissue is always freely permeated by a network of intercellular spaces.

b) Histological structure.

In section, the fat body appears as a collection of separate trophocytes which often abut one another or may be linked by oenocytes (Fig. 2.1). The trophocyte cytoplasm is homogenous and usually contains many vacuoles which in life are filled with fat. A large nucleus is present, which may be oval or much distorted and reticulate. The nucleus is light-staining but darker chromatin granules are present. Normally the trophocytes dwarf the oenocytes, especially in queens (Fig. 2.1). The nucleus of each oenocyte contains much chromatin. In contrast to trophocytes, however, the cytoplasm of oenocytes lacks vacuoles. The fat body was similar in appearance in all bumblebee species which were examined.

Fig. 2.1. Section of fat body of *B. terrestris* gyne to show general histological appearance.



---

c) Distribution of fat body tissue.

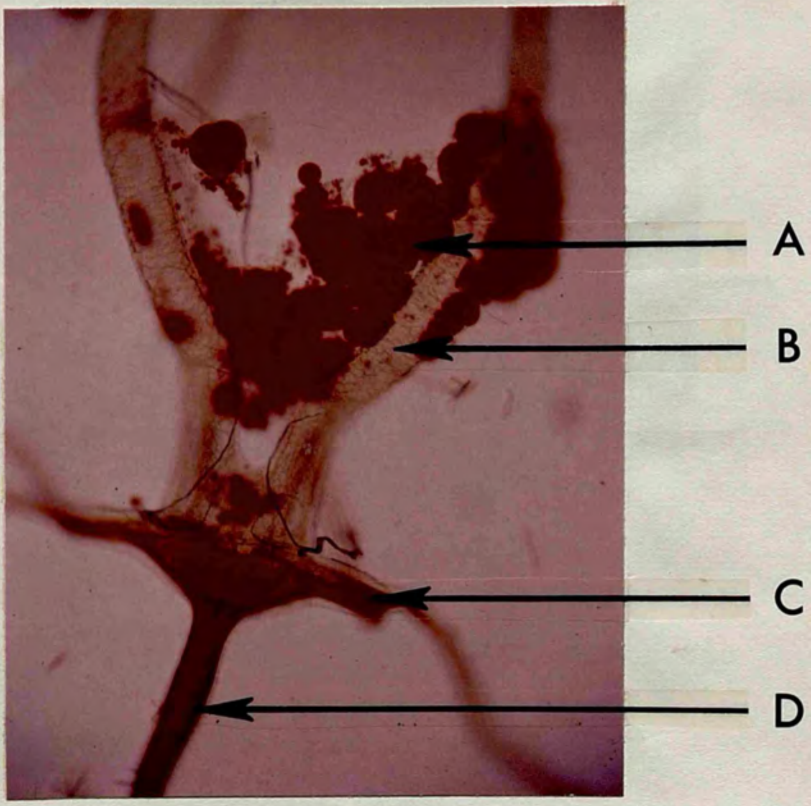
i. Head.

Several fat cells occur in the head, usually in small groups. Some are attached to the 5-branched salivary duct junction complex of the labial glands, forming a group of cells between the paired ducts which lead back towards the thoracic glands (Fig. 2.2). Fat cells may also occur adjacent to the mandibular glands and are also present amongst, and more commonly at the periphery of, the acini of the hypopharyngeal glands (Fig. 2.3). The latter fat cells are particularly easy to distinguish in freshly dissected material since they appear milky-opaque when viewed in distilled water or saline solution, whereas the acini of the hypopharyngeal glands are greyish in appearance and less opaque. If the dissected head of a bumblebee is immersed in a solution of Fettrot 7B in 70% ethanol, the fat cell contents are stained bright red within a few minutes, leaving the remaining tissue and salivarly glands, with the exception of any oil in the post-cerebral gland acini and associated ducts, unstained.

ii. Thorax.

Fat cells are also present in the thorax, and as with those in the head, their presence is simply demonstrated by the addition of a solution of Fettrot 7B. Several cells occur in the prothoracic region, and adjacent to the acini of the thoracic glands (Fig. 2.4), and there may be a sparce, single layer of fat cells beneath the epidermis of part of

Fig. 2.2. Whole mount of 5-duct junction of labial glands of *B. lucorum* queen stained with Fettrot 7B to show associated fat cells.



x 20 (approx.)

A - group of fat cells.

B - duct of thoracic gland.

C - duct of post-cerebral gland containing oil.

D - common salivary duct of labial glands.

Fig. 2.3. Section of fat cells and acini of hypopharyngeal gland of B. lapidarius gyne.

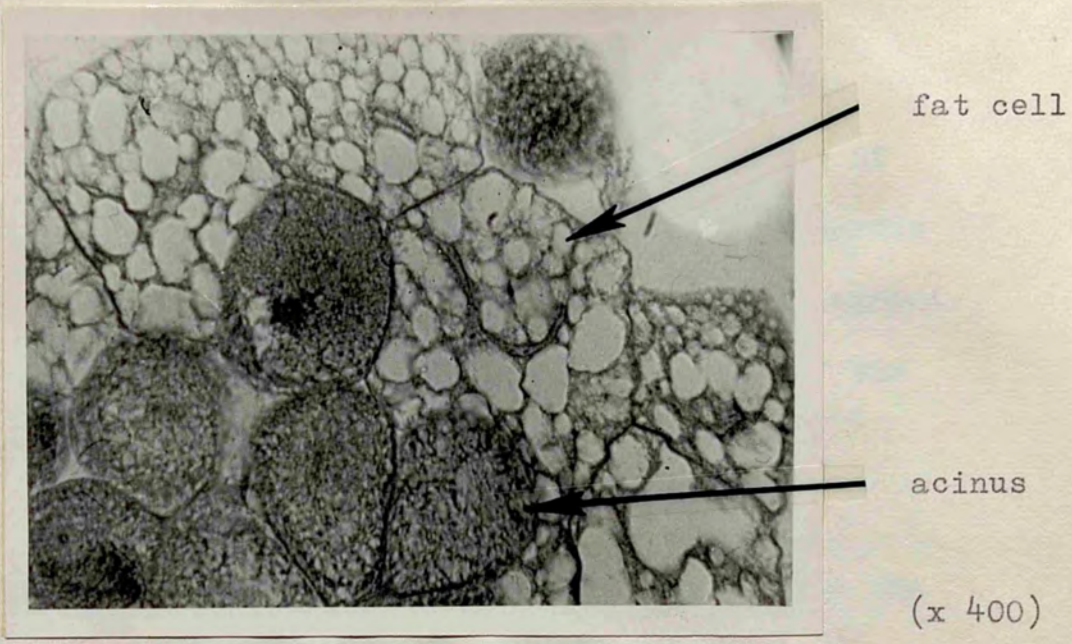
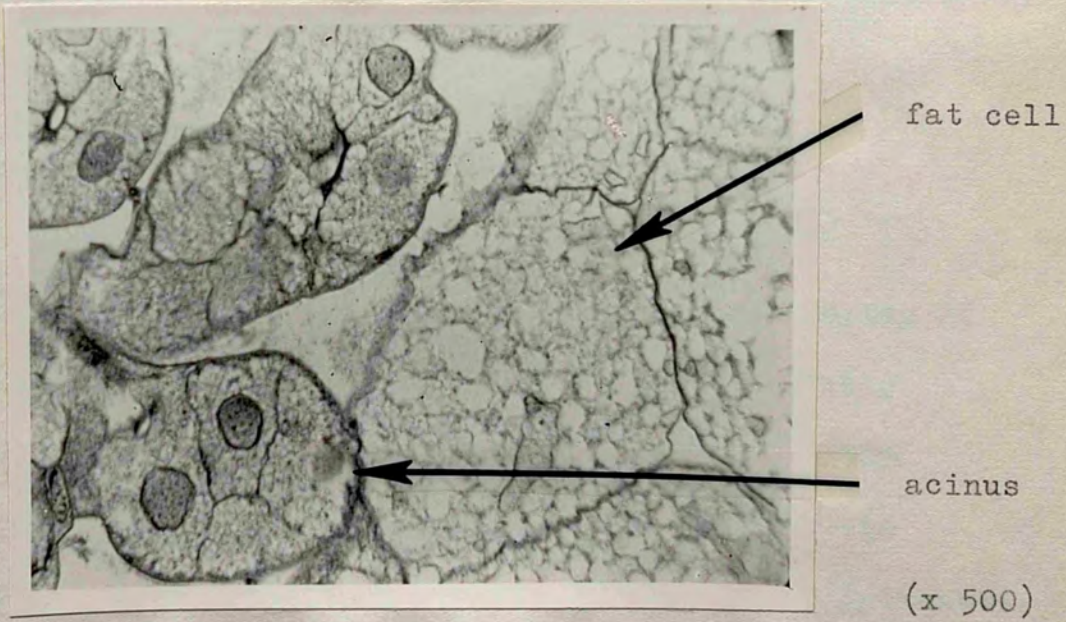


Fig. 2.4. Section of fat cells and acini of thoracic gland of B. lapidarius gyne.



the propodeal tergum.

iii. Abdomen.

The bulk of the fat body of adult bumblebees is located in the abdomen, occurring as clumps or layers of loosely aggregated tissue which lie beneath the epidermis in the dorsal and ventral regions of each abdominal segment. Fat body tissue also occurs laterally on each side of the abdomen beneath the epidermis. Unlike the condition found in certain insects, there is no fat body layer surrounding the gut. Coupland (1957) reports that in the locust, which has a fat body layer surrounding the gut, oenocytes are restricted to the peripheral fat body. In bumblebees, oenocytes were not found in the head or thorax and in females, they were apparently absent from much of the layer of fat body tissue overlying the poison sac. The thickness and extent of the fat body tissue varies considerably from bee to bee and is best developed in queens.

4. Histochemical observations.

a) Lipids.

The conspicuous globules present in the trophocytes of both fresh and formalin-fixed tissue were stained darkly after a few minutes in a solution of Sudan Black B in 70% ethanol. When ethylene glycol was used as a solvent the time taken for the accumulation of the stain was much increased. When stained with Nile blue sulphate, each globule took on a pink coloration which is due to the oxone form of the dye and considered as specific for neutral fats (Baker, 1958). None of the globules was stained blue by this stain, which indicated that acidic fats were not present. With Oil Red O, the fat globules were stained orange-red, while with Fettrot 7B each was stained bright red. Both of these stains are regarded as specific for neutral fat (Pearse, 1960). Fettrot 7B gave excellent results when employed as a saturated solution of the stain in 70% ethanol, and was used for the majority of tests. As previously mentioned, this stain was also used for locating fat cells in various parts of the body.

In paraffin-embedded tissue the lipids were, of course, removed but the sites of the individual fat globules were left as empty vacuoles and these were a useful guide to the storage state of the cells. Neutral fat globules were successfully demonstrated in frozen sections of fat body tissue that had been fixed in formol-calcium and embedded in gelatin before freezing. When gelatin-embedding was ommitted, satisfactory results were not obtained.

Neutral fat was demonstrated in the trophocytes of all categories of bumblebees, being most evident in gynes about to enter hibernation, or in those at the beginning of the hibernation period. In only a few instances was no fat demonstrated in the trophocytes. Quantitative results of the fat content of bumblebees are presented later.

b) Glycogen.

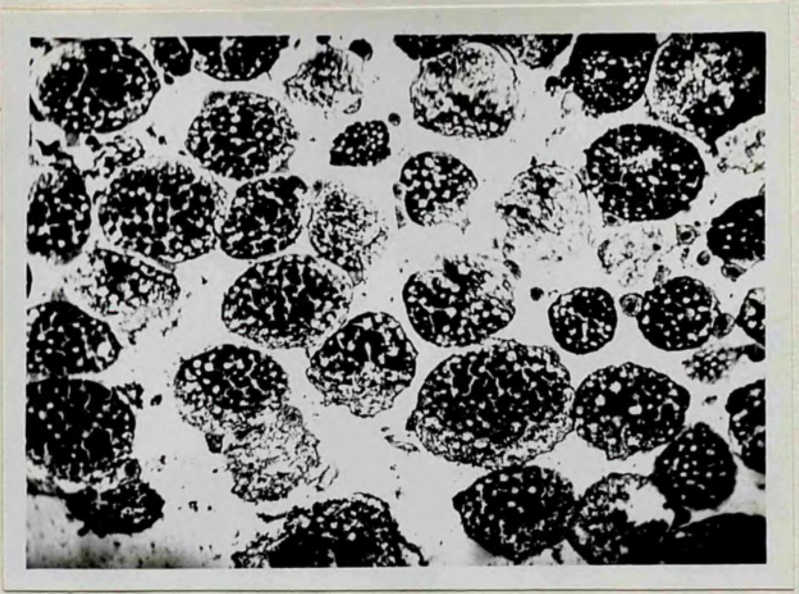
Glycogen was demonstrated in the trophocytes as being spread throughout the cytoplasm between the fat vacuoles (Fig. 2.5). In several instances the deposits were concentrated at one side of the cell. This was probably a consequence of poor fixation which had allowed of the phenomenon of streaming or polarization (see Casselman, 1959; Pearse, 1960). Slides coated with collodion were used with the PAS test since removal of glycogen from unprotected slides is known to be particularly rapid during the period of hydrolysis in periodic acid (Pearse, 1960). However, with Best's Carmine satisfactory results were obtained for both protected and unprotected slides.

To some extent the quantitative distribution of glycogen followed that of fat, in that the most intense staining reactions were obtained from trophocytes which contained masses of fat. However, although often when little fat was present, little glycogen was also demonstrated, this could not be taken as an unalterable rule since in several instances, bees had moderate deposits of glycogen in the trophocytes but little fat.

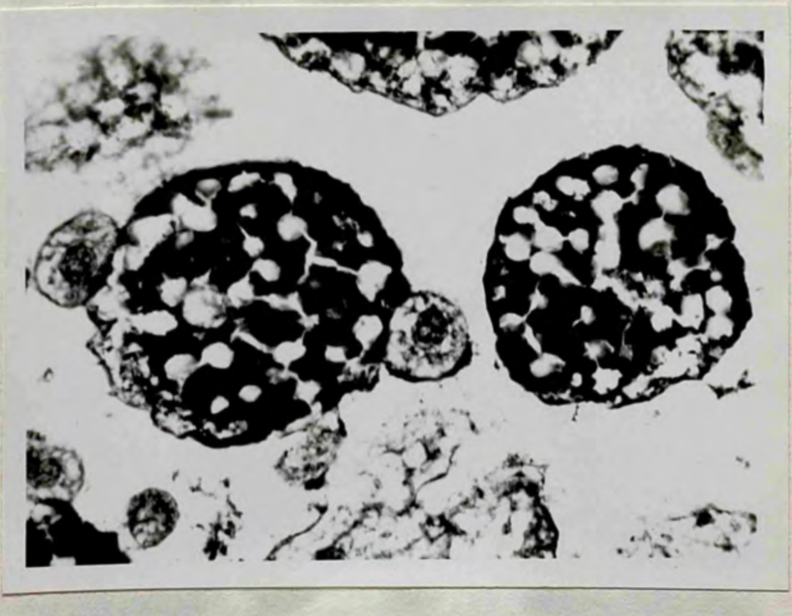
c) Protein.

In newly emerged bumblebees, the trophocytes contained granules which were stained reddish by the ninhydrin Schiff test. These granules were also stained dark blue-black by the Mercury Bromophenol Blue method (Fig. 2.7). These tests indicate that the deposits are composed of protein.

Fig. 2.5. Section of fat body of hibernating queen of *B. lucorum* stained with Best's Carmine to show glycogen deposits.

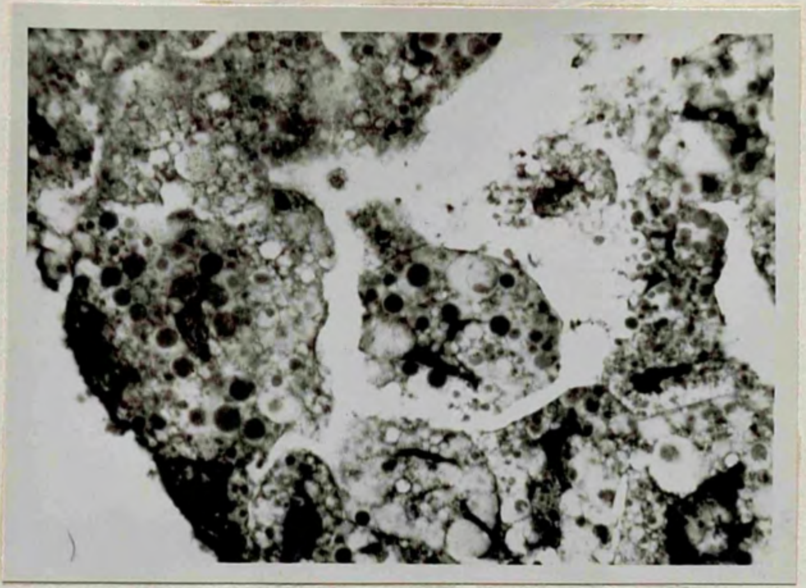


(x 100)



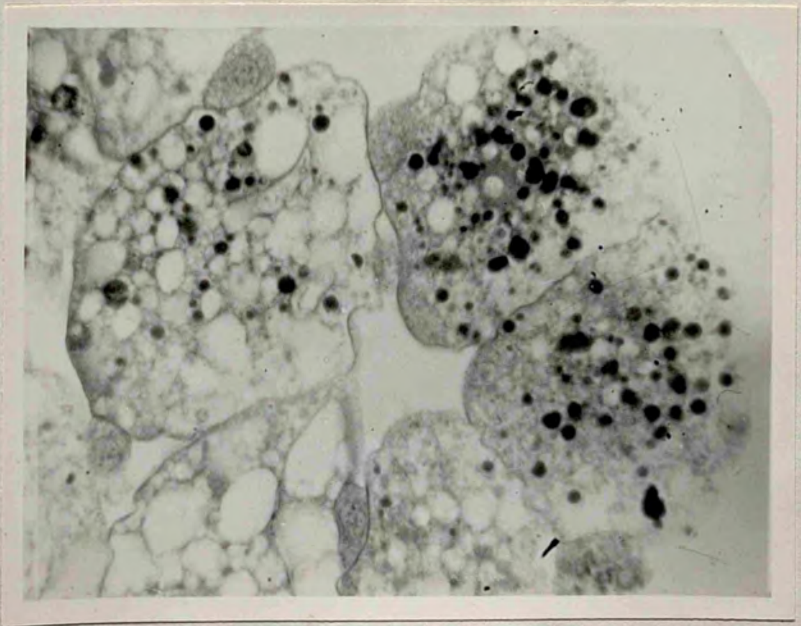
(x 400)

Fig. 2.6. Section of fat body of newly emerged *B. lucorum* male stained with Ehrlich's haematoxylin to show albumenoid granules.



(x 350)

Fig. 2.7. Section of fat body of callow *B. lucorum* male stained with Mercury-Bromophenol blue showing diminishing size of albumenoid granules.



(x 360)

These protein or albumenoid granules are also stained by haematoxylin (Fig. 2.6) and by eosin, and they attain a diameter of about 15 - 20 μ. They are most abundant in bees that have just completed the pupal ecdysis but have yet to emerge from the pupal cell. Although still persisting in young callows, where they are generally smaller in size (Fig. 2.7), they are absent from the trophocytes of older bumblebees. Reserves of protein were not found in the fat bodies of hibernating bumblebees.

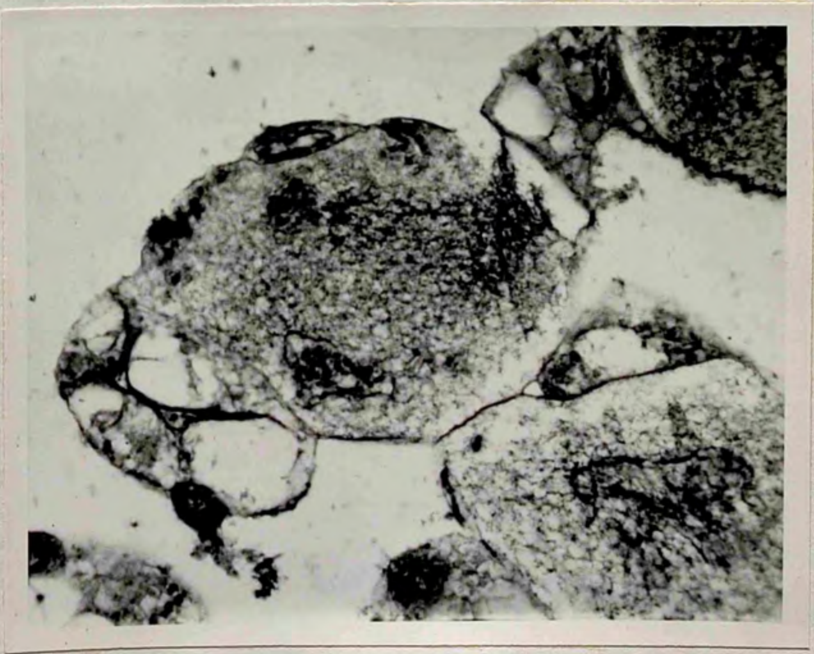
d) Uric acid.

Associated with the fat body of newly emerged adult bumblebees are clumps of white, opaque concretions which form irregularly rounded and loosely aggregated masses. These concretions have been noted previously in callow bumblebees by Cumber (1949b) and have also been described in callow honey bees (Haydak, 1957). They occur principally towards the outer regions of the abdominal fat body and also in the region of the gonads. These transitory aggregates are most evident in the youngest callows and have mostly been eliminated from the body after 2 or 3 days of adult life.

The concretions were stained black by the hexamine silver method and that they are composed of uric acid is confirmed by their being insoluble in acid, but soluble in dilute alkali and in dilute lithium carbonate. These deposits are contained in urate cells which have persisted from the larval stage (Fig. 2.8).

Fig. 2.8. Section of fat body of newly emerged B. agrorum gyne stained with Ehrlich's haematoxylin to show excretory or urate cells.

x 80 (approx.)



x 320 (approx.)

5. The fat body of bumblebees of various categories.

a) Callows.

The fat body of callow bumblebees has a greyish, watery appearance, and white deposits of uric acid in urate cells are scattered throughout the tissue. The trophocytes tend to be smaller and more spherical than in older individuals, and they contain fewer, but often larger, vacuoles. The nuclei of the trophocytes are generally somewhat distorted, and drawn out into long processes between adjacent vacuoles (Fig. 2.8). The oenocytes are also small in callows, and their large central nucleus often exceeds one half of the diameter of the cell in size.

In section, the urate cells still persisting from the pre-imaginal stage are visible scattered here and there amongst the adult tissue (Fig. 2.8). They have a regularly granular cytoplasm and a darker-staining nucleus which is not always separated from the surrounding cytoplasm by a distinct nuclear membrane.

b) Queens.

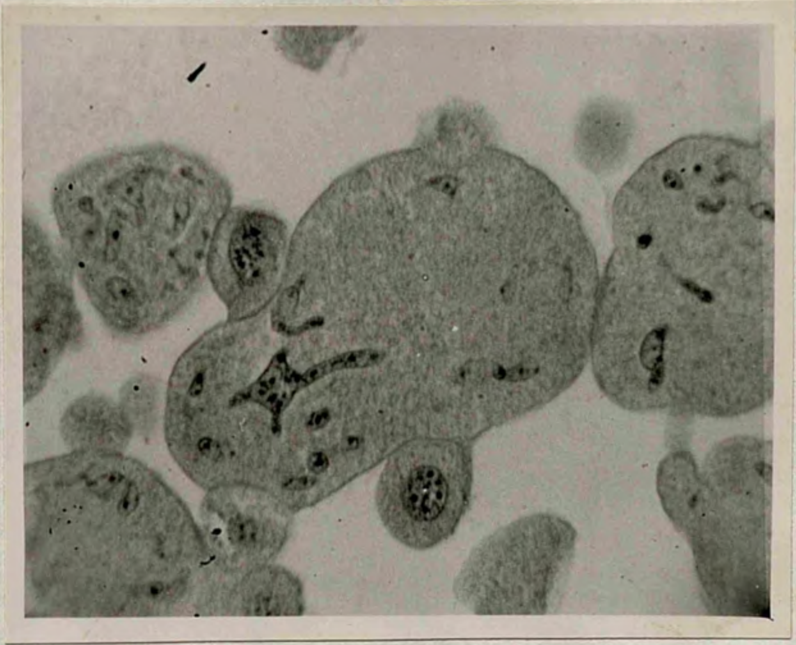
After the callow stage, the fat body develops rapidly and at the commencement of hibernation is at its maximum development. In large species such as <u>B. terrestris</u> and <u>P. vestalis</u> the trophocytes may exceed 250 x 200 μ in size, but more usually they have a maximum diameter of about 200 μ. The cytoplasm is evenly vacuolated and each vacuole is about

15 - 20 μ in diameter. Larger vacuoles may also occur in the cells, but these may, at least in some cases, be artefacts. In cells packed with fat vacuoles, the nuclei are so distorted that they are often difficult to locate, even in stained sections. In the spring, the fat body is reduced in bulk, but is still white and profuse (Cumber, 1954). The trophocytes of a gyne of *B. lapidarius* found excavating its hibernaculum on the 12th August, 1965, for example, measured up to 225 x 162 μ, and those of a queen of the same species which was captured in the early spring of the following year, had trophocytes not exceeding 100 x 80 μ in size.

In spring queens the trophocyte nucleus is visible in stained whole mounts as a reticulate structure, spread throughout the cell. In section, the nucleus is clearly seen but because of its reticulate form, appears to be broken into small, often branching, ribbon-like pieces. The nuclei retain this distorted form, inspite of the fact that there may be few or even no fat vacuoles present in the cytoplasm (Fig. 2.9).

In some spring queens relatively large stores of fat and glycogen were demonstrated in the trophocytes, but as the spring progressed there was a marked reduction in the number of queens still with moderate reserves. Although the trophocytes of late spring queens tend to be smaller than those of early spring queens, such a size difference is not usually very marked. On the other hand, an increase in the size of the oenocytes is quite obvious. An increase in the size of

Fig. 2.9. Section of fat body of early spring queen of B. ruderarius stained with Ehrlich's haematoxylin and showing homogenous trophocyte cytoplasm.



(x 370)

both cells and nuclei occurs. After hibernation, pigments begin to accumulate in the oenocytes of queens, so that as the spring progresses, there is a general darkening of the fat body tissue.

The fat body of foundress queens is usually relatively profuse but is always very dark in colour, due to the accumulation of pigments in the oenocytes. Although in old foundress queens the trophocyte nuclei may tend to be reticulate, they are often less irregular and concentrated more or less centrally in the cells.

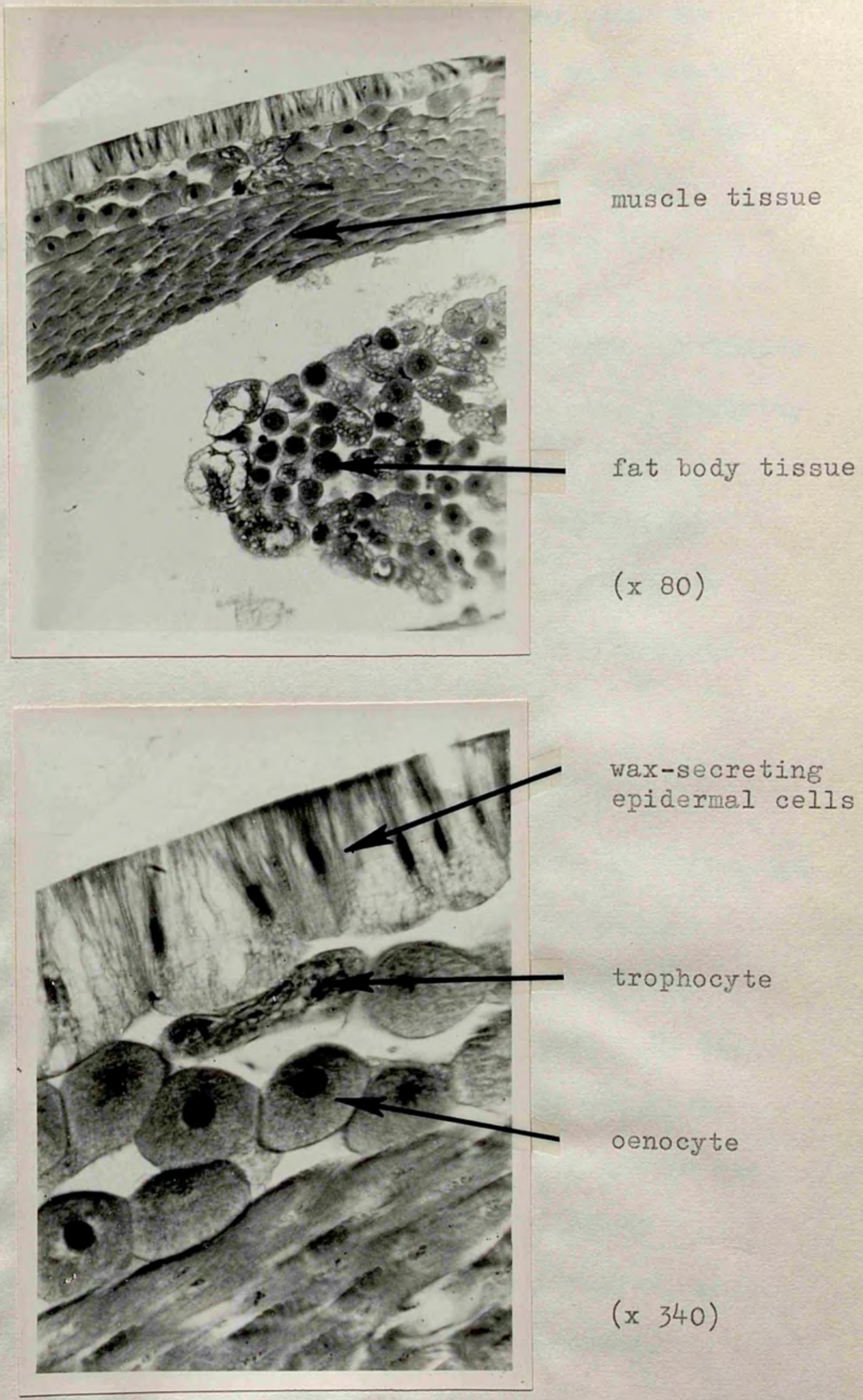
In wax-producing queens large numbers of enlarged oenocytes occur in close proximity to the wax-secreting epidermal cells of the abdomen. The trophocytes which are close to the epidermis in such regions, are usually small and much distorted (Fig. 2.10).

c) Workers.

Worker bumblebees generally only possess sparce fat bodies, but there is considerable variation. In some bees lobes of tissue may be relatively thick, but often the tissue is only one cell in thickness. Unlike the condition found in queens, pigments accumulate in the oenocytes shortly after the callow stage, so that workers generally possess yellowish or brownish fat body tissue.

Histologically the fat bodies of foragers and workers remaining in the nest are similar, although in the latter, especially in older individuals with developed ovaries, the

Fig. 2.10. Transverse section of part of abdomen of wax-producing *B. lucorum* queen.



Cuticle overlying epidermis not shown.

oenocytes are larger and more pigmented. Vacuoles in the trophocytes of workers are usually few in number, and the nuclei are usually irregularly lobate, or where the tissue is not particularly well developed, only slightly lobate or oval.

d) Males.

Although males may possess relatively profuse fat bodies, their development is not as great as that of queens. Normally the tissue is whitish or yellowish and in section more closely resembles that of queens than workers. Although oenocytes were occasionally quite large (exceeding 50 µ in diameter in some instances) the nuclei were generally small.

e) Parasitized bumblebees.

i. Queens parasitized by *Sphaerularia bombi* Dufour.

According to Palm (1948) there is little or no difference between the fat bodies of healthy and parasitized queens at or about the time of their emergence from hibernation. This observation was confirmed in the present work. By the early summer, shortly before the emergence of the parasitic worms from the host, the fat bodies of parasitized queens are noticeably different to those of healthy queens, being reduced to sparce whitish or yellowish layers of tissue, with small cells containing virtually no metabolic reserves.

ii. Bumblebees parasitized by *Syntretus splendidus* (Marshall).

In the early stages of parasitism, *Syntretus* has a marked effect upon the fat body of the host because following the release of the 1st instar parasite larvae into the haemocoel of the host, the dissociated embryonic membrane cells of the parasites begin to enlarge and accumulate nutrients from the host's body fluid. The fat bodies are therefore rapidly depleted of their reserves and become noticeably smaller in size and considerably distorted (Fig. 2.11).

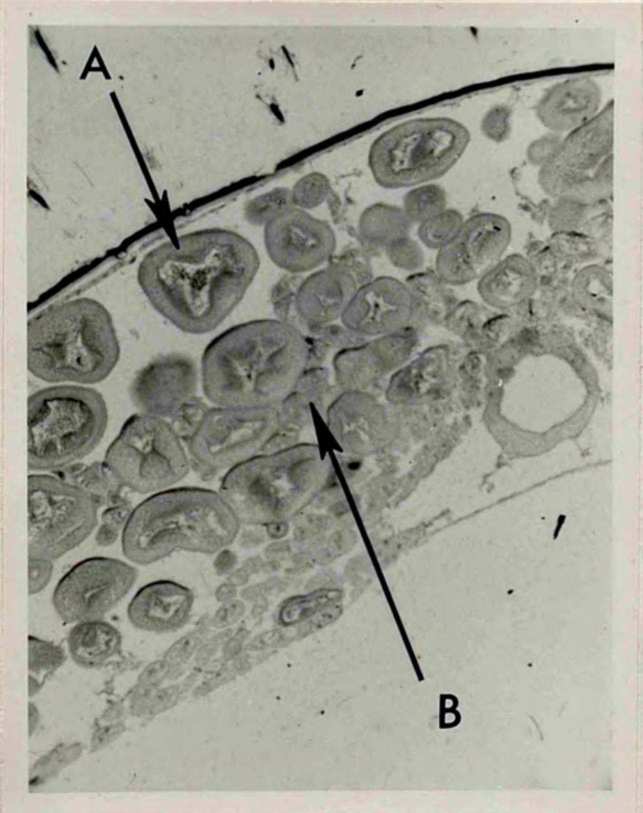
iii. Bumblebees infected by other parasites.

A few bumblebees parasitized by Conopid larvae were examined. All, however, contained relatively young parasites and no differences were observed between the fat bodies of healthy and parasitized bees. Towards the late stages of infection the fat bodies would probably be affected, either by the excessive drainage of their reserves, or by mechanical damage, or both.

The presence of the tracheal mite *Bombacarus buchneri* Stammer appeared to have no effect upon the fat body of the host, even when infection was severe.

The characteristics of the fat body of bumblebees of various categories are summarized in Table 9, and in Appendix Tables IV to IX the results of the measurements of the trophocytes and oenocytes of bumblebees of various categories and species are given.

Fig. 2.11. Transverse section of part of abdomen of *B. agrorum* worker parasitized by the Braconid *S. splendidus* showing dissociated embryonic membrane cells among reduced fat body tissue of host.



(x 100)

A - embryonic membrane cell of parasite.

B - fat body tissue of host.

Table 9 — Summary of some characteristics of the fat body of bumblebees of various categories.

Category of bumblebee	(A) Colour of tissue	(B) Size of cells		(C) Reserves present in trophocytes		
		Oenocytes	Trophocytes	Fat	Glycogen	Protein
Queens						
Callow	-	-	++	++/+++	-/+	+
Gynes	-	+/++	+++/++++	++++	+++	-
Hibernating	-	+/++	++/+++	++/+++	++/+++	-
Early spring	-	+	++	+/++	+/++	-
Late spring	+/++	++	++	+	+/++	-
Foundress	++/+++	++/+++	++	+	+/++	-
Old foundress	++++	++/+++	++	+	+	-
Workers						
Callow	-	-	+	+	-/+	+
House bees	+/+++	++	+	+	+/++	-
Laying	+++/++++	++/+++	+	+	+	-
Forager	+/++	+/++	+	+	+/++	-
Males	-					
Callow	-	-	+	+/+++	-/+	+
Wild (Mature)	+/++	+/+++	+/++	+/++	++	-

Key to symbols used

	(A)	(B)	(C)
-	whitish	very small	none
+	lemonish	small	small amount
++	yellowish	medium	medium amount
+++	golden brown	large	large amount
++++	dark brown	very large	very large amount

/ : indicates a range between two values.

---

6. Quantitative studies on the fat content of bumblebees.

a) Methods.

Bumblebees preserved in Pampel's fluid were dried to a constant weight in an oven at 96°C. and then extracted in a 500 ml. Soxhlet apparatus with 300 ml. of chloroform for a period of 6 hours, after which time no further fat could be extracted. Bees were then again dried to a constant weight in the 96°C. oven. Five or ten bumblebees were extracted at a time, depending upon the size of the individuals. In some instances head, thorax and abdomen were treated separately and in others whole bees were employed. The difference between the dry and extracted (dry) weight was taken as the amount of fat present in the body. Inevitably some material other than fat was included in the extracted fraction, but the total weight of these additional solids was probably small and therefore ignored.

To enable comparisons between bumblebees of various sizes to be made, the amount of fat extracted is expressed as a percentage of the total dry body weight. The statistical significance between samples was determined by Student's t-test.

b) Results.

The results obtained for bumblebees of various categories are given in Appendix Tables X to XXI. These results are summarized in Fig. 2.12 for workers, Fig. 2.13 for males, and Fig. 2.14 for queens.

i. Workers.

Although in some instances more than 10% of the total dry weight of newly emerged worker bumblebees was found to be fat, the actual fat content of the bees was never very high.

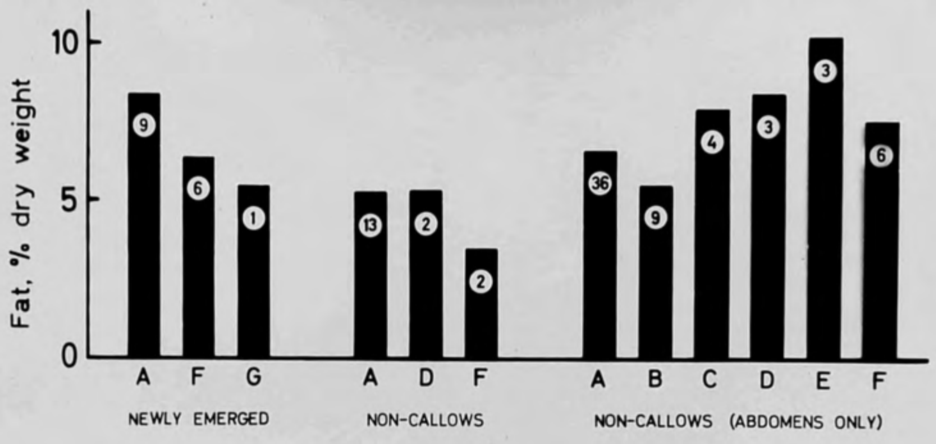
The fat content and percentage of fat in the dry weight of non-callow workers taken at random from colonies or captured in the field, showed considerable variation but was generally less than that of newly emerged bees (*B. terrestris* sample, $p < 0.02$; *B. agrorum* sample, $p < 0.05$). Since there was apparently no correlation between the % of fat in the dry weight of workers and the occupation of individual bees, it was considered unnecessary to subdivide the non-callow samples into groups such as foragers or house bees.

From large workers, only rarely was more than 10 mg. of fat extracted, while from small individuals often less than 1 mg. was obtained. The difference in the % of fat in the dry weight of abdomens of newly emerged and non-callow workers is significant in the case of *B. terrestris* ($p < 0.001$), but not in that of *B. agrorum* ($p > 0.05$).

ii. Males.

The mean % of fat in the dry weight of newly emerged males was higher than that of newly emerged workers of the same species. Although the difference was not significant in the case of *B. agrorum*, the difference between the *B. terrestris* samples was highly significant ($p < 0.001$).

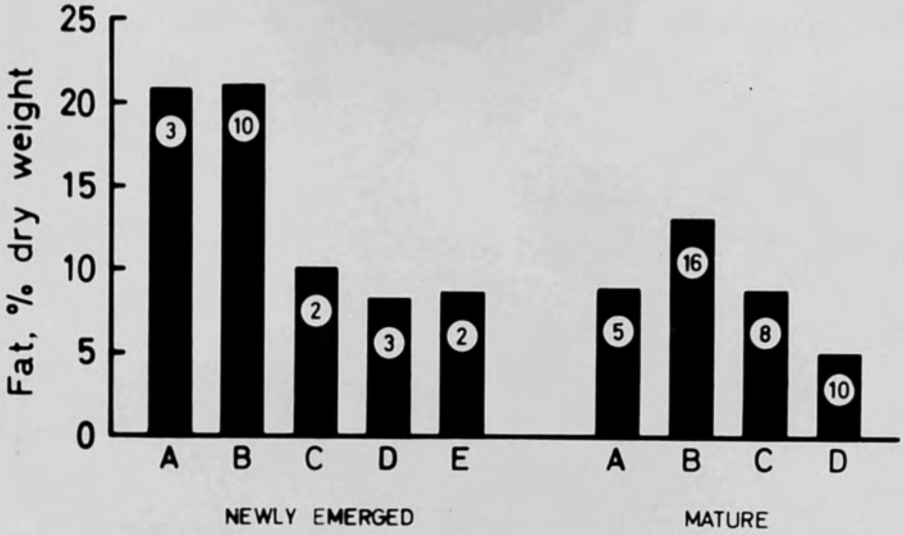
Fig. 2.12. Mean fat content of worker bumblebees of various species and categories.



A - B. terrestris
B - B. lucorum
C - B. lapidarius
D - B. hortorum
E - B. ruderarius
F - B. agrorum
G - B. humilis

The number of individuals contributing to each mean is given in each column.

Fig. 2.13. Mean fat content of male bumblebees of various species and categories.



A - B. terrestris

B - B. lucorum

C - B. hortorum

D - B. agrorum

E - B. humilis

The number of individuals contributing to each mean is given in each column.

Fat formed a significantly greater part ($p < 0.001$) of the total dry weight of newly emerged males of the "pollen-storers" *B. terrestris* and *B. lucorum*, than in the "pocket-makers" *B. hortorum*, *B. agrorum* and *B. humilis*.

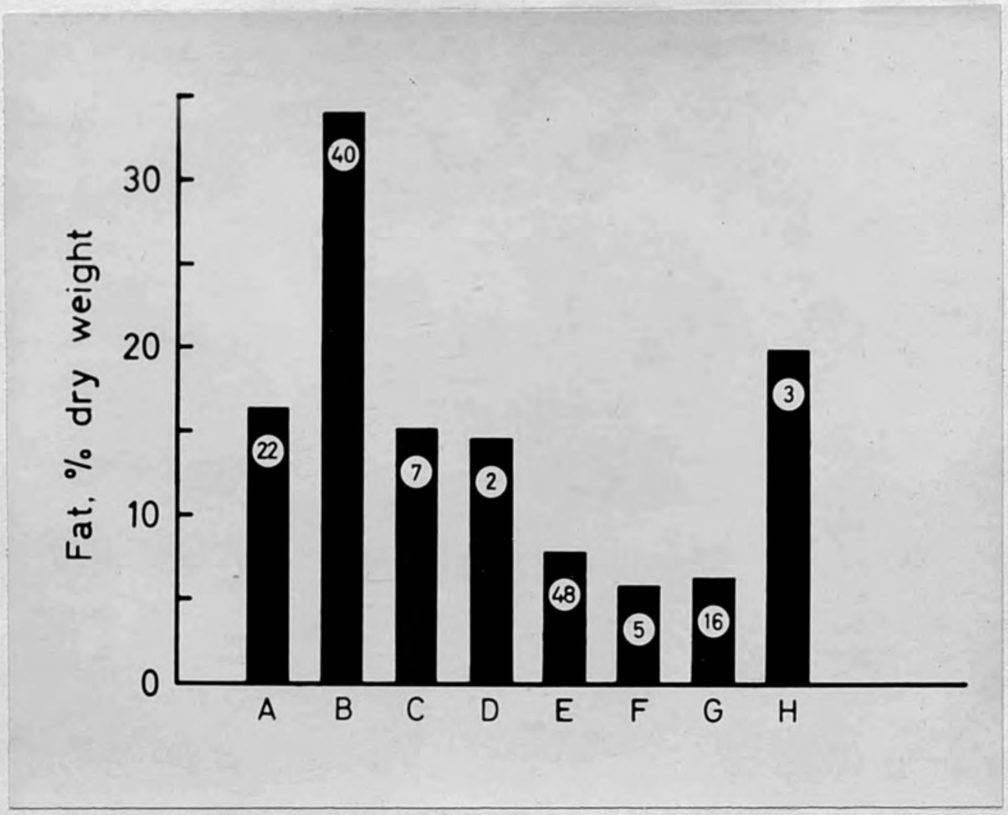
As in workers, the fat content and % of fat in the dry weight of mature males showed considerable variation. The difference between the % of fat in the dry weight of mature males of *B. terrestris* and *B. lucorum* was not significant, and nor was that between mature males of *B. hortorum* and *B. agrorum*. However, fat formed a significantly greater part ($p < 0.001$) of the dry weight of the *B. terrestris* and *B. lucorum* samples than of the *B. hortorum* and *B. agrorum* samples, as in newly emerged males.

iii. Queens.

Newly emerged queens of all species examined, whether "pollen-storers" or "pocket-makers", contained considerable quantities of fat which on average represented almost 17% of their total dry body weight, and was in some cases as much as 25%.

In gynes the fat content of the body was found to approach 50% of the dry weight. The most fat extracted from any one individual was 163.5 mg., which was obtained from a *B. terrestris* gyne. The more normal amount for this species, however, was about 100 mg.. On average, 34% of the dry weight of gynes was found to be fat. However, in some confined gynes, the fat bodies failed to develop and appeared worker-like.

Fig. 2.14. Mean fat content of queen bumblebees of various categories.



A - Newly emerged gynes.

B - Gynes (non-callows).

C - Hibernating queens (mid-winter).

D - Hibernating queens (spring).

E - Spring queens.

F - Spring queens parasitized by Sphaerularia.

G - Foundress queens.

H - Anomalous 'foundress' queens.

The number of individuals contributing to each mean is given in each column.

In these cases little fat was laid down. The physiology of such queens is obviously disturbed and such individuals have been excluded from the gyne samples.

The bulk of the fat stored in gynes — about 80% — is utilized during hibernation (Fig. 2.14: see also Fig. 1.17) and queens sampled within a day or two of their emergence from hibernation rarely contain more than 10% of fat in their total dry weight. At this time the fat content of spring queens parasitized by Sphaerularia was similar to that of spring queens, but some of this fat was probably present in the parasites.

In foundress queens fat did not exceed 9% of their total dry body weight. In some spring queens confined in laboratory nest boxes for the purpose of colony initiation, Medler (1962) reports that egg absorption occurs and that such queens will not found colonies. This phenomenon was noted in the present studies and it was found that in these anomalous 'foundress' queens, fat reserves may accumulate. The amount of fat found in such queens may be considerable (Fig. 2.14).

iv. The quantitative distribution of fat.

The amounts of fat extracted from the head, thorax and abdomen of queen bumblebees before and after hibernation are given in Appendix Table XXII, and summarized in Table 10. As expected, most fat is present in the abdomen, but in the autumn, the amount present in the thorax is often surprisingly high, exceeding 10 mg. in many cases.

Table 10 — The mean amount of fat in various parts of the body, expressed as a percentage of the total dry body weight.

Species	Autumn queens				Spring queens			
	(n)	Head	Thorax	Abdomen	(n)	Head	Thorax	Abdomen
B. lapidarius	(5)	1.7	12.3	82.7	(5)	0.5	2.0	3.6
B. terrestris	(2)	1.4	11.8	78.7	(3)	1.1	3.8	17.6
B. lucorum	(7)	1.4	8.8	79.1	(5)	0.8	2.0	12.5

(n) = Number of observations.

---

7. The fat content of bumblebees of various ages.

a) Methods.

In order to obtain quantitative data on the fat content of bumblebees of various ages, with special reference to the build up of the reserves in gynes, colonies were removed from the field to the laboratory where they were kept in nest boxes. The food reserves of each colony were supplemented by the addition of both sugar syrup and pollen. The latter was obtained from a pollen trap attached to a honey bee colony.

Bumblebees to be sampled were removed from the nest boxes on their emergence from their cocoons, or as soon after emergence as possible, when they were individually marked and returned to their respective boxes. The age in days at which individuals were to be sampled was determined by tabulating the age requirements for each experiment in a randomized list and allocating the bees to the relevant list in the order of their emergence from the cocoons. When bees reached the age allotted to them, they were again removed from the colonies, weighed, and preserved in Pampel's fluid. Subsequent treatment for the determination of their fat content was identical to that described earlier. Males and workers sampled in these experiments were taken from the same colonies which were producing experimental queens.

b) Results.

The results obtained for each experiment are summarized in Appendix Tables XXIII to XXX inclusive.

i. Gynes.

The reserves present in gynes are built up rapidly following the emergence of the bees from the cocoons, although in most cases (with the exception of the B. hortorum experiment) there is a latent period in the first 24 hours of adult life in which little change occurs. The results for individual queens of various species are given in Fig. 2.15.

Little or no fat was extracted from 10-day old gynes that were denied access to pollen from the time of their emergence from the pupa (Fig. 2.16). When callow gynes were removed from the same colonies and fed on a pollen-free diet, then although less fat was laid down than in control bees which were given access to pollen from the time of their emergence, some individuals occurred which after 10 days, contained substantially more fat than those taken from their colonies directly on their emergence. The former individuals had probably eaten some pollen before they were taken from their

Fig. 2.15. Fat content of gynes of various ages.

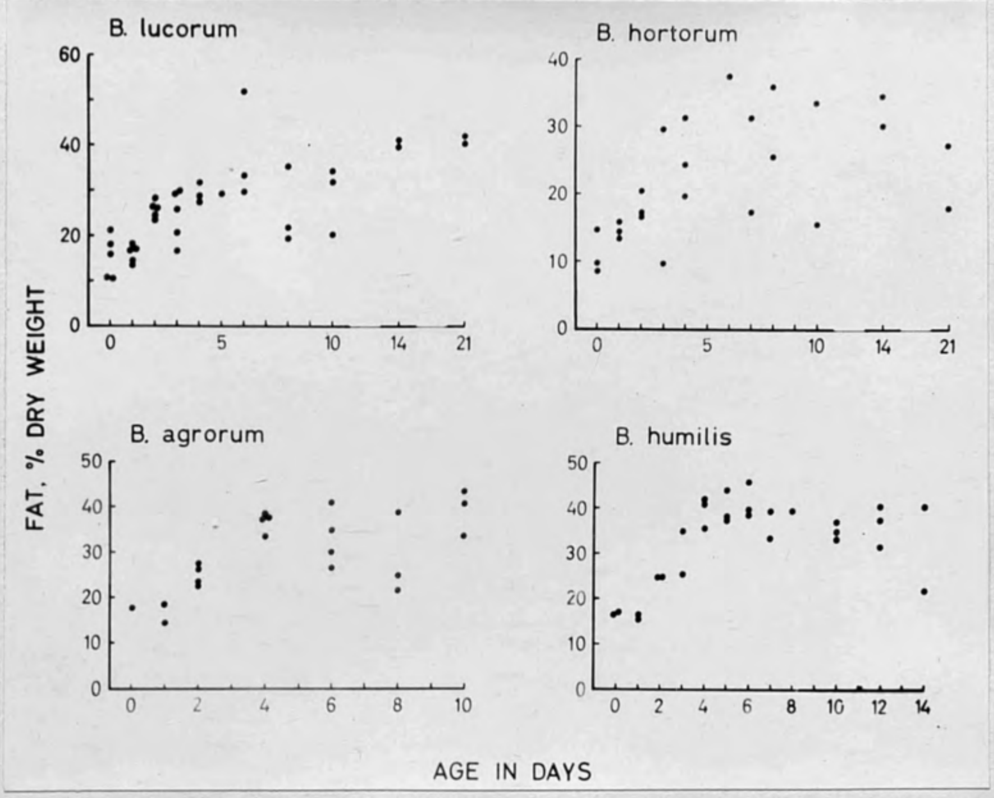
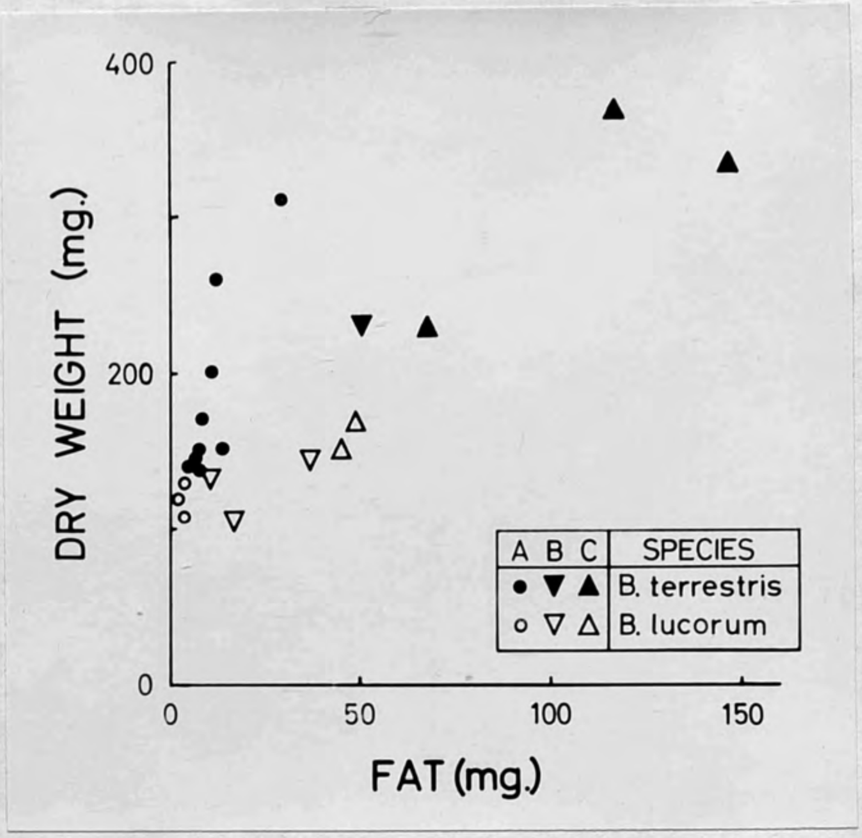


Fig. 2.16. Fat content of gynes fed on various diets.



A - Bees fed on pollen-free diet from time of emergence from pupa.
B - Bees fed on pollen-free diet from callow stage.
C - Control bees fed on both pollen and sugar syrup.

All bees fed for 10 days on 50% R.I. sugar syrup and kept caged at room temperature.

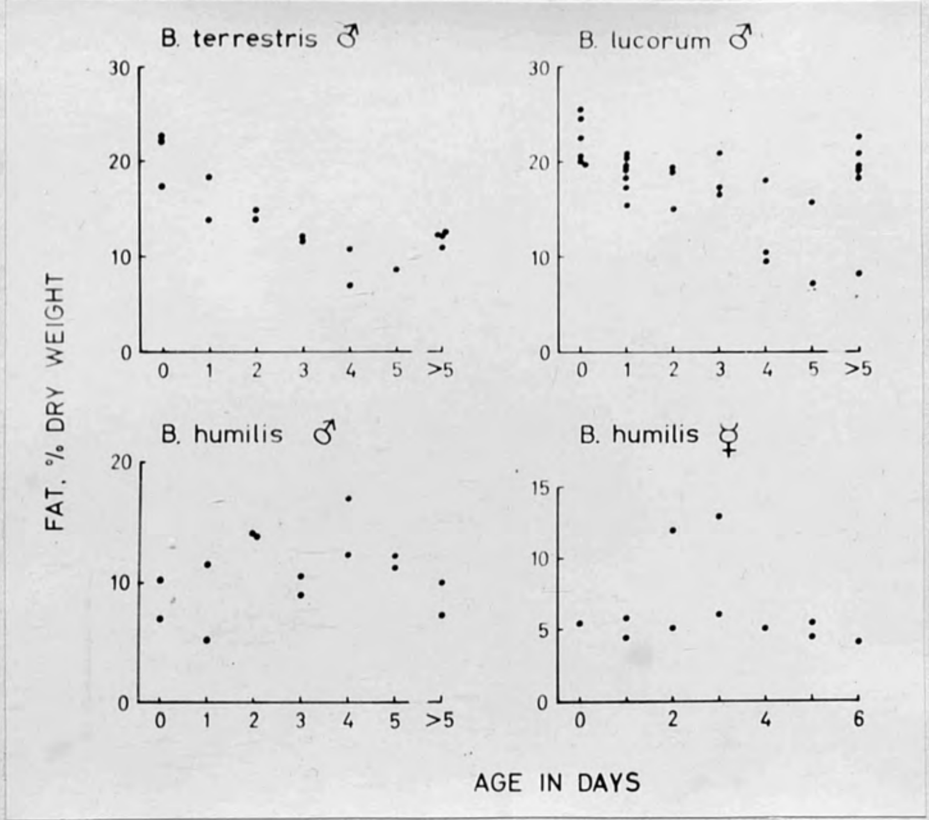
colonies. The difference between the distribution of the samples in Fig. 2.16 indicates the importance of pollen in the diet of newly emerged gynes.

Fat forms about 34% of the dry weight of gynes with well developed fat bodies (Fig. 2.14). This figure is attained by the experimental groups in the first 4 to 6 days of adult life. After this period the value fluctuates somewhat, although it is generally within the limits of the figures obtained for non-experimental gynes (Appendix Table XVI). So far as the actual fat content is concerned, this too attains the mean level of the non-experimental samples for comparable species within a period of 6 days from emergence. The exception to this is found in the B. hortorum experiment where the gynes never attained the level of fat content of the non-experimental samples, although the maximum was reached in 6 days.

ii. Males.

In both experiments involving males of "pollen-storers" (B. terrestris and B. lucorum) the % of fat in the dry weight of the bees was reduced following their emergence from the cocoon, and such a reduction persisted for the whole period of the experiment (Fig. 2.17). The amount of fat in the dry weight of older males from the same colonies as the experimental bees, which had been confined in the nest boxes during the experimental period, was similar to that of the 3-day old male sample, and higher than that of the 4- and 5- day old samples, but not significantly so.

Fig. 2.17. Fat content of male and worker bumblebees of various ages.



Results for males of B. humilis were different in that there was no reduction in the fat content following their emergence from the cocoon. However, these males were consistent with those of other "pocket-makers" (B. hortorum and B. agrorum (Fig. 2.13)) in having little fat in their bodies at the time of emergence.

iii. Workers.

Little fat was present in any of the experimental worker bees of B. humilis, irrespective of the age of the bees, and as shown in Fig. 2.17, unlike queens, there was no build up of fat reserves following their emergence from the cocoon. No opportunity was afforded to examine experimentally the fat content of young workers of "pollen-storers".

8. Discussion.

As well as being an important storage organ, the insect fat body is a centre of intermediary metabolism, performing many functions in both larvae and adults, and also during metamorphosis (Kilby, 1963; Wigglesworth, 1965; and others). The accumulation of pigments in oenocytes is widespread, and has been interpreted as indicating that these cells perform a regulatory or excretory function. Oenocytes are generally enlarged in adults with developed gonads and as in bumblebees, this enlargement may be particularly evident in females with developing ovaries (Wigglesworth, 1965). A correlation between the size of the oenocytes of young worker honey bees and the height of their wax-secreting epithelial cells has been observed by Kramer & Wigglesworth (1950), Freudenstein (1960), and others, and the fat body of honey bees is known to be concerned in the synthesis of wax secreted by the abdominal wax glands (Rösch, 1930; Reimann, 1952; Piek, 1964). It is likely that a similar relationship between the fat body and wax production occurs in female bumblebees.

The metabolic reserves stored in the fat body of adult bumblebees are glycogen and fat. Although albumenoid granules occur in the trophocytes of newly emerged bumblebees, protein is not stored as a food reserve in the trophocytes of hibernating queens as is mistakenly implied by Palm (1948). Protein is present in the trophocytes of overwintering honey bees (Köhler, 1921) but these deposits are not utilized as an important source of energy during the winter months but are eventually elaborated into brood food which is later fed to larvae as a secretion of the hypopharyngeal glands. The albumenoid granules in the trophocytes of young bumblebees, which are the remains of those protein substances elaborated during metamorphosis for use in the formation of adult tissues, show similar properties to the protein deposits in the fat bodies of honey bees (Schnelle, 1924) and other Hymenoptera (Schmieder, 1928) which also stain with both acidic and basic dyes.

The nature of the albumenoid granules in the fat bodies of insects has been discussed by Schmieder (loc. cit.) and Nair & George (1964), as has the synthesis and excretion of uric acid. The large vacuoles in the trophocytes of newly emerged bumblebees may possibly be associated in some way with the elimination of urates from the cells, following the deamination of amino acids during metamorphosis, since both the secretion and temporary storage of uric acid is known to occur in the insect fat body (Kilby, 1963; Stobbart & Shaw, 1964) and large vacuoles linked with urate production and excretion have previously been described in the fat cells of insects (see Wigglesworth, 1942, 1965). However, since the present investigations are not intended as a biochemical study these points will not be considered further.

The ability to lay down substantial food reserves in the fat body of adult bumblebees is restricted to queens and is an important caste difference. Cumber (1949b) has shown that while lavish feeding of autumn queens results in their increasing in weight, workers hardly gain in weight at all. Although the fat bodies of workers do not enlarge, under favourable conditions the feeding of even forager workers may enable their ovaries to develop (Free, 1957).

Normally the feeding of spring queens results in the rapid development of their ovaries but not the replenishment of fat body reserves. Bumblebees are inefficient at digesting pollen (Bailey, 1954) and it is probable that normally the majority of metabolites elaborated from the food of spring queens is used up by the day to day activities of the queens and by the nutritional requirements of the developing ovaries, so that little or none will be available for storage in the fat body. The substantial reserves of fat found in the anomalous 'foundress' queens with degenerate ovaries, probably accumulated because of the reduction in the nutritional requirements of the ovaries, so that once more metabolites were available for storage. These anomalous queens, like those which did not develop normally in the autumn, were probably affected by the unnatural conditions under which they were kept.

The quantitative differences in the amount of fat present in newly emerged bumblebees are related to the trophic conditions under which each was reared. Queens of all species examined, and males of "pollen-storers", all contained a relatively high proportion of fat in their bodies at the time of their emergence from the pupa, which indicates that during larval development adequate food was fed to them. Males of "pocket-makers" contained little fat in their bodies on their emergence from the pupa, suggesting that such bees are less well fed during the larval stage. So far as workers were concerned, such a difference was less obvious, but there was still a tendency for newly emerged workers of the "pollen-storers" to contain a greater proportion of fat than workers of the "pocket-makers", suggesting that the latter are less well fed.

The rapid reduction in the fat content of males of <u>B. terrestris</u> and <u>B. lucorum</u> following their emergence from the pupa may be explained by the reserves and metabolites of post-emergence feeding being utilized during maturation.

The higher value found in older males may indicate that maturity is reached so that once again metabolites may accumulate in the fat body. If this is so, then the position is similar to that suggested to explain the presence of substantial fat reserves in the anomalous 'foundress' queens. The results of the experiment with males of B. humilis do not follow this pattern, but these bees and all other males of "pocket-makers" contained little fat in their bodies at any time. Whether males with greater reserves of fat in their bodies at the time of their emergence from the pupa mature earlier than individuals which must rely mainly on post-emergence feeding for supplying the nutritional needs of the developing gonads, is not known, and there are apparently no data available in the literature to indicate at what age male bumblebees of various species become sexually mature.

Many insects lay down reserves of fat and glycogen in their fat bodies before their entry into hibernation (Lees, 1955) and according to El-Hariri (1966) glycogen instead of fat is consumed by hibernating Coccinellid beetles during periods of very low temperature, although in these insects, in common with many others, fat is the most important stored source of energy. Since glycogen is abundant in the trophocytes of hibernating bumblebees this reserve may also be important for their survival during periods of colder weather.

The presence of viable fat bodies in male bumblebees is in direct contrast to the condition found in drone honey bees which possess little or no fat body tissue (Freudenstein, 1924; Haydak, 1957). Unlike drone honey bees which are entirely dependent upon their parental colonies for their day to day nutritional requirements, male bumblebees, once they leave their colonies, exist quite independently in the field, and normally do not return to them (Free & Butler, 1959) although occasionally males of some species may do so (Frison, 1917, 1928). The fat body reserves are, therefore, probably important in sustaining male bumblebees in the field during, for example, periods of inactivity such as when at rest on flower heads during the night or during inclement weather. Foraging workers may also, on occasions, remain in the field overnight (Plath, 1934; Free, 1955a) and here too fat body reserves may be important for their survival.

PART III : THE INCIPIENT COLONIES OF BUMBLEBEES, WITH SPECIAL REFERENCE TO THOSE OF BOMBUS AGRORUM (FABR.).

1. Introduction.

Little information on naturally occurring bumblebee colonies at an early stage of development has been published, as previous workers have usually not found colonies in any numbers, until the latter are reasonably well established. Much of the knowledge of the early stages of colony development has been obtained from colonies founded in artificial domiciles or from laboratory initiated colonies, although Sladen (1912), Plath (1934), and others, have provided some data on natural incipient colonies of a few species.

Typically an incipient colony consists of a wax honey pot which is placed towards the nest entrance and a wax covered brood clump which, at least in the early developmental stages, consists of a lump of pollen or bee-bread (basically pollen, moistened with honey) upon which the larvae feed. The top of the brood clump is usually depressed along one axis, in line with the honey pot and nest entrance, forming an incubation groove along which the queen lies whilst incubating her brood (Sladen, 1912; Frison, 1929; Plath, 1934; and others). Such a colony is produced by a single foundress queen.

Several incipient colonies of surface-nesting species, mostly at the pupal stage of development, were obtained in 1965 and 1966, and it was considered that an analysis of this material would be of interest. In 1967, incipient colonies were collected in order to obtain information on the early incipient stages of natural colonies.

2. Methods.

a) Location of incipient colonies.

In order to find incipient colonies of surface-nesting species, searches were made adjacent to likely looking nesting sites for foraging queens with corbicular pollen. Usually only queens with reasonably large pollen loads were kept under observation as such bees were probably about to return to their nests. When a queen left for her colony, she was followed, or if this was not practical, the direction taken by the queen was noted (see later — "lining" method). In several instances queens foraging on Lamium album L., Aesculus hippocastanum L., Sarothamus sp., and Rhododendron sp., were subsequently followed directly to their nests. Queens returning to their colonies have a characteristic, heavy flight (Sladen, 1912), and often fly reasonably straight. One B. agrorum queen, for example, was followed across a field from a flower on which she had been foraging, to her nest more than 100 metres away, and at no time did she deviate more than a few degrees from a direct line to her nest and she was never more than 1.5 metres above the ground.

Coville (1890) considered that the method of "lining" was impractical for locating bumblebee colonies and Plath (1934) states that he was never able to find a colony in this way. However, this method was successfully employed in the present work to locate some incipient colonies, by lining on foraging queens returning to their nests. Also in the spring small colonies of both surface and underground-nesting species were found by lining on foraging workers, but the method was not successful later in the season.

Many incipient colonies were found by waiting in likely nesting areas for queens to return from foraging or for others to leave their nests. This method was most successful when a known foraging direction to, for example, an isolated patch of Lamium, or a lone Horse-Chestnut tree, was known. A few nests were also found by chance.

b) Management of material.

Having located a colony, the queen was captured. The nest was then placed in a pill box for transit back to the laboratory. Once in the laboratory the colonies were examined and the nest material discarded. Examination of young brood clumps was carried out under a dissecting microscope since with the naked eye it was often difficult to distinguish wax from pollen. Colonies at an early developmental stage were usually not kept, but most of those in the pupal stage were retained in order to obtain data on the 1st brood adults produced. These latter colonies were placed in small nest boxes and anchored to the floor of their box with melted bees wax. When the wax had hardened, each foundress queen was introduced. Queens began to incubate their brood clumps within a few minutes of being introduced. The boxes were kept at room temperature, and each was provided with a supply of pollen and sugar syrup, the latter being kept in a gravity feeder which was protruded through one wall of the box.

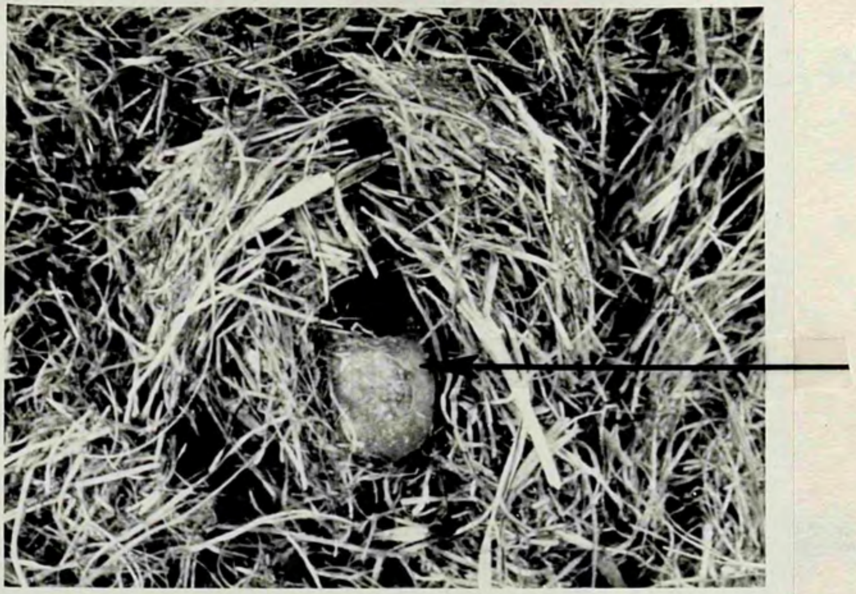
Boxes with pupal clumps were examined several times each day and newly emerged adults were removed, weighed on a Metler balance and their left fore wing (following Cumber, 1949b) measured to the nearest half millimetre, from the centre of the tegula to the wing tip. The cell from which each individual had emerged was noted. Bees were then marked and returned to their respective boxes. Foundress queens were weighed and measured after several days in confinement. If a queen died or was not captured, a wild queen of the same species was introduced into the queenless box.

3. Results.

Seventy seven incipient colonies, belonging to 5 species (B. pratorum, 2; B. ruderarius, 1; B. hortorum, 22; B. agrorum, 45; B. humilis, 7), were obtained during 1965, 1966 and 1967 (Appendix Table I). The number of eggs or larvae in incipient colonies (excluding those with incomplete egg clumps) of various species is given in Table 11, and the number of pupal cells present in incipient colonies is given in Table 12.

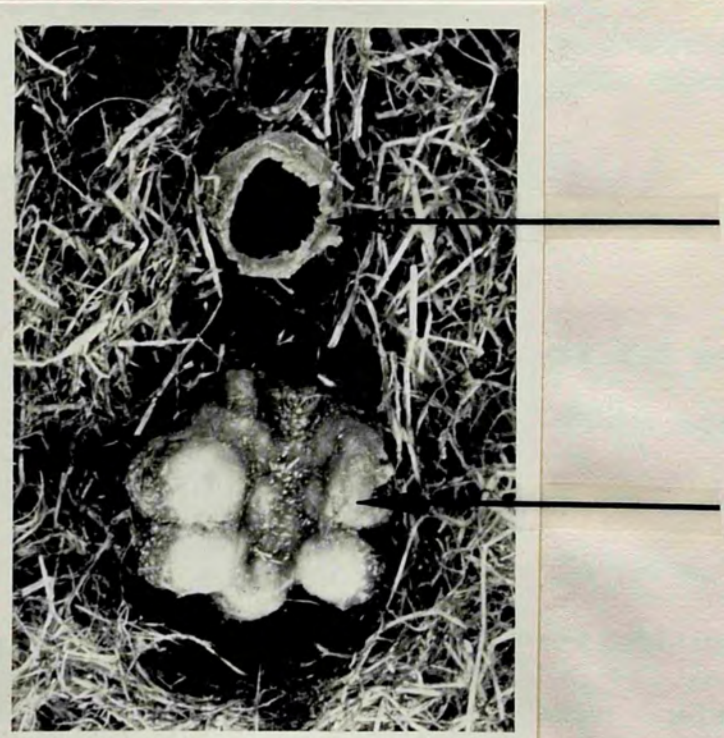
In all nests examined, the honey pot was found at the entrance to the nest chamber (Fig. 3.1), and all incubation grooves were orientated with their long axis in line with the nest entrance and honey pot (Fig. 3.2).

Fig. 3.1. B. agrorum (Col. 130) nest with some nest material removed to show honey pot at entrance to nest chamber.



(x 1)

Fig. 3.2. B. hortorum (Col. 143) incipient colony at pupal stage in plan view.



(x 1)

Table 11 — Number of eggs or larvae in completed incipient brood clumps of various species.

Species	Number present														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B. pratorum	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
B. hortorum	-	-	-	-	1	-	1	1	-	1	2	1	2	2	-
B. agrorum	-	-	1	-	1	1	4	3	1	-	-	-	-	-	-
B. humilis	-	-	-	-	-	-	1	2	-	-	1	-	-	-	-

Table 12 — Number of pupal cells in incipient brood clumps of various species.§

Species	Number present												
	1	2	3	4	5	6	7	8	9	10	11	12	13
B. ruderarius	-	-	-	-	-	1	-	-	1	-	-	-	-
B. hortorum	-	-	-	-	-	-	-	2	1	-	3	1	-
B. agrorum	1	-	1	-	2	9	7	10	2	1	-	-	-
B. humilis	-	-	-	1	-	-	2	-	-	-	-	-	-

§ :- Includes some data obtained from colonies past the incipient stage, but where incipient cells were still discernible.

Several "nests" were found before any wax had been secreted or pollen collected by the foundress queen. These have not been included in the colony totals and have not been plotted on the distribution map (Appendix Map 'A'). In each, the nest material had been manipulated to form a central nest chamber and the inner parts were reasonably dry and warm. Although these nests were left in situ with their queens, all but two were subsequently deserted.

In a few cases in colonies which were too advanced to be classified as incipient, data on the incipient pupal cell arrangement were obtained.

Data from the various species are dealt with separately, and for convenience, the order of treatment is not arranged systematically. All figures of incipient clumps in plan view are orientated with the nest entrance (and honey pot) towards the top of the page.

a) Data from *B. agrorum* colonies.

i. Pre-brood stage.

One colony was found before any eggs had been laid. A small quantity of pollen was present in the centre of the floor of the nest chamber, but there was no sign of a honey pot.

ii. Egg stage.

Unfinished egg clumps were found in three colonies. In Col. 127 one egg had been laid, but a honey pot had not been initiated (Fig. 3.3). Colonies 121 (Fig. 3.4) and 124 (Fig. 3.5 a) both contained two eggs. Although in the former colony there was no sign of a honey pot, in the latter the floor of a honey pot was under construction. In all three clumps the eggs were covered over by wax, but some of the pollen lump was still exposed. The queens of these colonies were still collecting pollen, which indicated that egg laying had begun before the pollen lump was completed.

Fig. 3.3. *B. agrorum* (Col. 127). Incomplete incipient egg clump with single egg.

(x 1.5 approx.)

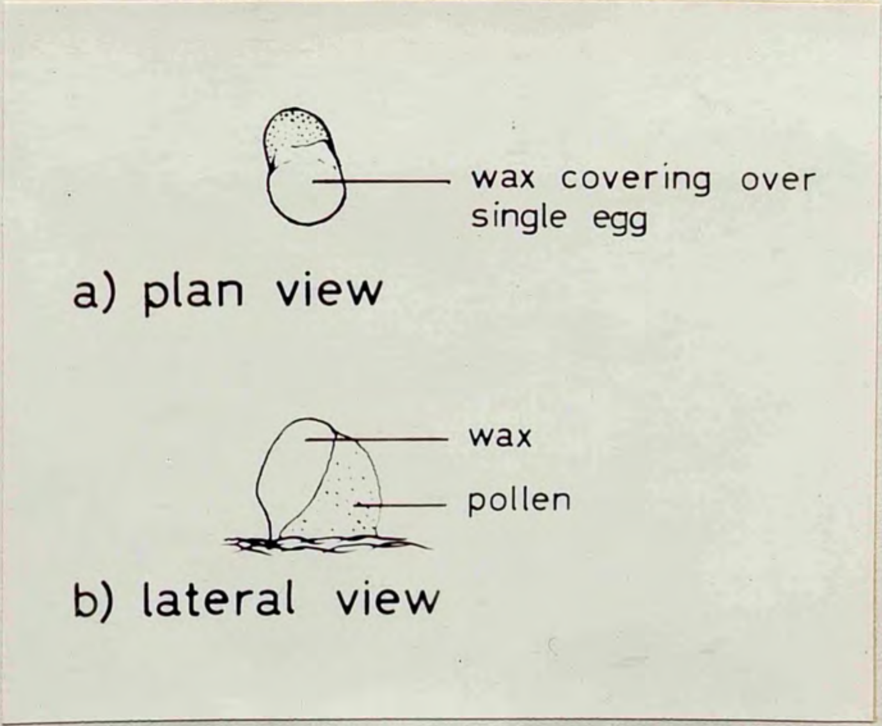


Fig. 3.4. B. agrorum (Col. 121). Incomplete incipient egg clump with two eggs.

(x 1.5 approx.)

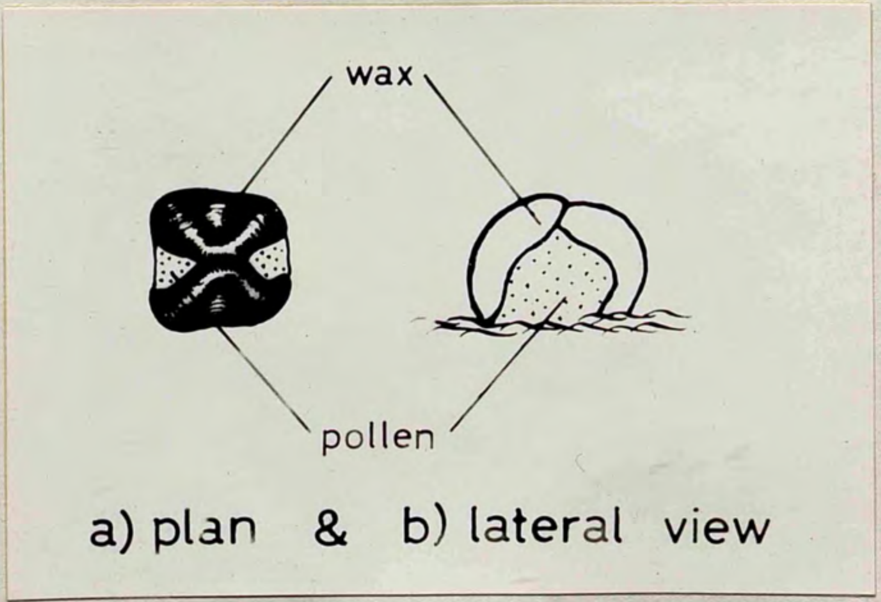


Fig. 3.5. B. agrorum (Col. 124). a) Incomplete and b) complete incipient egg clump.

a) With 2 eggs.

b) With 7 eggs.

(x 1.5)

Colony 124 was left in situ in the field with its queen and examined one day later. Seven eggs were then present and the egg clump was completely covered with wax. The honey pot was also nearing completion (Fig. 3.5 b). No further eggs were laid in this colony.

Completed egg clumps were found in six colonies. Although the precise size and shape of the egg clumps varied according to the number and arrangement of egg present, the outline of each was generally rounded and tended to be concave on the leading and hind edges (Fig. 3.6). The clumps in <u>B. agrorum</u> measured approximately 6 x 5 mm. and were about 4 mm. in height. At each corner, beneath each clump, were thin pillars of wax which merged with the wax canopy of the clump and anchored it to the nest material on the floor of the nest chamber. The distance from the centre of the honey pot to the centre of the egg clump ranged from 12 to 18 mm..

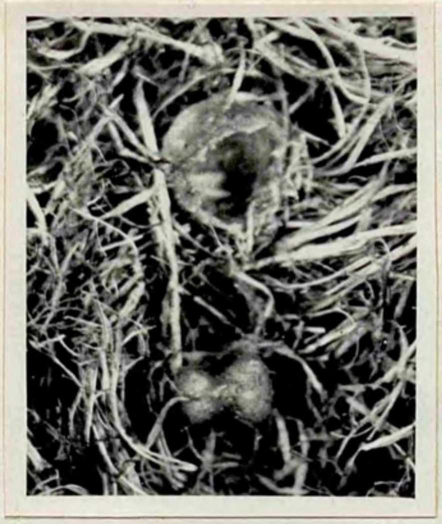
The number of eggs present, and their arrangement in each case, is shown in Fig. 3.7. Eggs were orientated more or less vertically although they were slightly inclined towards the central pollen lump around which they were laid. The eggs were not, therefore, lying upon a bed of pollen. The pollen lump was relatively small and shaped like a four sided pyramid. Observations suggest that the arrangement of eggs, as found in Cols. 51 and 117, with a central row of 2 eggs (each against one face of the pollen lump) and two lateral rows of 3 eggs (each row against the lateral faces of the pollen lump) is typical of this species. Further, in other colonies where fewer than 8 eggs were present the pattern was similar to the arrangement described above with one or more eggs deleted. In Col. 115, where there were 9 eggs, the ninth egg was lying on top of the pollen lump while the remaining eight eggs were arranged as in Cols. 51 and 117 (Fig. 3.7).

iii. Larval stage.

Three clumps containing young larvae were obtained. Each larva was feeding upon the pollen lump which was being added to by the queen from below, via lateral pollen pockets. The larvae in each clump were arranged as shown in Fig. 3.8. Those in Col. 125 weighed 10.8/10.4/8.1/8.0/7.7/7.1 & 6.4 mg., and the clump measured approximately 9 x 8 mm., and was 11 mm. high. The larvae in Col. 130 weighed 8.6/8.2/4.9/3.1/2.8/2.2 & 2.0 mg.. This clump measured 7 x 6.5 mm., and was 6 mm. high. The top of each clump was depressed slightly along one axis, forming a shallow incubation groove. Colony 1 contained 7 slightly more advanced larvae, but they were not weighed. The clump measured 16 x 14 mm., and was 13 mm. high. In this clump a distinct incubation groove was evident. In all three clumps the centrally placed larvae were the largest individuals (Fig. 3.8).

One colony with more advanced larvae was obtained (Col. 119). There were only 3 larvae, all of similar size, in the clump and each was enclosed in a separate cell within the brood clump. There was no incubation groove in this colony.

Fig. 3.6. Completed incipient egg clumps of B. agrorum.



Col. 129 (6)

Col. 128 (7)

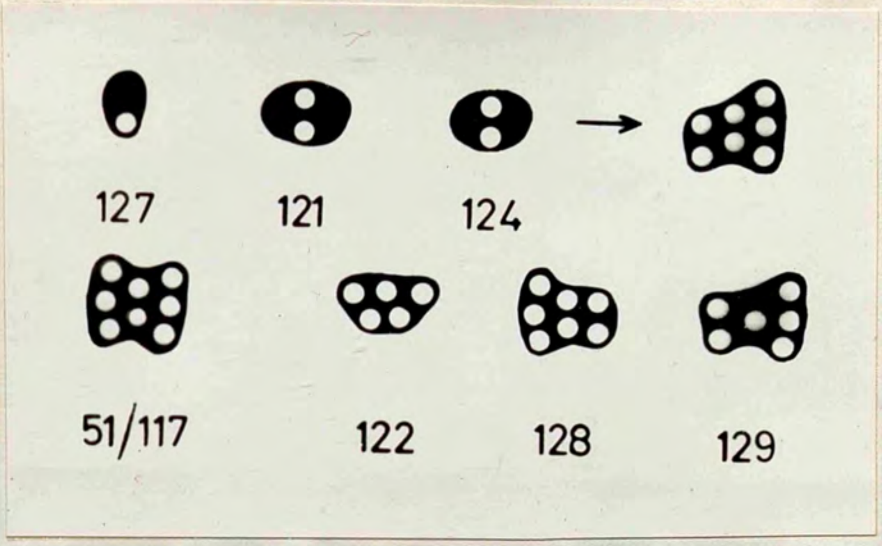
Col. 117 (8)

Col. 115 (9)

Number of eggs given in parenthesis.

(x 2 approx.)

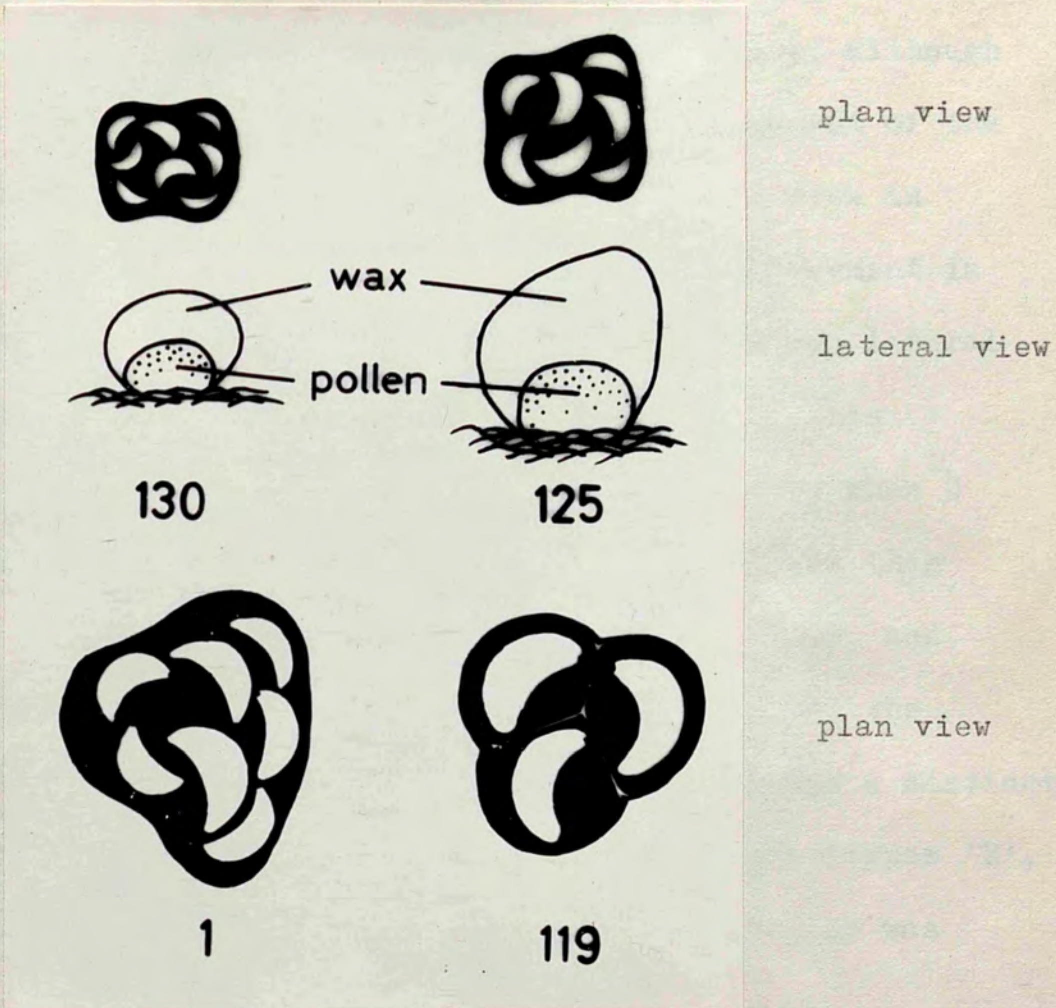
Fig. 3.7. Arrangement of eggs in incipient clumps of *B. agrorum*.



115

Colony number given beneath each clump.

Fig. 3.8. Sketches of incipient larval clumps of *B. agrorum*.



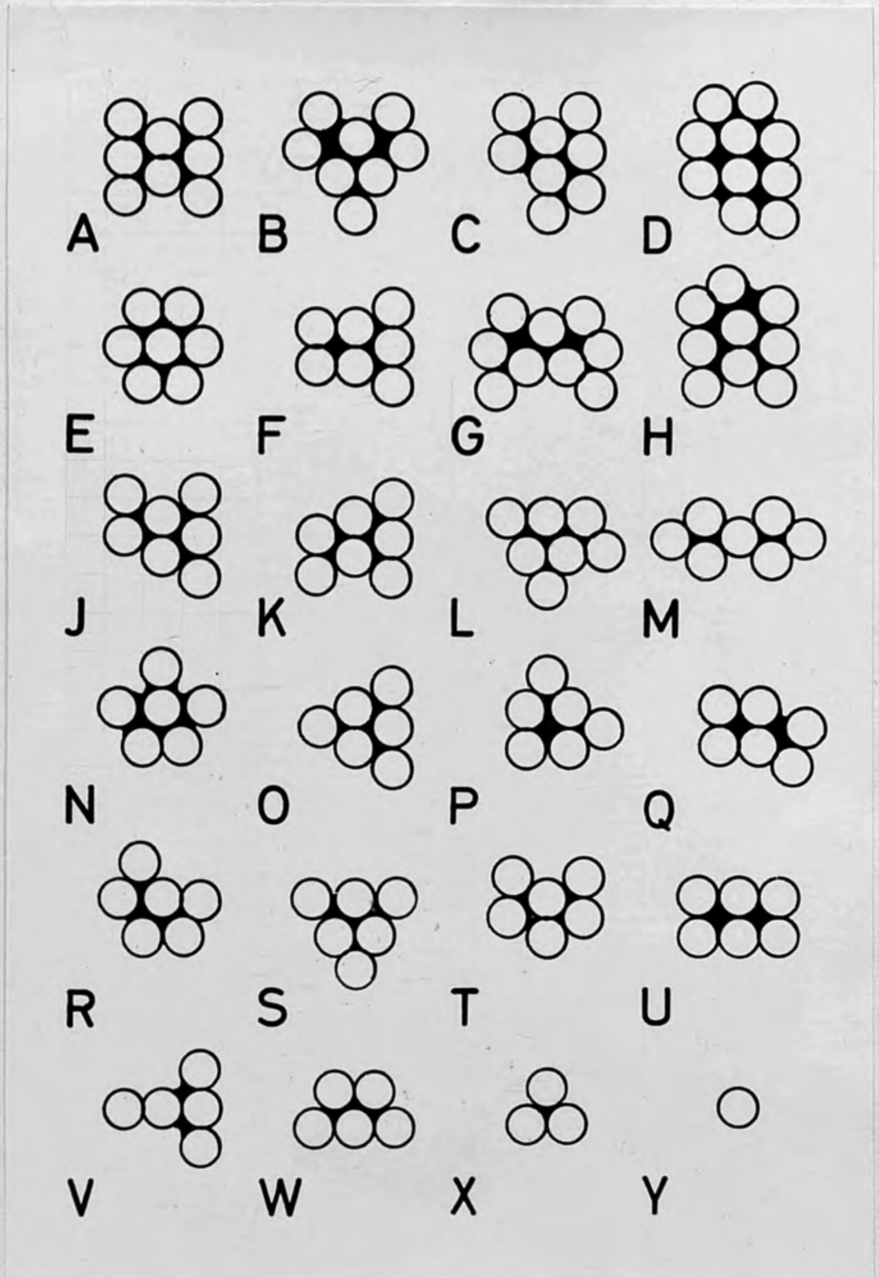
Colony number given beneath each clump.

iv. Pupal stage.

Data from 33 pupal clumps of B. agrorum were obtained. In most clumps (Table 12) there were 8 pupal cells (mean, 7). Only rarely were more than 8 cells present in a clump, although 6 or 7 cells frequently occurred. The cell arrangement of the various clumps in the pupal stage is shown in plan view in Fig. 3.9. Type 'A' appeared to be the typical arrangement in this species. In this type, cells are arranged in two lateral rows of 3 cells each and a central row of 2 cells. This arrangement is identical to that of eggs in egg clumps when 8 are present. The central row of pupal cells is lower than the rest, so forming the incubation groove of the clump, and the lateral cells tend to be inclined somewhat towards the centre of the clump (Fig. 3.10). In most pupal clumps a distinct incubation groove was present, but in a few instances (types 'E', 'N', 'W', 'X' and 'Y'(Fig. 3.9)) little or no impression was formed.

Egg cells were present on the lateral pupal cells of some colonies, but not in those headed by foundress queens parasitized by Syntretus splendidus. In Col. 3 (Fig. 3.10) some second generation larvae were developing, yet only two 1st brood adults had emerged. More usually, most if not all of the first generation workers were available to assist the queen in the rearing of the next brood batch.

Fig. 3.9. Arrangement of cells in incipient pupal clumps of *B. agrorum*.

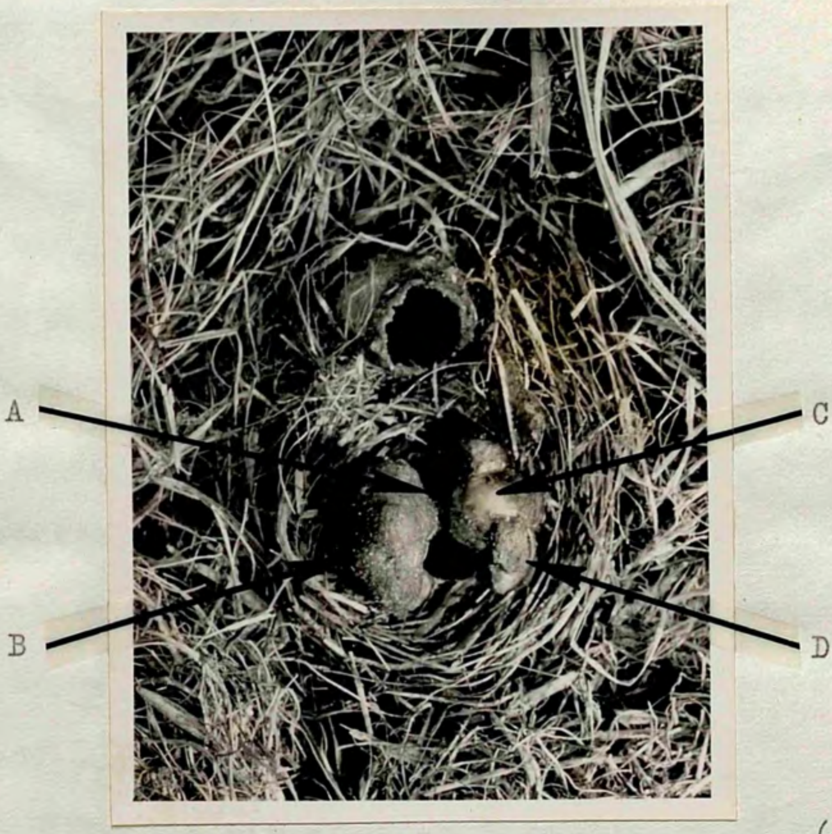


Clumps are shown in plan view and orientated with the nest entrance towards the top of the page. For simplicity each cell is drawn the same size.

One colony of each type was found except for the following:-

A - 8 colonies; E - 2 colonies; O - 2 colonies.

Fig. 3.10. Incipient colony of B. agrorum (Col. 3) at late stage.



(x 1)

A - Central row of 2 emerged cells under incubation groove.

B - 2nd brood larval clump with lateral pollen pocket.

C - Lateral pupae yet to emerge.

D - 2nd brood egg cell.

---

v. Details of 1st brood adults.

The size of bees, their order of emergence, and the position in the clump from which each emerged, are given for each colony in Appendix Table XXXI.

All first generation adults produced were workers with the exception of one male which was reared in Col. 6.

It was possible in most pupal clumps to distinguish arbitrarily a set of "central" cells and a set of "peripheral" cells. Such a division was based on the relative position of the cells to one another, bearing in mind the probable original centre of the larval food lump. Bees emerging from central cells and peripheral cells are referred to in the following account as "central" and "peripheral" bees respectively.

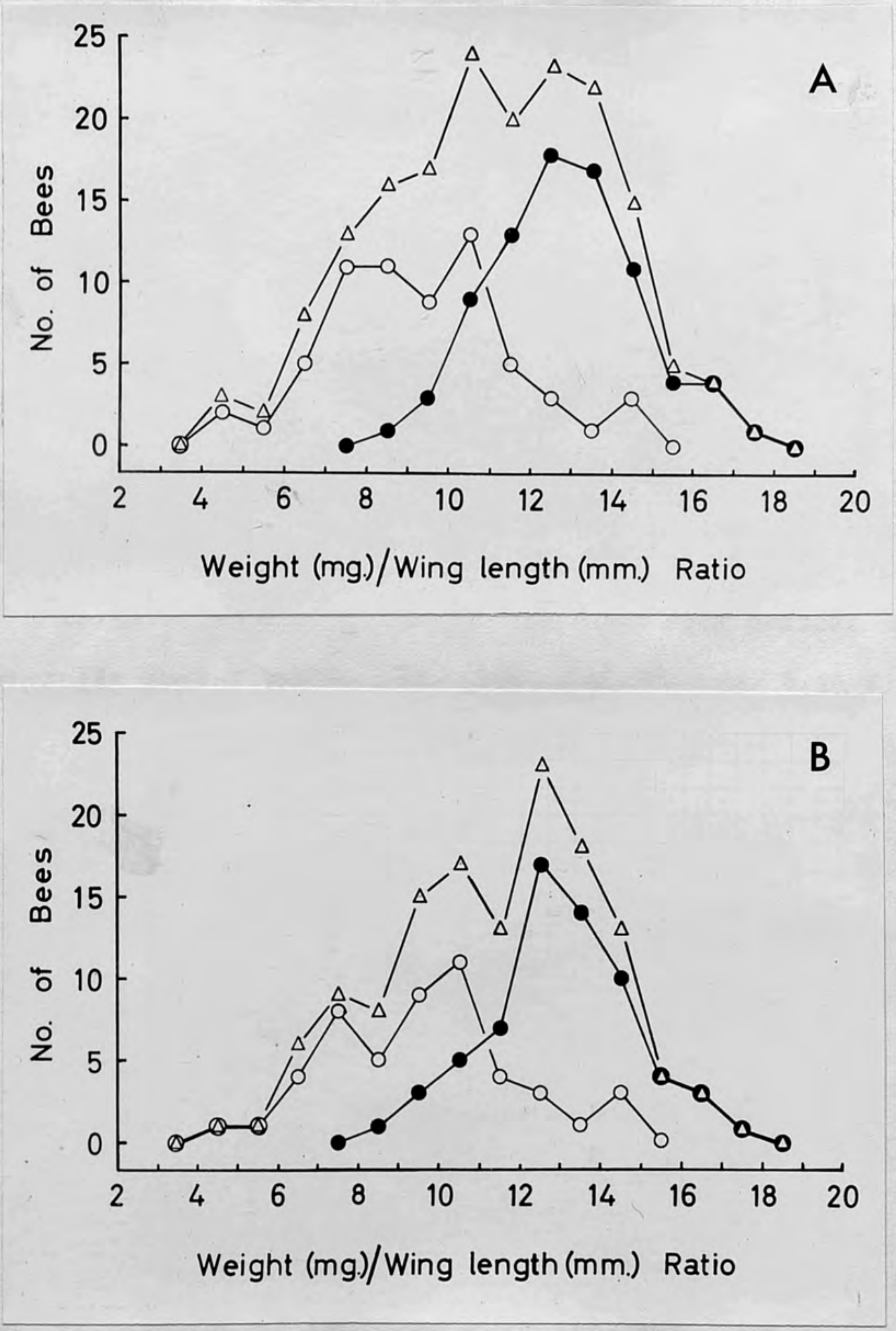
Central bees were significantly heavier than peripheral bees ($p < 0.001$). A frequency distribution by weight for the progeny of healthy (unparasitized) queens is given in Table 13. As shown in the Table there is a similar bimodality in the weight distribution of the bees for both the 1965 and 1966 samples, which is clearly attributable to the position in the clump where they were reared.

The progeny of healthy queens only are included in Table 13 since it is known (see Part IV) that such bees are significantly heavier than the progeny of <u>Syntretus</u>-parasitized queens and the effect of their inclusion would be to mask the bimodal distribution. If, however, weight is divided by wing length, a bimodality is restored which is similar in distribution whether the progeny of *Syntretus*-parasitized queens are included or not (Fig. 3.11). As with the frequency distribution based on weight, the bimodality is clearly a result of the size difference between peripheral and central bees. Although the difference between the size (weight/wing length) of the progeny of healthy and parasitized queens is not significant ($p > 0.4$), a significant difference does exist between the size of all central and all peripheral bees ($p < 0.01$).

Table 13 — Frequency distribution by weight of bees which emerged from incipient brood clumps of healthy B. agrorum queens.

Weight (mg.)	1965	1966	Bees from central cells	Bees from peripheral cells
0 - 19	-	-	-	-
20 - 39	-	2	-	2
40 - 59	5	4	-	9
60 - 79	7	9	3	13
80 - 99	10	12	5	17
100 - 119	8	9	15	2
120 - 139	13	13	23	3
140 - 159	8	9	14	3
160 - 179	2	4	5	1
180 - 199	1	-	1	-
200 - 219	-	-	-	-

Fig. 3.11. Size of adults produced in incipient colonies of B. agrorum.



A - Progeny of healthy and Syntretus-infected queens.

B - Progeny of healthy queens only.

△ - central + peripheral bees

● - central bees only

○ - peripheral bees only

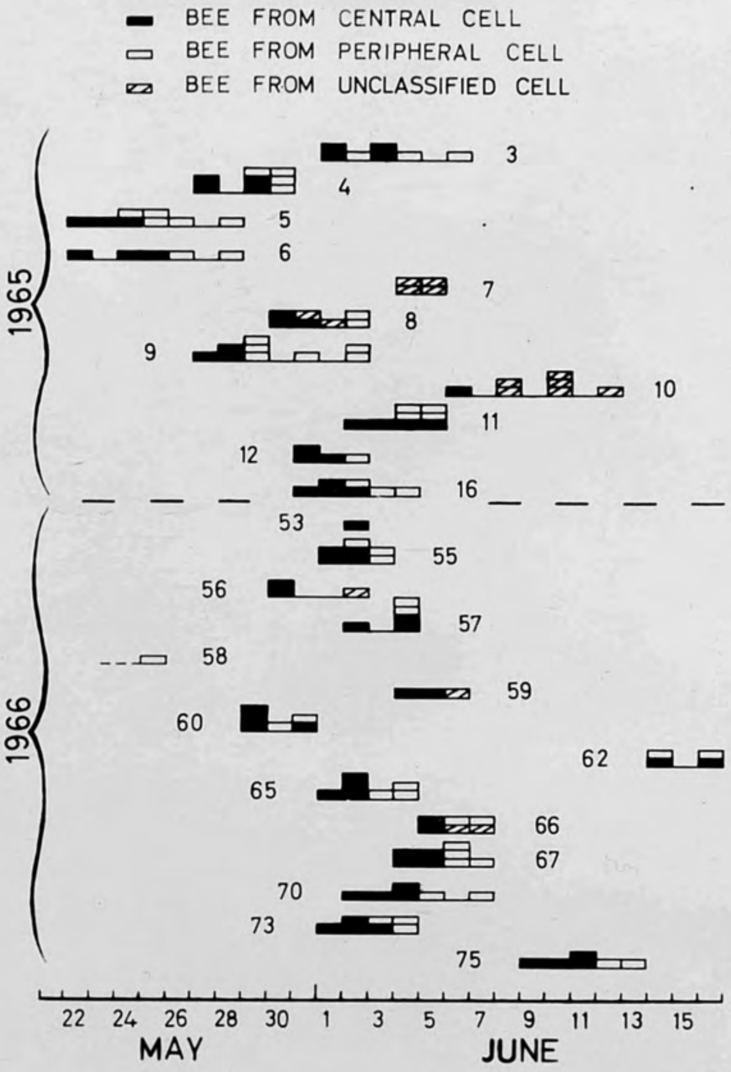
Information on the order and time of emergence of individual bees (Appendix Table XXXI) is summarized for each colony, where details are known, in Fig. 3.12. The number of bees emerging from brood clumps each day, is shown for both 1965 and 1966 samples in Fig. 3.13. The highest 3-day mean of emergence calculable for 1965 occurs from the 31st May to the 2nd June, while that for 1966 occurs from the 2nd to the 4th June. The highest 5-day means occur from the 31st May to the 4th June (1965) and from the 2nd to the 6th June (1966). Although bees tended to emerge later in 1966 than in 1965, the difference between years was not significant ($p > 0.3$).

Figs. 3.14 and 3.15 show that bees from central cells generally emerge before peripheral bees. Fig. 3.15 further indicates that larger (heavier) bees tend to emerge before smaller (lighter) bees. The plotted regression line is for all bees and the coefficient $b = -13.661 \pm 0.289$. The coefficients (b) for central bees only and peripheral bees only, are -1.095 ± 0.123 and -15.929 ± 0.136 respectively.

vi. Foundress queens.

The largest foundress queen of B. agrorum weighed 468 mg. (wing length, 14 mm.), and the smallest 234 mg. (w.l., 13 mm.) (Appendix Table XXXIV). No correlation was found between the size of a queen and the size or number of bees produced in an incipient brood clump. As stated previously, however, queens parasitized by Syntretus did produce smaller bees.

Fig. 3.12. Summary of data of adult emergence from incipient colonies of *B. agrorum*.



Colony reference number given against each emergence histogram.

Fig. 3.13. Period of emergence of adults from incipient colonies of *B. agrorum*.

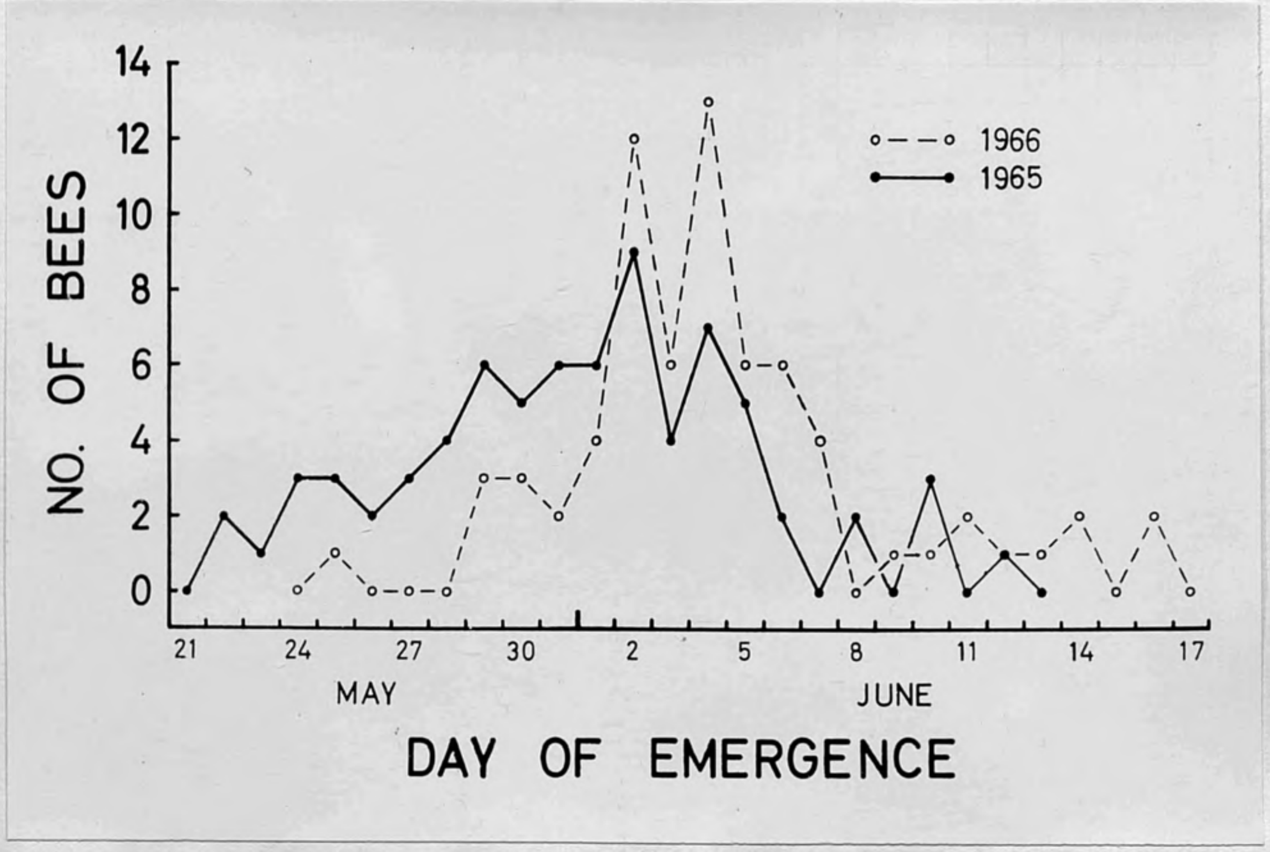
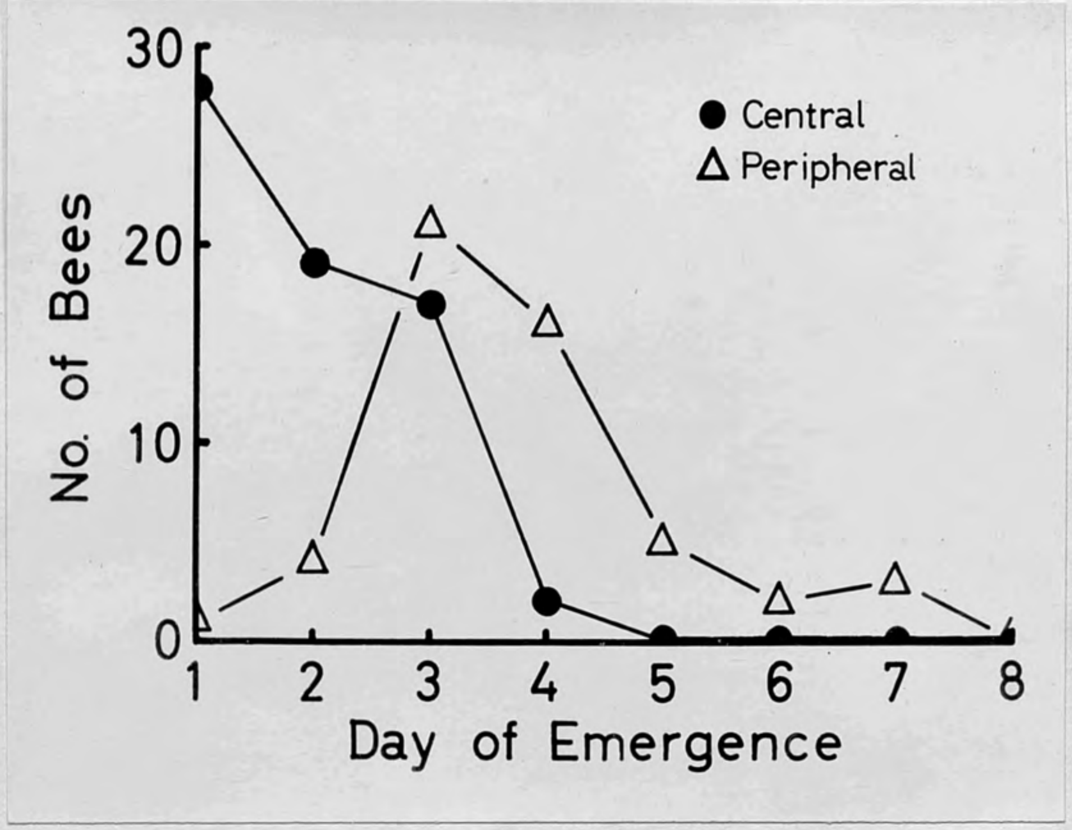
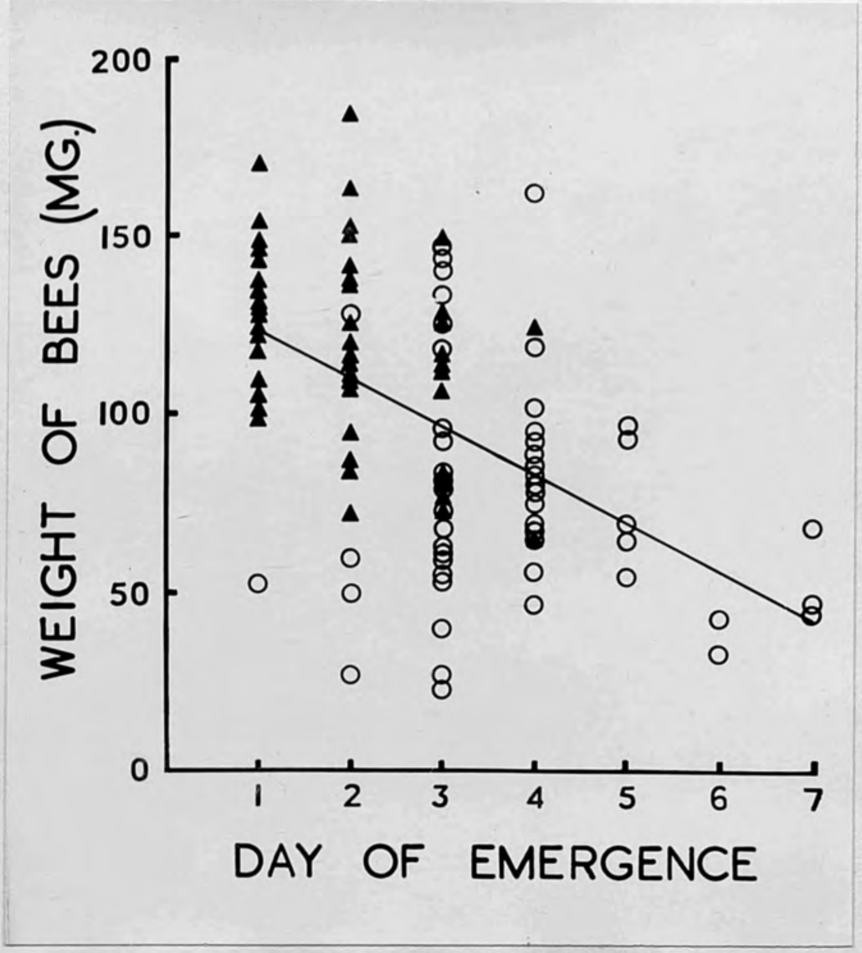


Fig. 3.14. Emergence of adults from incipient pupal clumps of *B. agrorum*.



The day on which the first bee from each clump emerged is recorded under 'day 1' as are other individuals of the same clump emerging on that day. Subsequent emergences are recorded under the appropriate day in relation to the date of emergence of the first bee in the clump.

Fig. 3.15. Relationship between weight and time of emergence of adults from incipient colonies of *B. agrorum*.



Bees emerging from central cells are represented by triangles and bees from peripheral cells by circles. The plotted regression line is for all bees.

b) Data from B. humilis colonies.

i. Egg stage.

The egg clumps of B. humilis were identical in appearance to those of B. agrorum (Fig. 3.16). In two cases 8 eggs were present which were arranged in the same way as the 8-egg type of B. agrorum, but in a third clump (Col. 144) there were 11 eggs arranged as a central row of 2 eggs and lateral rows of 4 and 5 eggs. Egg orientation was the same as in B. agrorum.

ii. Larval stage.

Only one larval clump was found. This measured 12 mm. x 7.5 mm. and contained seven larvae which were feeding upon a bed of pollen (Fig. 3.17). The larvae weighed 59.5/39.4/20.8/18.6/18.0/10.5 & 7.1 mg., and those in the centre of the clump were the largest. As with the larval clumps of B. agrorum, there were lateral pollen pockets beneath the clump.

iii. Pupal stage.

Three colonies in the pupal stage were found. There were 7 cells in two of these and 4 in the other, and the cell arrangement was as shown in Fig. 3.18.

iv. 1st brood adults.

The size and order of emergence of adults and the position in the clump from which each emerged is given for Col. 116 in Appendix Table XXXII. As with B. agrorum, the larger bees emerged first and from the central cells of the brood clump.

Fig. 3.16. Egg clumps of incipient colonies of *B. humilis*.

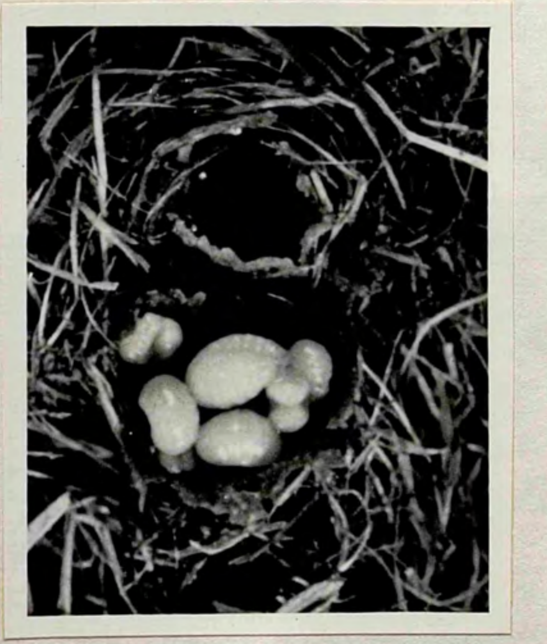
Col. 149 (8)

Col. 144 (11)

Number of eggs given in parenthesis.

(x 2 approx.)

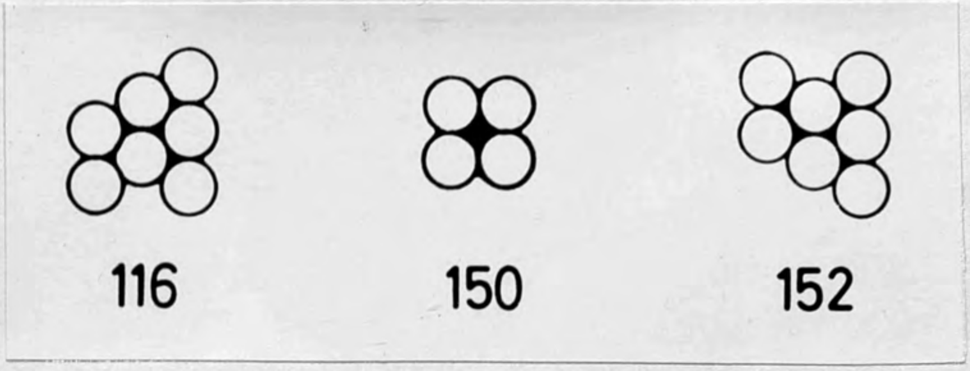
Fig. 3.17. Larval clump of incipient colony of B. humilis (Col. 145).



In lower picture clump has been broken open to display larvae.

(x 2)

Fig. 3.18. Arrangement of cells in incipient pupal clumps of *B. humilis*.



Clumps are shown in plan view and orientated with the nest entrance towards the top of the page. For simplicity each cell is drawn the same size. The colony number is given beneath each clump.

c) Data from B. pratorum colonies.

i. Egg stage.

The initial egg clump of Col. 114 is shown in Fig. 3.19. It measured 6.5 x 6 mm. and was 3 mm. in height. Unlike the clumps of B. agrorum and B. humilis it was longer than wide. Also, the enclosed pollen lump was larger than those found in egg clumps of the previously mentioned species and it was not pyramidal in form but more or less cushion-shaped. The eggs were almost vertically placed but inclined slightly towards the pollen lump. There were 5 eggs against the leading edge of the pollen lump and 5 along the rearward edge, but none laterally. The honey pot of this colony was complete (Fig. 3.19).

ii. Larval stage.

Only one incipient colony of B. pratorum (Col. 113) was found at the larval stage. This colony contained a small brood clump measuring about 9 x 8 mm. which was 4 mm. in height. Unlike the egg clump of Col. 114, the clump was noticeably depressed centrally along one axis (Fig. 3.20). Within the clump were 10 small larvae which were feeding upon the central pollen lump.

Fig. 3.19. Incipient egg clump of B. pratorum (Col. 114).

(x 1.5 approx.)

Fig. 3.20. Incipient larval clump of B. pratorum (Col. 113) at very early stage of development.

(x 1.5 approx.)

d) Data from B. hortorum colonies.

i. Pre-brood stage.

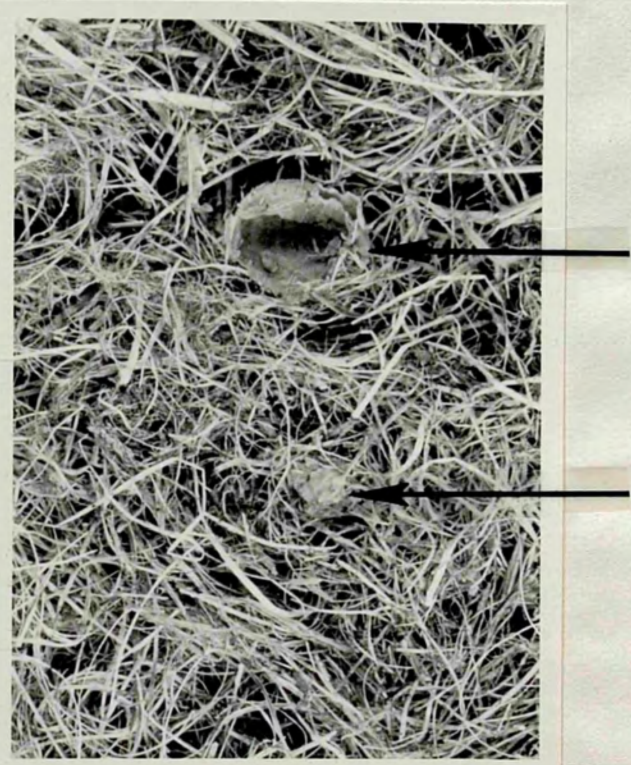
In Col. 118 a small quantity of pollen was found in the centre of the floor of the nest chamber. A honey pot was also under construction (Fig. 3.21). At the time of capture, the foundress queen had just returned to the nest with more pollen.

ii. Egg stage.

Three colonies with incomplete egg clumps were found (Fig. 3.22). They contained 8, 3 and 4 eggs, which were arranged as shown in Fig. 3.23. In Col. 120 some pollen was still exposed to view, but in the other two colonies the pollen was covered by a layer of wax. In all three cases a honey pot had been started, but in none was it as advanced as that in Col. 118.

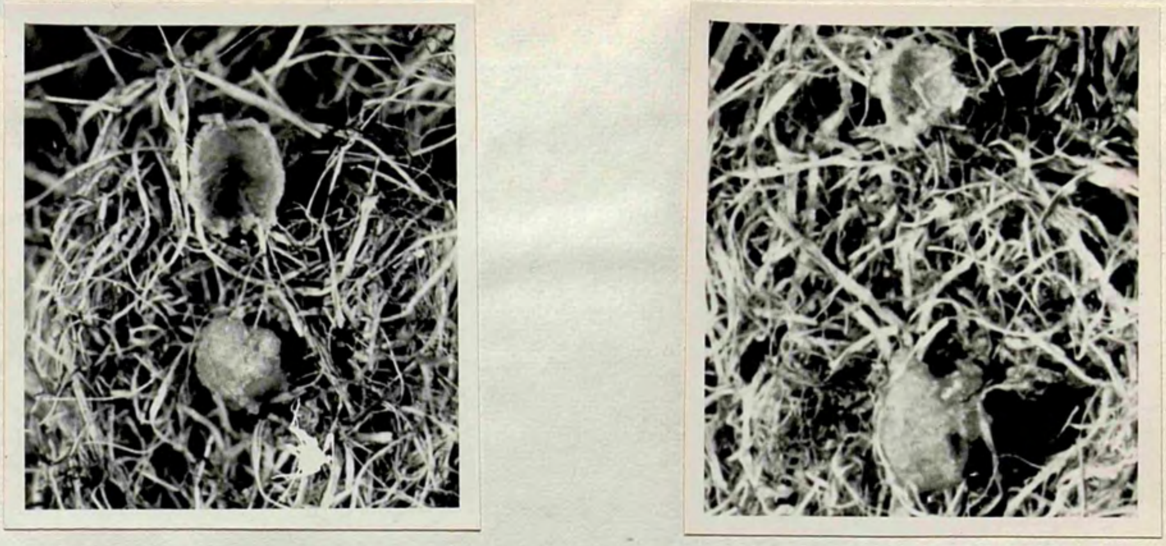
Complete egg clumps were found in four colonies. The eggs in these were arranged as shown in Fig. 3.23, and numbered from 8 to 14 per clump. As with all previous species studied, the eggs of <u>B. hortorum</u> were more or less vertically placed. They were, however, slightly inclined towards the anterior end of the clump. Characteristically in this species, each egg occupied a separate position within the pollen lump and they were not arranged around the perimeter. Most eggs were roofed over by pollen and since the pollen lump was deeper anteriorly the eggs were not all found at the same level. Although the unfinished clumps of Cols. 132 and 135 lacked any definite shape, that of Col. 120, which was slightly more

Fig. 3.21. Pre-brood stage of incipient colony of *B. hortorum* (Col. 118).



(x 1.5)

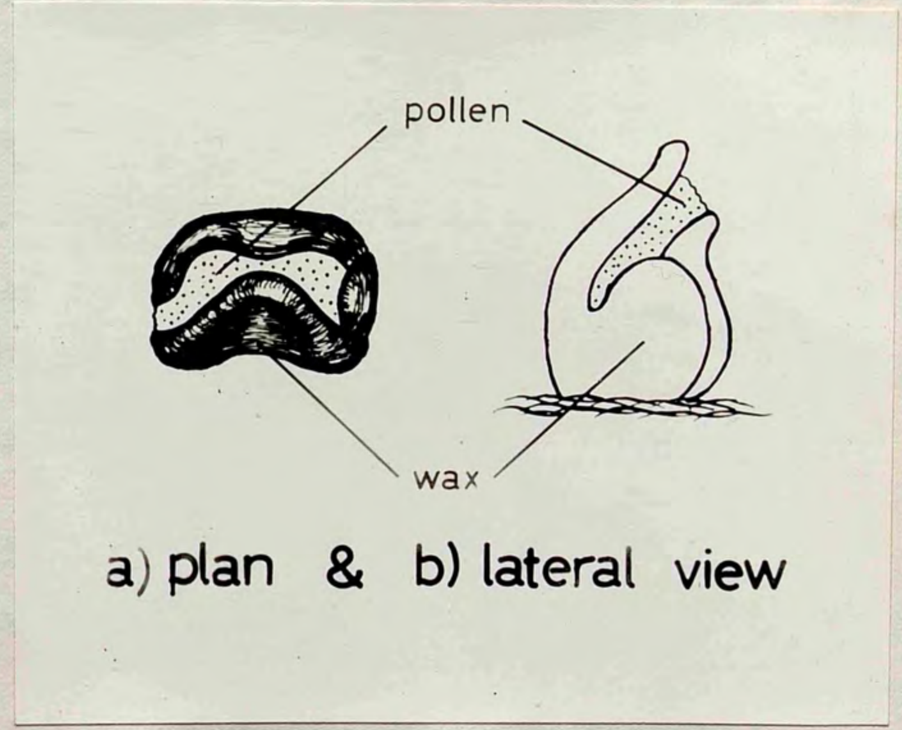
Fig. 3.22. Incomplete incipient egg clumps of B. hortorum.



(x 1.5 approx.)

Col. 132 (3)

Col. 135 (4)



Col. 120 (8)

Number of eggs given in parenthesis

advanced, was approaching the typical shape of a completed egg clump of this species.

The structure of a completed egg clump of B. hortorum is quite different to that of all other species examined. The leading edge — that nearest to the honey pot — is concave and narrower than the similarly shaped hind edge, while the sides are slightly convex, as seen in plan view. The anterior part of the clump is thicker than the posterior, and the top of the clump is noticeably depressed centrally along one axis, forming the initials of an incubation groove. The depression, on its longer rearward slope, is surrounded by a horseshoe-shaped ridge formed by the higher parts of the clump (Fig. 3.24).

The smallest egg clump (Col. 151) measured approximately 7 x 7 mm. and the largest (Col. 136), 10 x 8 mm., while the maximum height of the clumps ranged from 7 to 10 mm.. The distance between the centre of the egg clumps and the centre of the honey pots ranged from 17 to 22 mm..

iii. Larval stage.

Two colonies with very young larval clumps were found. In both, some eggs had still to eclose and after 36 hrs. in confinement, all had done so. Externally the clumps were identical in appearance to egg clumps. The larvae were occupying separate cavities within the pollen lump and there was a wide range in size between individuals. The arrangement of the larvae was very irregular and no distinct pattern in size distribution within the clumps was established (Fig. 3.23). Although listed under this section, Col. 133 probably contained only eggs when first found and photographed. The photograph is, therefore, included with those of completed egg clumps (Fig. 3.24).

Several clumps at a late larval stage were obtained. As shown in Fig. 3.25, the positions of the larvae are visible externally and there is a wide size range and irregular arrangement of individuals (Fig. 3.23 D). None of these clumps was followed to the pupal stage.

Significantly more ($p < 0.01$) eggs and larvae were found in incipient clumps of B. hortorum than in those of B. agrorum (Table 11).

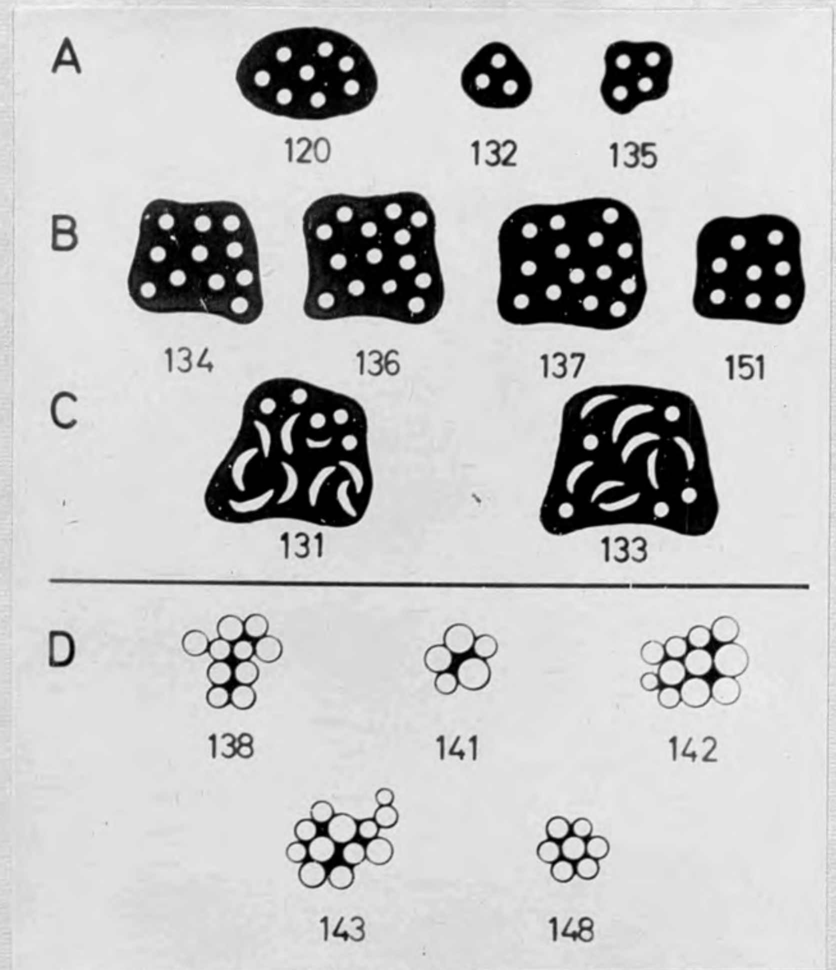
iv. Pupal stage.

Seven incipient pupal clumps of B. hortorum were obtained. The arrangement of the cells in these clumps is shown in Fig. 3.26. More cells ($p < 0.01$) were present in the pupal clumps of this species (mean = 10) than in B. agrorum (Table 12).

v. 1st brood adults.

Although the larger bees tended to emerge from the central cells of the brood batches, there appeared to be no correlation between the size of the bees and the order in which they emerged. Data for each colony, where details are known, are given in Appendix Table XXXIII.

Fig. 3.23. Sketches of incipient egg and larval clumps of *B. hortorum* to show arrangement of individuals in plan view.



N.B. Sketches not to scale.

A - Incomplete egg clumps.

B - Completed egg clumps.

C - Young larval clumps.

D - Older larval clumps - relative sizes and positions of larval cells shown.

Colony number given beneath each clump.

Fig. 3.24. Completed incipient egg clumps of *B. hortorum*.

Col. 133 (13) Col. 151 (8)

Plan view

Col. 134 (11)

Side view

Number of eggs given in parenthesis

(x 1.5 approx.)

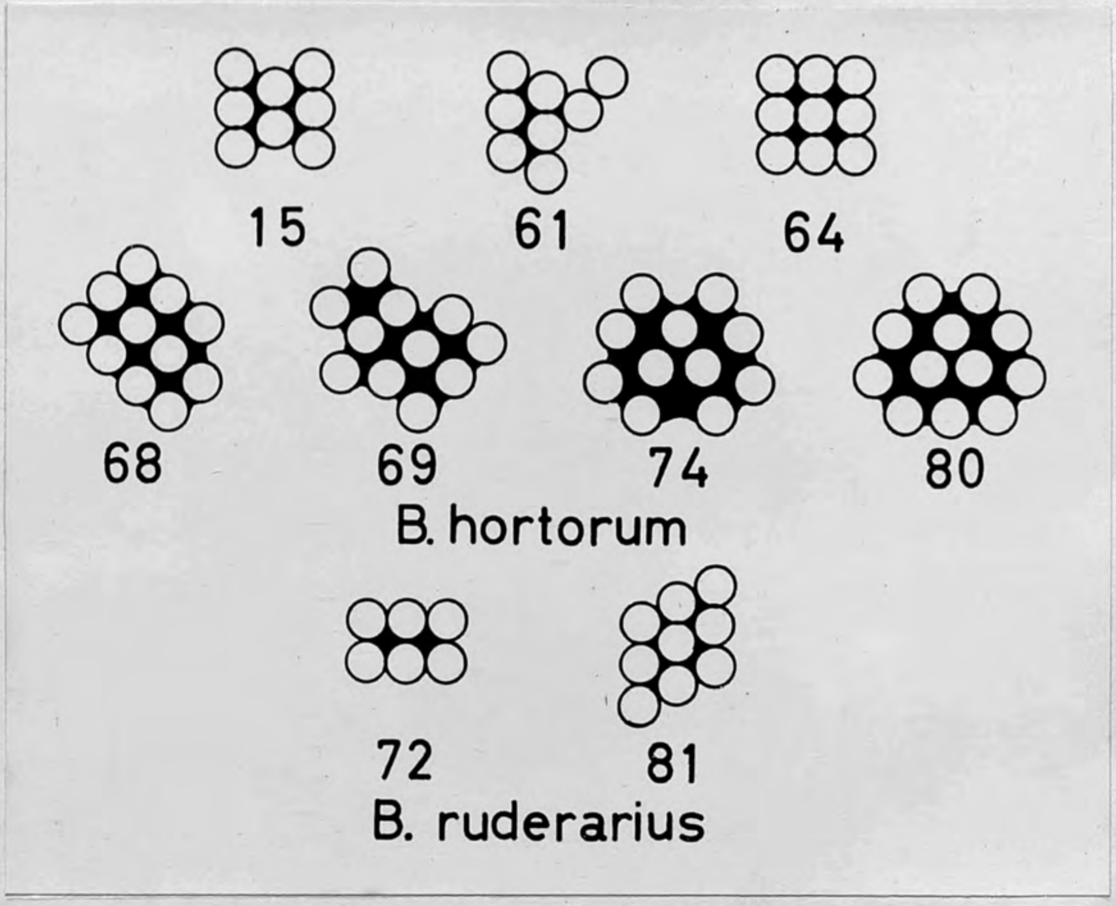
Fig. 3.25. Larval clumps of incipient colonies of *B. hortorum*.

Col. 138 (10)

Col. 142 (11) (x 1.5 approx.)

Number of larvae given in parenthesis.

Fig. 3.26. Arrangement of cells in incipient pupal clumps of *B. hortorum* and *B. ruderarius*.



Clumps are shown in plan view and orientated with the nest entrance towards the top of the page. For simplicity each cell is drawn the same size. Colony numbers are given beneath each clump.

e) Data from B. ruderarius colonies.

The cell arrangement of the incipient pupal clumps of two colonies is shown in Fig. 3.26. One of these contained 6 cells and the other, 9. Only one of these colonies was still at an incipient stage when found. Details of the adults produced in incipient colonies of this species were not obtained.

4. Discussion.

Sladen (1912) has described the founding and early development of a bumblebee colony, and Plath (1934) has given an account of nest founding by American species. According to Sladen, whose observations principally concern B. lapidarius, the queen, having formed a cavity within the nest material, first forms a lump of pollen paste and then constructs an egg cell upon it. Other writers, including Plath (1934), Richards (1953), and Free & Butler (1959), state that, at least in some species, an egg cell is constructed first and pollen then deposited in it prior to egg laying.

Evidence from the present work indicates that in B. hortorum and B. agrorum, before wax is deposited, a start is made in building the pollen lump. Further, in these species an egg cell as such, is not formed. For this reason the term "egg clump" has been used throughout the present account as this more closely describes the observed condition, not only in these two species, but also in B. pratorum and B. humilis. In B. hortorum and B. agrorum, it was demonstrated that egg laying may begin before the pollen lump is completed.

Although it is possible that some species may form an egg cell and then deposit pollen in it, apparently none of the published evidence in support of this method refers to observations made on natural colonies. Probably, such a method only occurs under artificial conditions when changes in natural behaviour could be expected, especially since queens are not then able to forage normally.

In the case of *B. lapidarius* and *B. hortorum*, Sladen (1912) found that "construction of the honey pot was not begun until after the lump of pollen had been made." He further considered that in most cases (species) it was probable that construction of the honey pot followed formation of the brood clump. Jordan (1936) reports that normally a honey pot is made before an egg cell, but gives no evidence to support his view, yet apparently a queen of *B. muscorum* which he induced to found a colony in a field nest box, constructed her honey pot after formation of the brood clump. Other writers also state that the honey pot is constructed first (Plath, 1934; Michener & Michener, 1951; Richards, 1953; and others), but again if, as seems likely, this evidence is also based upon observations on artificially initiated colonies, it may not be a reliable guide to the sequence of events which occurs under natural conditions.

Data obtained from natural incipient colonies in the present work show that in both *B. hortorum* and *B. agrorum*, honey pot and brood clump construction may occur concurrently, but that normally initiation of the brood clump precedes formation of the honey pot.

The number of eggs laid in the incipient colonies of various species is, to judge from the estimates of different authors, quite varied. Putnam (1864) states that from 7 to 14 eggs are laid. Sladen (1912) observed that "the eggs are sometimes laid in two lots, separated by an interval of a day or two. Their number varies from 8 to 16; generally it is about 12." Plath (1934) gives the number of eggs laid as being about 8, and Richards (1953) states that there are from 6 to 10 eggs in the incipient clump. Free & Butler (1959) consider that between 8 and 14 eggs are laid, depending upon the species and individual queen.

The present data indicate that in B. agrorum the usual number of eggs laid is 8, probably one from each ovariole. This number is probably characteristic of other small bumblebee species such as B. ruderarius and B. humilis, although exceptions will inevitably occur. B. pratorum apparently lays more eggs, and B. hortorum evidently lays about 12 or 14. These or higher numbers are probably typical of more prolific species such as B. lapidarius, B. terrestris and B. lucorum.

The observation that the eggs are not all laid in one sitting (Sladen, 1912) is confirmed in the case of B. hortorum and B. agrorum, but details of the time interval between the laying of different batches and the number of sittings taken, were not obtained.

The more or less vertical orientation of the eggs found in all incipient egg clumps of B. pratorum, B. hortorum, B. agrorum and B. humilis, is of interest since eggs in subsequent batches in these and other species are laid more or less horizontally. The latter egg batches, however, are deposited in actual egg cells and the observed absence of egg cells at the commencement of colony development in all of the above mentioned species, probably accounts for this difference. Sladen (1912) describes the eggs in an incipient colony of B. lapidarius as being more or less horizontally placed and laid in a bundle on top of the pollen lump. Sladen also describes finding 14 eggs in an incipient colony of B. ruderatus, each laid in a separate bed in the pollen lump. This agrees with the present observations on the closely related species B. hortorum, where the eggs were found more or less vertically in separate beds in the initial pollen lump. Sladen does not mention, however, whether the eggs in the B. ruderatus colony were lying flat or upright.

Usually there is only one egg cell or clump associated with the initial pollen lump, but Frison (1930) states that in B. americanorum there are several. Frison further reports that in this species only 2 to 5 eggs are laid per cell in the early part of the season, which probably accounts for this unusual condition. Rau (1941), however, describes an incipient colony of this species that had a single egg-bearing mass containing 8 eggs and 1 small larva. Interestingly, Rau does not describe the clump as an egg cell and found that the eggs were individually placed and so tightly packed in the pollen, that a needle was needed to remove them. This description is similar to the condition found in <u>B. hortorum</u>.

According to Brian (1951) only about 30 to 40% of the eggs laid in a mature bumblebee colony will produce adults. Cumber's (1949b) observations also indicate that many more eggs are laid than will attain the adult stage. Since in <u>B. agrorum</u> colonies 7 or 8 eggs were generally laid in the incipient clump and a similar number of 1st brood adults were normally produced, it is suggested that under normal conditions the incipient colony of a bumblebee develops with few or no brood mortalities. This suggestion is backed by the results from other species. The abnormally low number of adults produced by some colonies is no doubt due to brood mortalities and not to there having been fewer eggs laid, but most incipient colonies are evidently quite successful and high mortalities are certainly not characteristic.

Probably the usual number of eggs laid in an incipient colony is an optimum for the species which, under normal conditions and without brood mortalities, will give rise to an initial worker force of conveniently sized adults. There will be no advantage in having larger, and inevitably fewer, 1st brood workers than can efficiently carry out the tasks required of them, and clearly there is no point in producing a large initial worker force of dwarf bees, since the foraging potential of such individuals would not be very high and to maintain them would be a drain on the reserves of the colony.

The bimodality in the size of the 1st brood adults from the colonies of B. agrorum studied, is of interest, as it provides a basis from the outset for a division of labour. It is already known that the foragers tend to be the larger individuals of a colony (Richards, 1946; Cumber, 1949b; Brian, 1952; Free, 1955b) and in the confined incipient colonies of B. agrorum which were allowed access to the outside world after all the adults had emerged, it was found that the larger bees usually became the foragers. No bimodality would be expected to exist much after the 1st generation since the ranges in the size of adults from the different larval clumps will overlap.

The statement of previous workers (Sladen, 1912; Plath, 1934; Richards, 1953; Free, 1955c; and others) that bees reared at the centre of the incipient brood clump are the first to emerge, is confirmed by the present work. That these bees tend to be the larger individuals is also demonstrated, at least in B. agrorum and B. humilis colonies. Free (1955c) states that in his laboratory initiated colonies of B. pratorum the bees from the central cells of the brood clump emerged first, but that these bees were not necessarily the largest individuals. This was also true of some of the natural clumps of B. hortorum studied in 1965 and 1966, and was thought to be due to the spatial irregularity in the distribution of individuals in some clumps, probably initiated at the egg stage. In laboratory initiated colonies of several species, including those of B. pratorum, B. hortorum and B. agrorum, it was found that pupal clumps were often very asymmetrical and as in Free's colonies, the bees from the central cells tended to emerge first, but were not necessarily the larger individuals. The reason for the inferior development of these clumps was considered to be due mainly to the inadequate supply of pollen to the larvae, particularly at the onset of development. In those colonies provided with the largest initial pollen lumps, development tended to be better, but no precise data on this point were obtained.

Typically, natural brood clumps at the pupal stage are compact and usually symmetrically arranged, with the incubation groove forming the axis of symmetry. The 8-cell pattern (Type 'A', Fig. 3.9) considered as typical of B. agrorum, has also been described by Sladen (1912) as being the normal form for B. derhamellus Kirby (= B. ruderarius (Müller)), and it is probably also predominant in other species which rear this number of individuals. In species which normally produce more 1st brood bees, such as B. hortorum, B. ruderatus, and probably most "pollen-storers", an arrangement of cells similar to that found in Col. 74 or Col. 80 (B. hortorum, Fig. 3.27), may be usual, but insufficient data on the more prolific species have been obtained for the typical patterns of such species to be ascertained.

PART IV : SYNTRETUS SPLENDIDUS (MARSHALL)(BRACONIDAE: EUPHORINAE) AS A PARASITE OF ADULT BUMBLEBEES.

1. Introduction.

Studies have been made of a previously little known hymenopterous parasite of adult bumblebees. Larvae of presumably the same insect have been recorded in bumblebees in America by Plath (1934), and in England by Legge (1937) and Cumber (1949a), but since the adult parasite was never reared, its systematic position and identity has, until now, been in doubt. These isolated reports appear to be the only previous records of the parasite in bumblebees.

Specimens obtained in the present studies were found to be Braconids and members of the sub-family Euphorinae. The adults were subsequently identified by Mr. G.E.J. Nixon of the Commonwealth Institute of Entomology, as Syntretus splendidus (Marshall) (= Microctonus splendidus Marshall).

Most of the Euphorinae are internal parasites of adult Coleoptera, although adults or nymphs of other insects, including certain species of Hemiptera and Psocoptera, have also been recorded as hosts (see Muesebeck, 1936, 1963). The only previous host record for a member of the genus Syntretus Förster, is that referring to the association of S. lyctaea Cole and adults of the Ichneumonid Phaeogenes invisor Thunberg (Cole, 1959). No other species of Syntretus has, until now, been asigned to its host.

Biological studies of Euphorine parasites, including various species of the genus Microctonus Wesmael and Perilitus Nees have been made by Speyer (1925), Balduf (1926), Jackson (1928), Smith (1952), Loan & Holdaway (1961) and others, but no account of the life history of a member of the genus Syntretus exists. A study of this parasite was therefore considered desirable, and further, from the economic aspect, since the parasite may be, at least in some areas, a potential threat to bumblebee populations which has previously been overlooked, an account of the relationship between S. splendidus and its host was thought to be of some importance.

2. Methods of obtaining material.

The parasite was first found when larvae in their final instar appeared in the nest box of a B. agrorum queen which was confined in the laboratory with her natural incipient brood clump. The larvae were particularly active and wriggled about in the box both on and under the corrugated cardboard floor cover. Although considerable quantities of silk were being trailed along by the larvae no cocoons were constructed and unfortunately all of the larvae subsequently died. The queen, although still alive following the appearance of the larvae, died the next day.

Subsequently, all other boxes with confined bumblebees were kept under observation for any sign of the parasite. Further full-grown larvae eventually made their appearance, and these were transferred to glass dishes which had been filled to a depth of about 1 cm. with washed and dried soil. Later larval batches were allowed to drop directly into soil-containing dishes from boxes or cages in which bumblebees were housed, the flooring of such boxes having been partially replaced by wire gauze. Unless larvae were transferred to the soil-containing dishes soon after their emergence from the host, they invariably died. Such larvae normally failed to burrow into the soil and perished on the surface without forming cocoons.

From these cultures several adult parasites were obtained, but although adults of Syntretus were sought for in the field and in bumblebee nests throughout the spring and summer, none was found.

The immature stages of the parasite were obtained from dissections of field samples of adult bumblebees. Unless otherwise stated, all observations and field samples were made within the area covered by Appendix Map 'A'. Drawings were made on squared paper with the aid of a squared eye piece in a binocular dissecting microscope. Live material in 0.8% saline solution was used whenever possible.

3. Observations.

a) Categories of host that are parasitized.

Details of the extent of parasitism among bumblebees in 1965, 1966 and 1967 are given in Appendix Tables XXXV, XXXVI, and XXXVII respectively. Seven Bombus species

(*B. lapidarius*, *B. terrestris*, *B. lucorum*, *B. pratorum*, *B. ruderarius*, *B. agrorum* and *B. humilis*) and one *Psithyrus* species (*P. vestalis*) were found to contain parasites. Surprisingly, no cases of parasitism in the common species *B. hortorum* were recorded.

Without exception, parasitized bumblebees were foraging bees or individuals that had recently foraged; that is either spring queens, young foundress queens (most with incipient colonies), forager workers, or wild males. Inspite of many dissections no sign of *S. splendidus* was found in house bees, old foundress queens, gynes, hibernating queens, or queens recently emerged from hibernation.

b) Number of parasites per host.

The number of parasites found in individual bumblebees (Appendix Tables XXXV, XXXVI and XXXVII) is summarized as a frequency distribution in Table 14. In one forager of *B. terrestris* a solitary 1st instar larva was found, but all other records were of gregarious associations of parasites. The highest number found in any individual bumblebee was seventy seven 2nd instar larvae, from a *P. vestalis* queen. There were significantly more parasites in queens than in workers ($p<0.001$), the means being 33.2 and 19.4 individuals respectively. As shown in Table 15 queens and workers of larger host species tended to contain more parasites than those of smaller host species. The mean number of parasites in all infected bumblebees was 23.2 individuals. Since in bumblebees containing few parasitic individuals, the parasites were, in some cases, in the early stages of development, it is evident that cannibalism or competition on the part of the developing larvae is not the cause of the difference. It is probable, therefore, that the female parasite to some extent regulates the number of eggs laid, according to the size of the host.

Table 14 — Number of parasites per host.

No. of parasites	Frequency in host Queens	Workers	Males
1 - 5	1	3	-
6 - 10	2	12	1
11 - 15	1	15	-
16 - 20	3	13	-
21 - 25	-	11	-
26 - 30	7	5	1
31 - 35	5	4	-
36 - 40	-	3	-
41 - 45	2	1	-
46 - 50	3	2	-
51 - 55	-	1	-
56 - 60	1	-	-
61 - 65	2	-	-
66 - 70	-	-	-
71 - 75	-	-	-
76 - 80	1	-	-

Table 15 — Mean number of parasites in various host species.

Species	Queens	Workers	Males
B. lapidarius	54.5 (2)	6.7 (3)	—
B. terrestris	—	26.1 (20)	—
B. lucorum	—	20.2 (21)	16.5 (2)
B. pratorum	30.5 (4)	13.7 (11)	—
B. ruderarius	28.0 (1)	—	—
B. agrorum	29.0 (18)	16.0 (15)	—
B. humilis	35.0 (2)	—	—
P. vestalis	77.0 (1)	—	—

(Number of hosts in each case, is given in parenthesis).

c) Distribution of parasites within host.

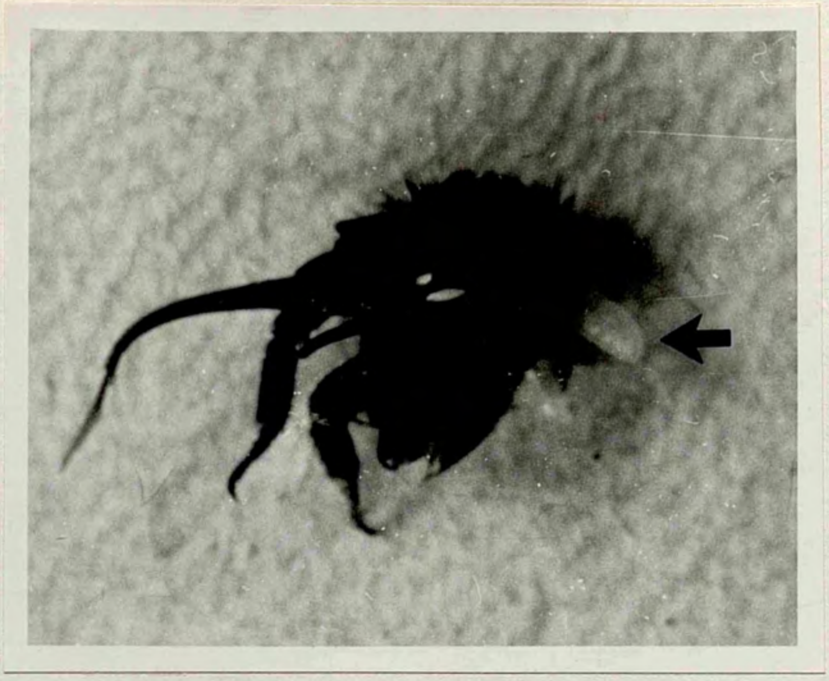
Larvae normally develop in the abdomen of the host (Fig. 4.1), but in the egg or early larval stages, many parasites may be present in the head and/or thorax (see Appendix Table XXXVII). By the second larval instar, most parasites occur in the abdomen of the host (Table 16), although occasionally older larvae may occur elsewhere. In a parasitized B. ruderarius queen two full-grown 4th instar larvae were found in the thorax and one of these was partially invading the neck region of the host (Fig. 4.2). This particular queen died before the emergence of any final instar larvae from her body.

Fig. 4.1. Larvae of *S. splendidus* in abdomen of host.

(x 6)

Tergites of abdomen and attached viscera removed to show larvae in body cavity.

Fig. 4.2. 4th instar larva of S. splendidus invading neck region of host.



(x 7)

Photograph shows head and prothorax of B. ruderarius queen, the meso- and metathorax having been dissected away.

Table 16 — Distribution of parasites of various stages within host.

Parasitic stage or larval instar	Number of hosts	% of hosts with parasites in Abdomen only	% of hosts with parasites in Abdomen & head &/or thorax.
Eggs	20	5	95
Eggs & 1st	11	36	64
1st	13	23	77
1st & 2nd	5	40	60
2nd	10	90	10
2nd & 3rd	2	100	-
3rd	3	100	-
3rd & 4th	5	80	20
4th	18	94	6

(Hosts from which 5th instar larvae were emerging are not included in the above.)

---

d) Oviposition.

No opportunity was afforded to study oviposition by S. splendidus into bumblebees but consideration of the categories of host parasitized, suggests that oviposition occurs in the field and not within bumblebee nests.

Jackson (1928) states that in the genus Perilitus the apex of the abdomen is the most favoured region of the host body for insertion of the ovipositor, although McColloch (1918) reports that Perilitus eleodis Viereck oviposits in the sutures between the legs and body of the host. According to Seitner & Nötzl (1925) the Euphorid Cosmophorus henscheli Ruschka deposits its eggs into the mesothoracic region of the beetle host, and Smith (1952) found that Microctonus vittatae Muesebeck oviposits into the thoracic region of the host, via the neck. The presence of large numbers of eggs and 1st instar larvae in the head or thorax of infected bumblebees, suggests that S. splendidus oviposits into the thoracic region, probably through the neck. Unlike the case in several Euphorine parasites, the eggs of S. splendidus lie freely in the host's haemocoel and are not attached to the viscera by a pedicel.

The full complement of eggs of gregarious, endoparasitic species is usually deposited into the host with one insertion of the ovipositor, and according to Tower (1915) the Braconid Apanteles militaris Walsh deposits up to 72 eggs in about one second.

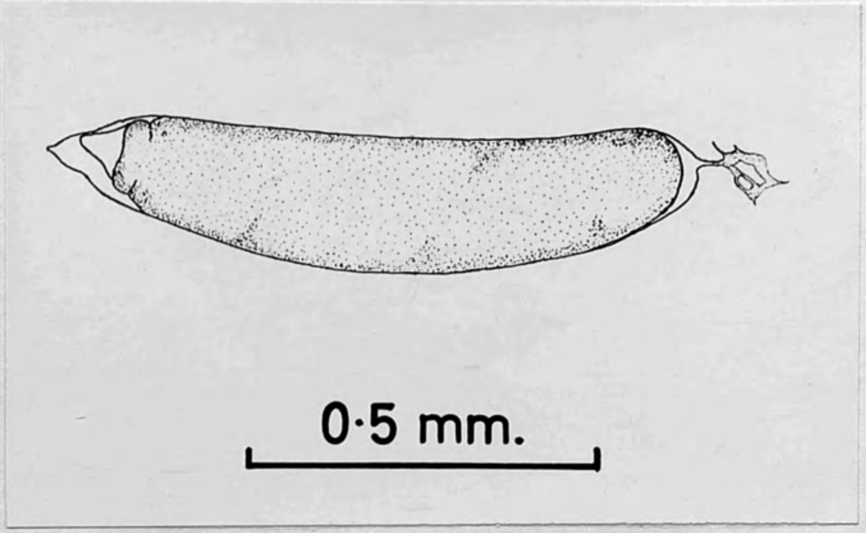
Infection of bumblebees by S. splendidus probably occurs during flower visitation by the host, since at such a time a foraging bumblebee is most vulnerable to attack.

e) Multiparasitism.

A forager of B. lucorum, dissected on the 29th June, 1965, contained several eggs and newly emerged 1st instar larvae of S. splendidus and a single egg of a Conopid fly. This latter egg is illustrated in Fig. 4.3 (cf. egg of S. splendidus, Fig. 4.19). A forager of B. agrorum captured on the 4th July, 1966, contained sixteen 2nd instar larvae of S. splendidus and also a single Conopid larva.

More than 60 bumblebee queens parasitized by the nematode Sphaerularia bombi, collected during May and June, 1967, were dissected but none was also parasitized by Syntretus.

Fig. 4.3. Egg of Conopid fly found in bumblebee forager which was also parasitized by *S. splendidus*.



f) Superinfection.

The B. lucorum forager dissected on the 23rd June, 1967 (Appendix Table XXXVII), was probably parasitized twice, since this bee contained four 4th instar larvae of S. splendidus and ten 1st instar larvae.

With solitary parasites, superinfection is readily observed. The present writer, for example, found two young Conopid larvae in a foraging worker of B. agrorum in 1966, and Smith (1952) recorded 14% of superinfection of beetles by M. vittatae in field samples taken during 1948 and 1949. However, since S. splendidus is gregarious, cases of superinfection by this parasite will be difficult to detect and its extent under field conditions hard to measure.

g) Hyperparasitism.

S. lyctaea is predominately a hyperparasite since its host is itself a parasite on Tortrix viridana Linn. (Cole, 1959).

Since S. splendidus occasionally parasitizes Psithyrus species, it also, at times, classifies as a hyperparasite. On the grounds of host availability, however, Bombus must be regarded as the principal host genus.

h) Morphology of the immature stages of S. splendidus.

i. Egg (Fig. 4.19).

Spherical to oval, slightly flattened laterally. No pedicel. Chorion smooth and transparent: colour hyaline. Newly laid egg small (smallest and youngest found — age not known precisely — measured 0.19 x 0.12 mm.); enlarges considerably as development proceeds. Embryo clearly visible surrounded by transparent embryonic membrane. Unfortunately, ovarian eggs were not examined.

ii. 1st instar larva (Fig. 4.4).

Size (n = 32): 1.8 - 2.7 (2.2) x 0.17 - 0.32 (0.23) mm.. §

Approaches caudate type of Clausen (1940). Head and 13 segments visible. Last segment elongated as ventral, caudal process. Tail at first blunt, becoming more pointed following eclosion. Pair of unsclerotized, falcate mandibles with tips overlapping in median line: two pairs of setae situated around oral cavity dorso-laterally, directed orally (Fig. 4.5). Head capsule unsclerotized. Mouth ventro-anterior. Short oesophagus opens into narrow, muscular pharynx. Intestine a simple wide tube; connection to hind gut blocked by solid core of cells (Fig. 4.6). Anus directed dorso-posteriorly. On emergence from embryonic membrane, proctodeum slightly evaginated, later forming a distinct vesicle. Vesicle never wider than preceding body segment. Pair of labial glands present, opening ventrally near mouth, extending backwards and dorsally for a short distance on either side of the gut, then bifurcating; each branch terminating anterior to the paired genital glands in abdominal segment 8. Pair of Malpighian tubules extend sinuously forward ventro-laterally as diverticula from hind gut, reaching as far as abdominal segment 2. Brain and ventral nerve cord composed of sub-oesophageal ganglion and 12 segmental ganglia. No visible tracheal system. Cuticle smooth: colour hyaline.

§ n = No. of observations. Size range given, followed by mean in parenthesis, for both length and width of specimens.

Fig. 4.4. Outline of 1st instar larva of *S. splendidus*.

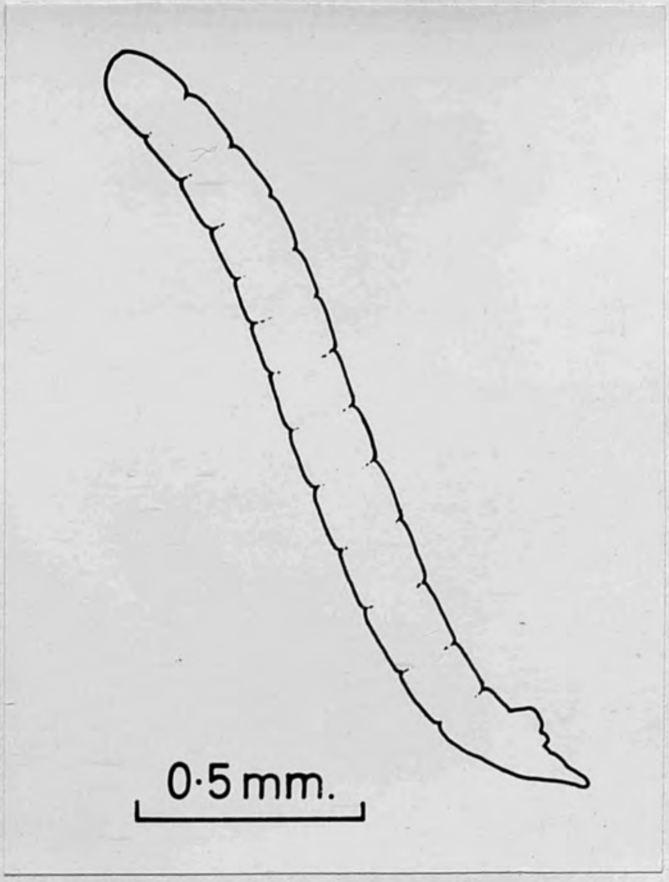


Fig. 4.5. Ventro-anterior view of head of 1st instar larva, slightly squashed dorso-ventrally.

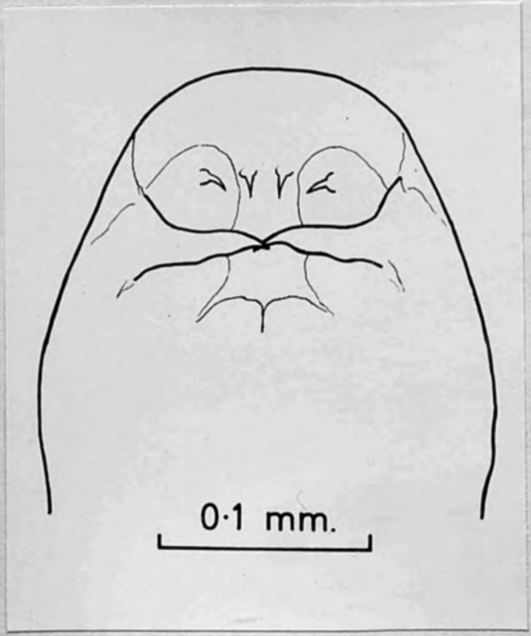
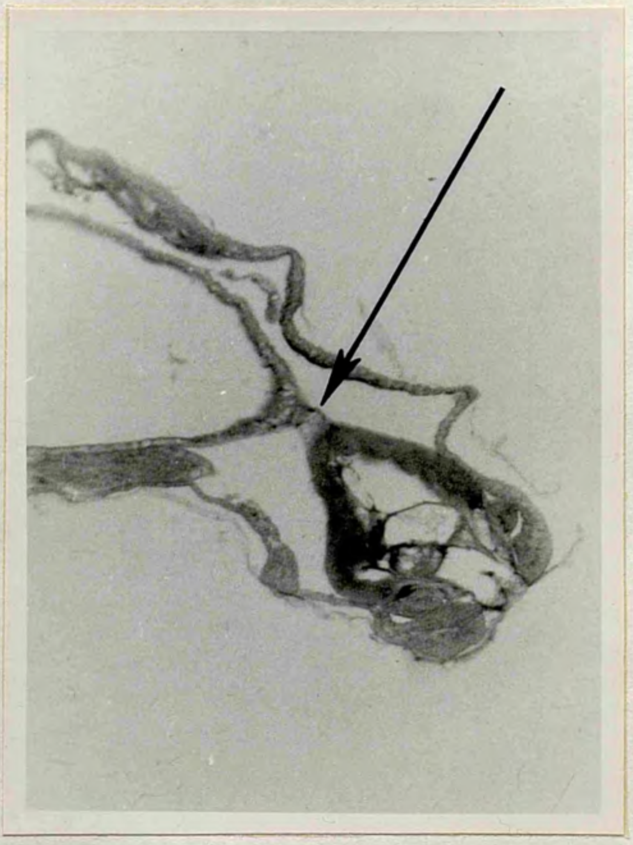


Fig. 4.6. Median longitudinal section of posterior abdominal region of 1st instar larva to show solid core of cells between mid- and hind-gut.



(x 150)

iii. 2nd instar larva (Fig. 4.7).

Size (n = 18): 2.5 - 3.8 (3.1) x 0.3 - 0.7 (0.5) mm..

Body gently arched with ventral surface concave. Central body segments distinctly deeper than others. Caudal process relatively smaller and more conical than in previous instar. Mouth and cephalic setae directed anteriorly. Anal vesicle reduced or absent. Anus directed posteriorly. Mandibles not detected. Tracheal system present, but no spiracles. Colour hyaline.

iv. 3rd instar larva (Fig. 4.8).

Size (n = 12): 2.9 - 4.0 (3.6) x 0.59 - 0.84 (0.72) mm..

Similar to previous instar, but caudal process reduced to small rounded protrusion. Mandibles small, poorly sclerotized, thorn-shaped and widely spaced (Fig. 4.9). Colour hyaline-whitish.

v. 4th instar larva (Fig. 4.10).

Size (n = 20): 4.2 - 5.2 (4.6) x 0.8 - 1.1 (1.0) mm..

Larva sausage-shaped and more grub-like than earlier instars. Mandibles and facial supports sclerotized. Labial and maxillary palpi present but antennae not observed. Nine pairs of spiracles present; one pair on the mesothorax and one pair on each of the first eight abdominal segments. Opaque, white uric acid deposits visible through transparent cuticle (Fig. 4.1). Colour whitish.

vi. 5th instar larva (Fig. 4.11).

Size (n = 16): 3.2 - 4.3 (3.9) x 0.9 - 1.4 (1.2) mm..

Larva of the hymenopteriform type (Clausen, 1940), with more squat appearance than previous instar. Lateral ampullae present. Unlike all previous instars, body extensively covered in short setae (Fig. 4.12). Longer,

Fig. 4.7. Right lateral view of young 2nd instar larva.

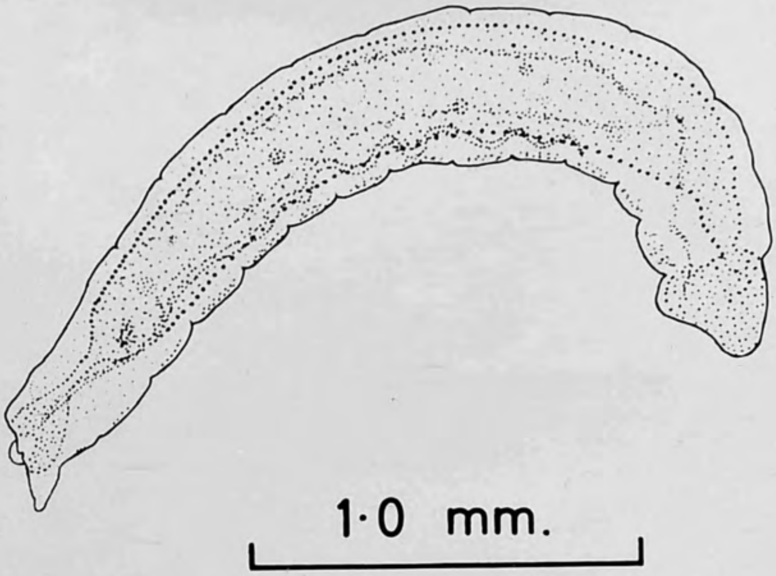


Fig. 4.8. Outline of 3rd instar larva - left side view.

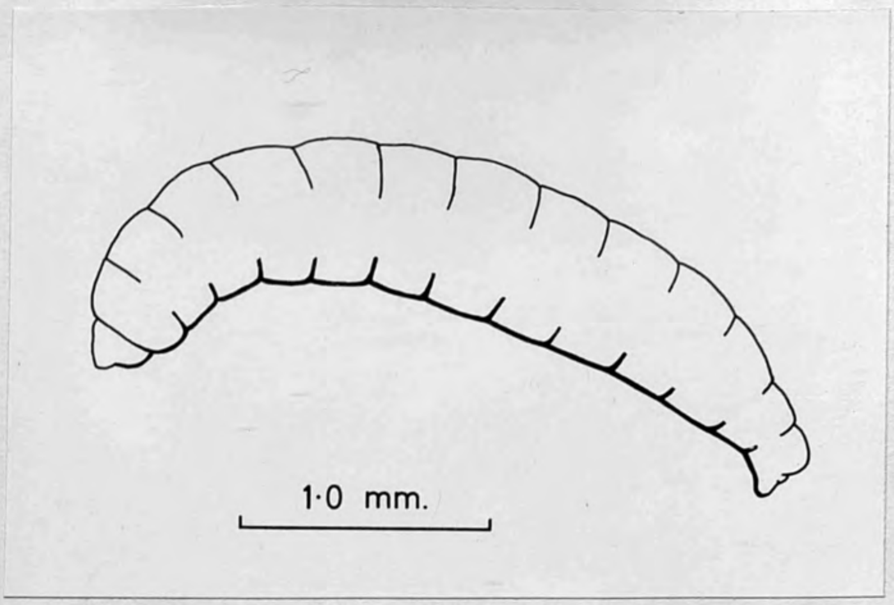


Fig. 4.9. Ventro-anterior view of head of 3rd instar larva.

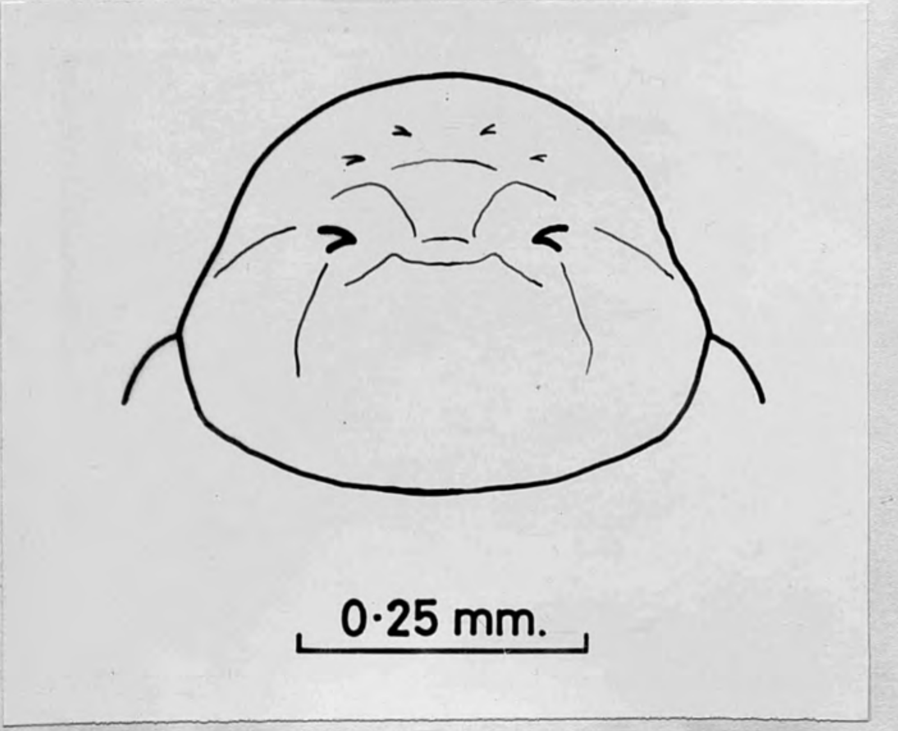


Fig. 4.10. Left lateral view of full-grown 4th instar larva.

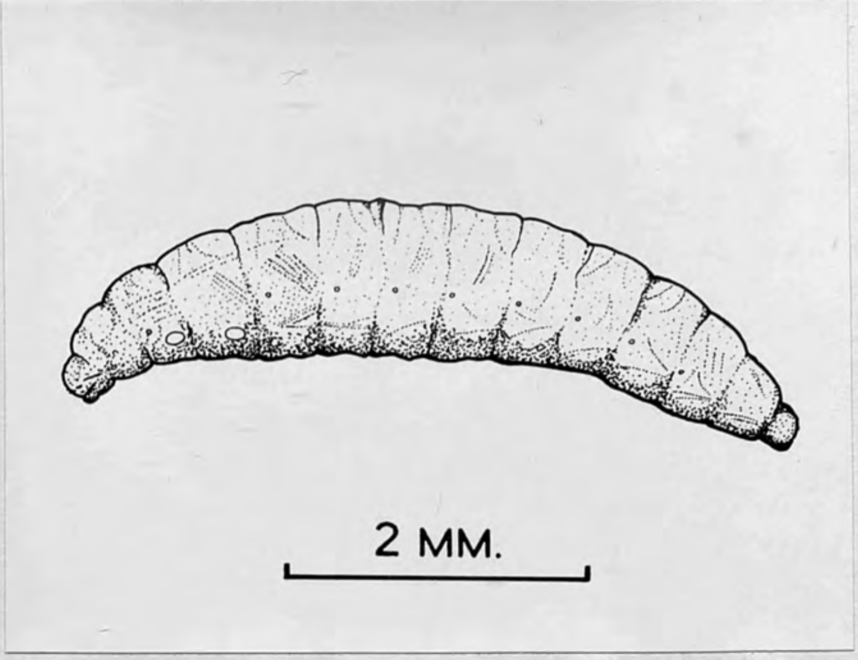


Fig. 4.11. Left lateral view of 5th instar larva.

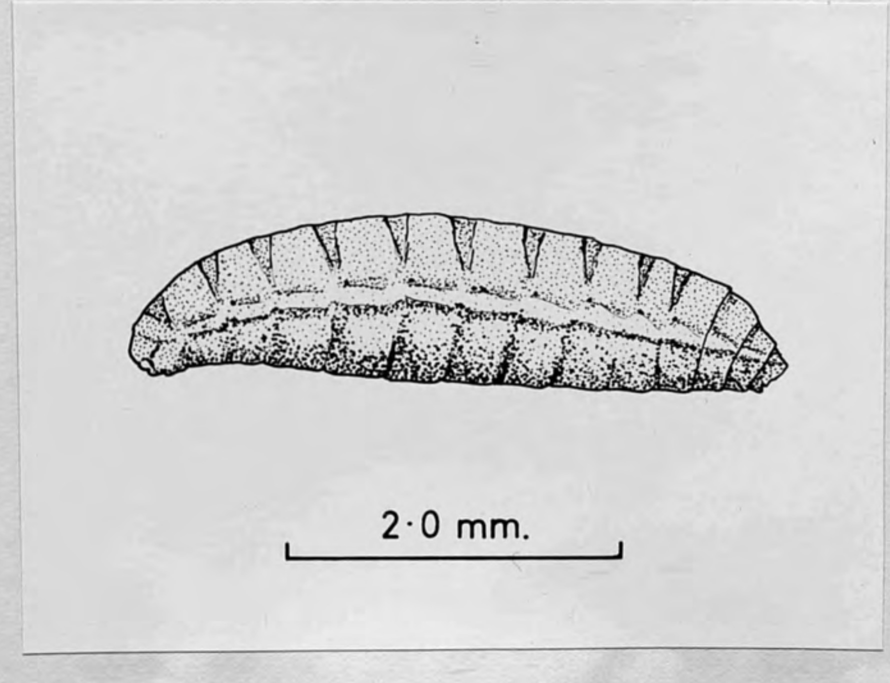
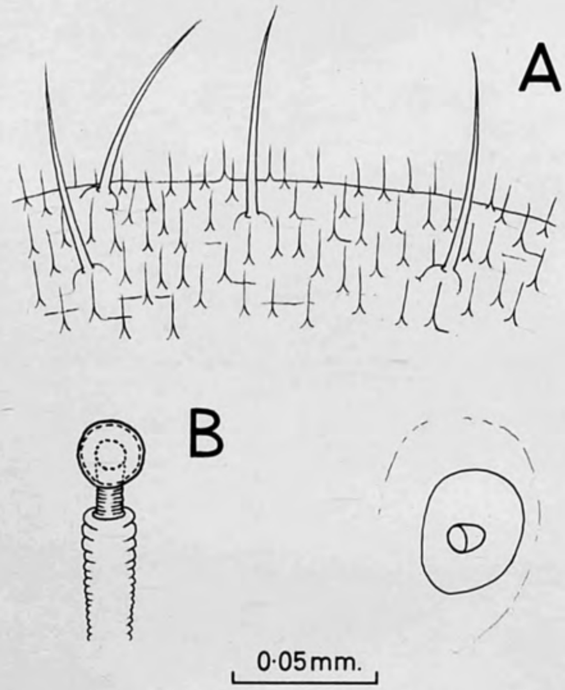


Fig. 4.12. Various morphological characters of final instar larva as seen from mounted cast skin.



A - Part of dorsal area of last abdominal segment showing large and small setae.
B - Spiracle.
C - Antenna.

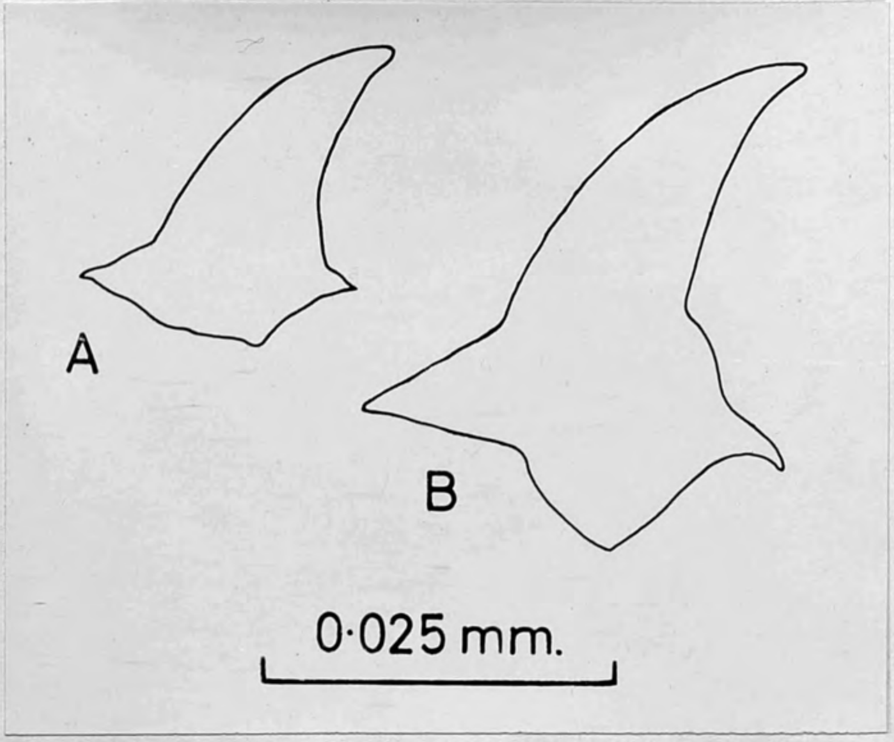
tubercular-based setae present in transverse bands over the body and on post-labium. Mandibles similar to, but larger than, those of previous instar (Fig. 4.13). Mouthparts (Fig. 4.14) similar to those of Syntretus lyctaea as illustrated by Cole (1959), except that the ventral part of the labial sclerite in S. splendidus is broad and weakly sclerotized. The absence of a hypostomal spur, the straight stipital sclerite, the weakly sclerotized hypostoma, and the presence of large setae on the post-labium, may be diagnostic of the genus. Cole does not describe the antennae of S. lyctaea. Those of S. splendidus are indistinct and papilliform. The spiracular closing apparatus, as in S. lyctaea (Cole, 1959) and other Euphorinae (Short, 1952) is close to the atrium (Fig. 4.12). Colour dirty creamish-white, slightly darker mid-dorsally.

vii. Pupa (Fig. 4.15).

Size (Female)(n = 3): 3.1 - 3.3 (3.20) x 1.2 - 1.3 (1.23) mm..
(Male) (n = 2): 2.2 - 2.5 (2.25) x 0.7 - 0.8 (0.75) mm..

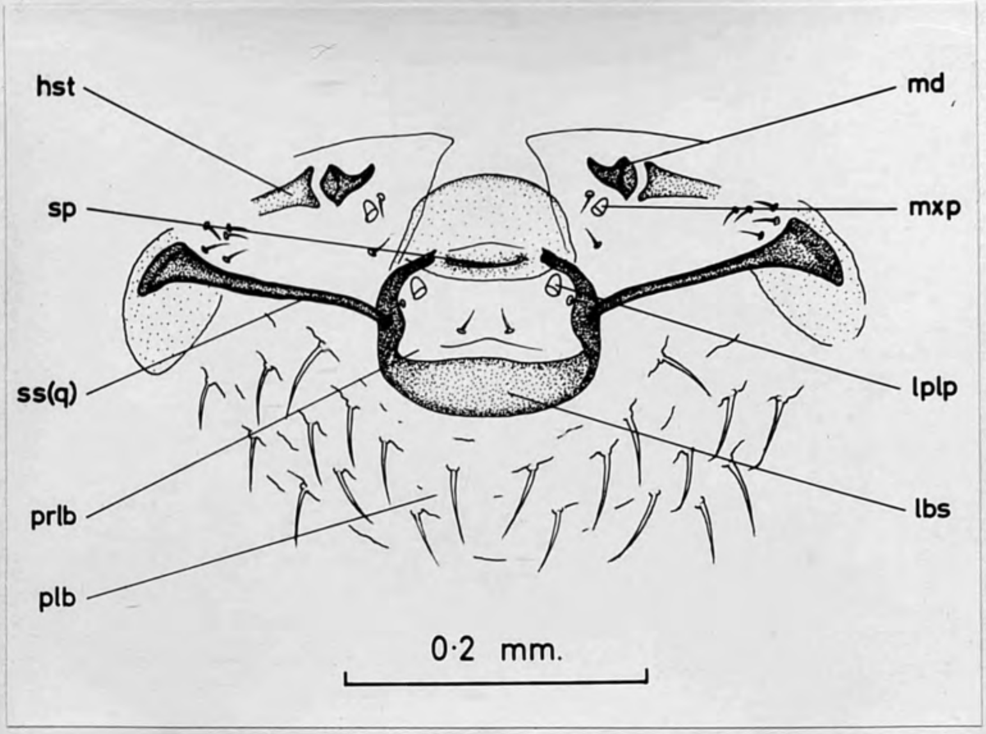
Creamish-white at first, darkening to golden- or blackish-brown as adult insect develops. Wings creamish-white to grey. Head, thorax and petiole darker than abdomen. Female lighter and larger than male. In male, opposing limb sheaths meet distally such that each tarsal sheath loops back along the opposite limb sheath. Tarsal sheaths of hind limbs are looped together on dorsal side of abdomen. In female, opposite limb sheaths are separate and hind limbs remain ventral. Ovipositor curves over to dorsal side of abdomen.

Fig. 4.13. Outlines of larval mandibles.



A - Mandible of 4th instar larva.
B - Mandible of 5th instar larva.

Fig. 4.14. Mouthparts of final instar larva.

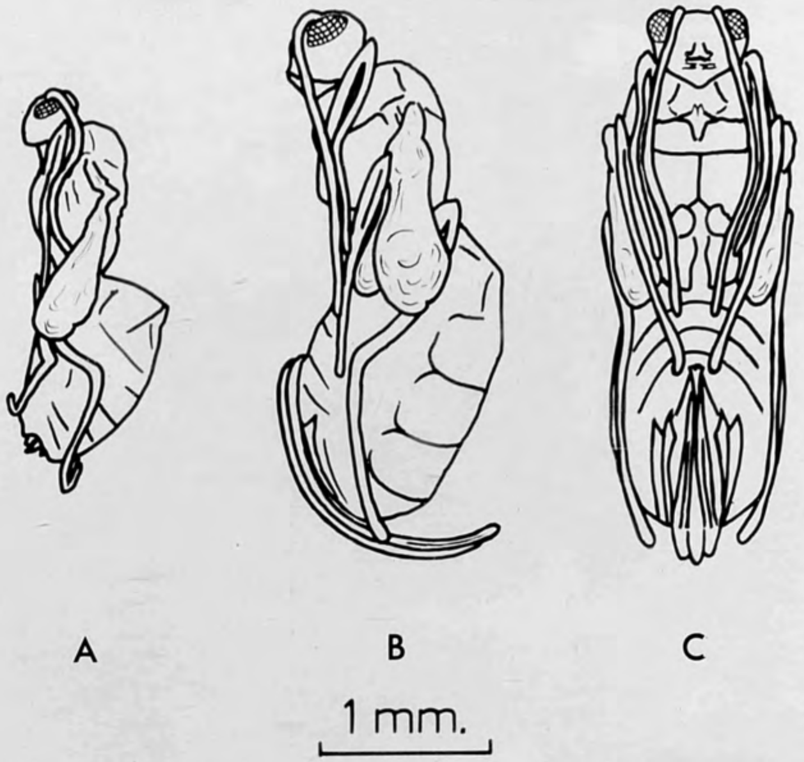


Nomenclature (following Short (1952)) is as follows:-

hst ..... hypostoma
lbs ..... labial sclerite
lplp .... labial palp
md ...... mandible
mxp ..... maxillary palp
plb ..... post-labium
prlb .... pre-labium
sp ...... silk press
ss(q) ... stipital sclerite

Drawn from cast skin mounted on a slide and therefore morphologically inaccurate.

Fig. 4.15. The pupa of *S. splendidus*.



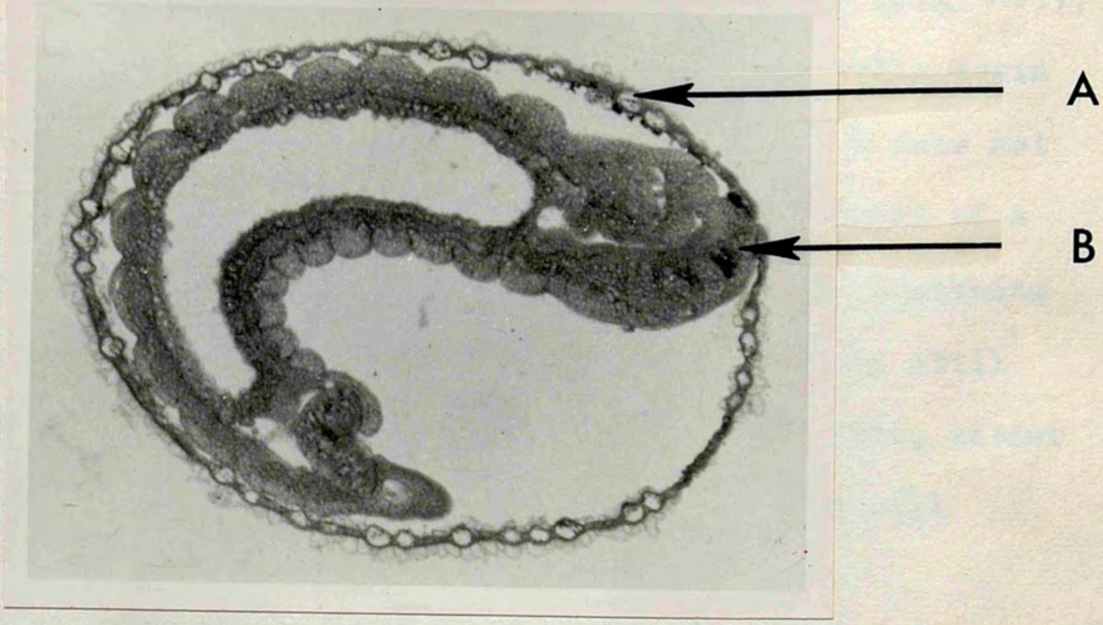
A - Male pupa; left lateral view.
B - Female pupa; left lateral view.
C - Female pupa; ventral view.

j) The embryonic membrane.

In many Braconids, including members of the Euphorinae, the embryonic membrane or trophamnion persists after eclosion of the egg, as an envelope which may partially or completely enclose the first instar larva. Jackson (1928, 1935) has provided much information on the embryonic membrane and its fate in parasitic Hymenoptera, with special reference to Perilitus rutilus Nees. In this, and many other species, the cells of the embryonic membrane dissociate following the eclosion of the egg, and continue to exist independently in the body of the host. During this time they increase considerably in size and accumulate food material from the host's body fluid. These cells are utilized by the developing parasitic larvae as a source of food, especially in the later instars.

In S. splendidus the actual time of eclosion has not been observed but in many instances larvae surrounded by a sac-like embryonic membrane, apparently no longer enclosed in a chorion, were found. At this stage the individual cells of the membrane are more or less hexagonal in outline and, because of the darker nuclear contents, the positioning of individual cells is visible without staining. The nuclei are more or less spherical and have a diameter of about 20 µ. The cells, in plan view, measure about 25 to 30 µ across. As seen in section, the membrane bulges somewhat in the nuclear region of each cell (Fig. 4.16). The cytoplasm is homogenous and lacks vacuoles.

Fig. 4.16. Section of "egg" with well developed embryo.



(x 140)

A - Embryonic membrane.
B - Embryo in median longitudinal section.

At about the time of the emergence of the larva from the embryonic membrane, the cells begin to round off and along, with the nuclei, increase in size. Initially, enlargement of the nuclei seems greater than that of the actual cells (Fig. 4.17, A and B). Following the escape of the larva the cells begin to dissociate. The phenomenon of cell dissociation does not take place all at the same time. In places, the cells of a recently evacuated membrane may be well rounded and beginning to part, while in others, divisions between cells may still be slight. Eventually, however, the cells break free, either individually or in small groups, until finally each cell becomes dissociated.

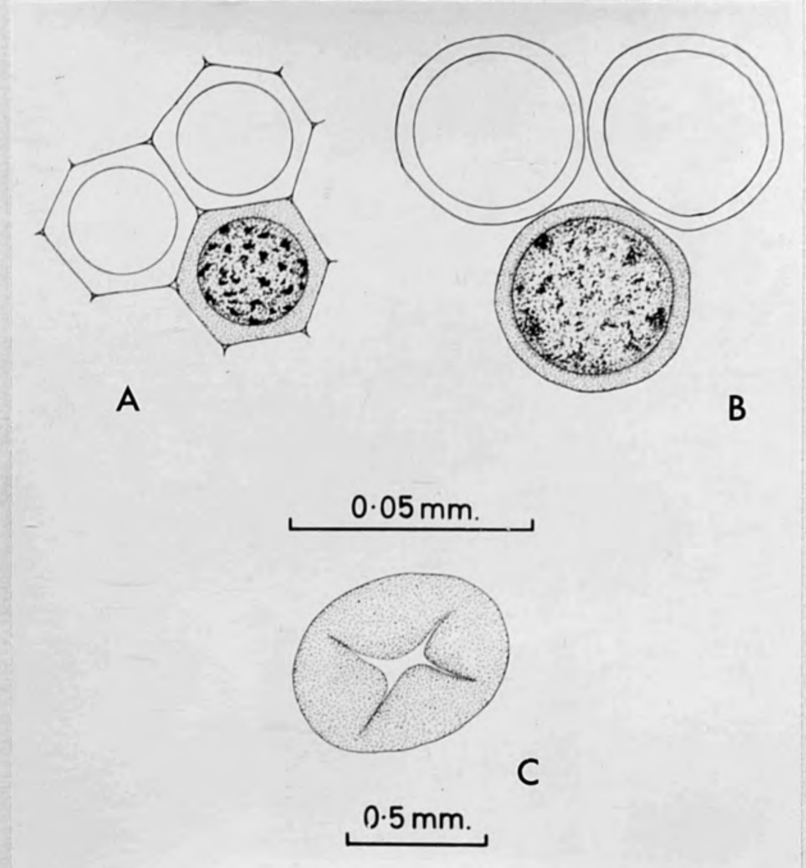
Following dissociation of the cells, the nuclei become more irregular (Fig. 4.18), and large cells at the final stages of their development have contracted nuclei which are drawn out into thin processes which extend into the surrounding cytoplasm (Fig. 4.17, C). The cells are particularly numerous between the fat body layers and epidermis of the host's abdomen (see Fig. 2.11 on page 95). Although the dissociated cells occur mainly in the abdomen of the host, most parasitized bumblebees contain some cells in the head and thorax, even when parasites are confined to the abdomen.

At first, the dissociated cells are hyaline in appearance and in hosts with 1st instar larvae or very young 2nd instar parasites, they are not immediately obvious, although reaching a diameter of about 0.09 mm.. However, except in the final stages of infection when most or all of the cells have disappeared, when the abdomen of a parasitized bumblebee containing older larvae is punctured in the dissecting dish, a stream of white, opaque globules of various sizes — the embryonic membrane cells — issues through the vent. Legge (1937) makes no mention of these cells, although the inference from his description is that he was dealing with immature Syntretus larvae. Cumber (1949a), however, found "masses" of the cells in the two parasitized bumblebees he dissected, although he did not realize their origin or significance. Cumber records the maximum diameter of these cells as about 1 mm.. The largest observed by the present writer, however, did not exceed 0.8 mm. in diameter.

During their period of hypertrophy, the dissociated embryonic membrane cells of S. splendidus accumulate reserves of fat from the host, and they are readily stained with fat stains such as Fettrot 7B. The fat is present as minute droplets within the cytoplasm. Glycogen deposits were also demonstrated in these cells.

In Perilitus, Jackson (1924) found that in the final stages of hypertrophy, the embryonic membrane cells tended to rupture and release their fat contents into the haemolymph of the host. This phenomenon was also observed in S. splendidus. Although small cells are swallowed whole by the developing larvae, large cells are probably broken and then their contents swallowed.

Fig. 4.17. Embryonic membrane cells at various developmental stages.



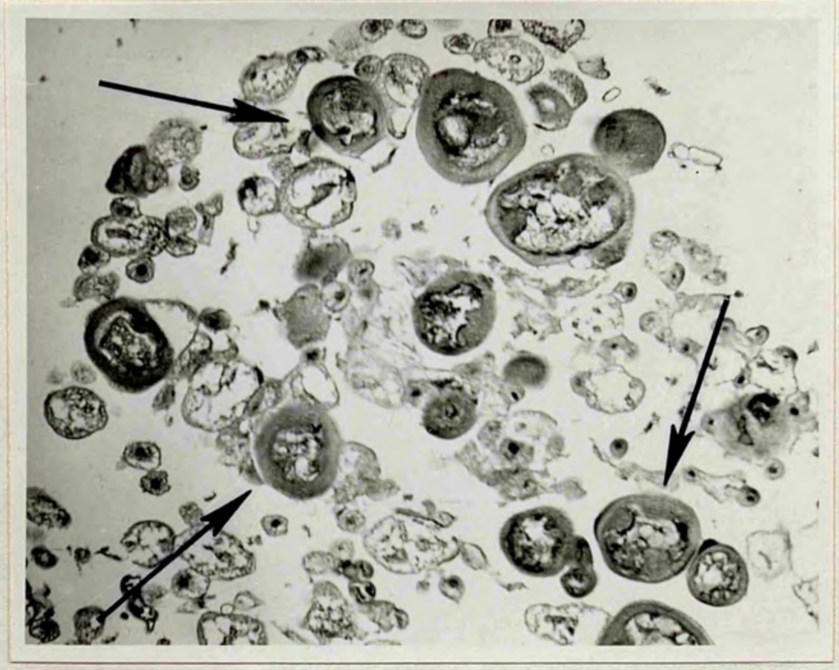
A - Prior to dissociation.

B - At about the time of dissociation.

C - When more or less fully grown.

A & B - surface view: C - median section.

Fig. 4.18. Section of dissociated embryonic membrane cells among host fat body tissue.



(x 140)

(x 560)

k) Development of the parasite.

Following their deposition into the host, there is a considerable and rapid increase in the size of the parasite eggs. Fig. 4.19 illustrates a range of sizes of eggs from one host where the largest is 25 times larger by volume than the smallest. The volume of the eggs may be calculated from the following:-

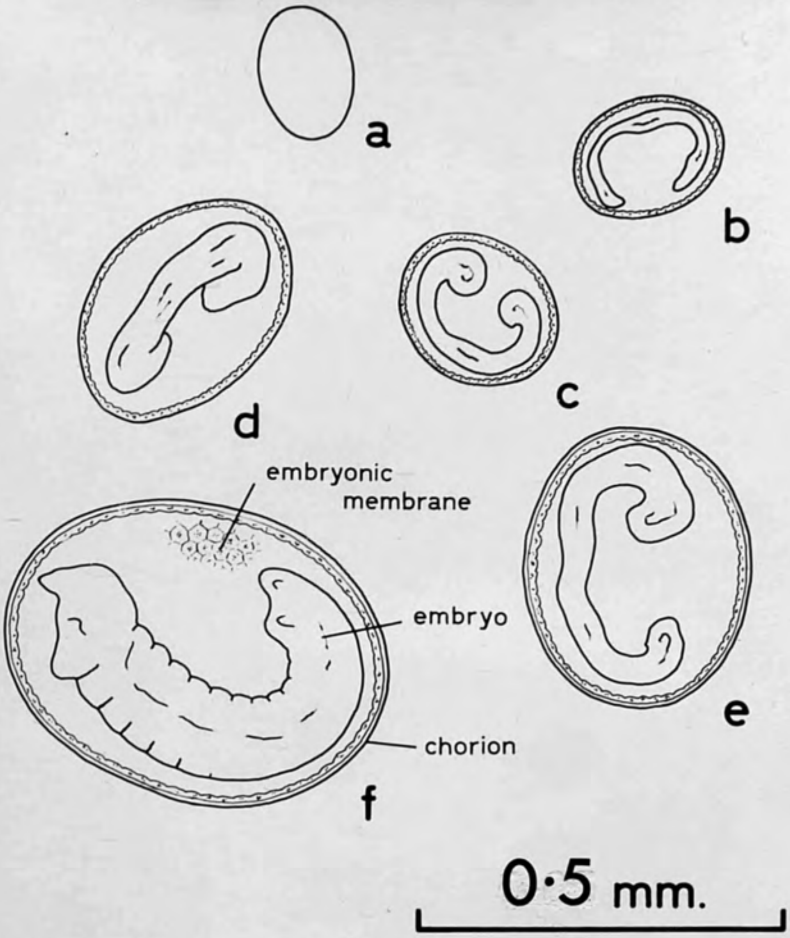
$$V = \frac{\pi B^2 A}{6}$$

where B is the width and A the length of the egg.

The largest parasite "egg" found in any bumblebee measured approximately 1.0 x 0.8 mm., which was more than 230 times larger by volume than the smallest egg found in any bumblebee. In this, and similar large "eggs", however, a chorion could not be distinguished around the embryonic membrane and so strictly speaking this stage is no longer an egg since eclosion-proper must have occurred. Presumably the stretching of the chorion cannot keep up with the expansion of the egg, and so the chorion breaks before the larva escapes from the embryonic membrane.

The young embryo develops with both the anterior and posterior ends incurved (Fig. 4.19). Later, the head end straightens and eventually the larva becomes more or less 'C'-shaped. The ventral surface of the developing larva is the convex side and at this time, the internal structures of the body are clearly visible (Fig. 4.20). In this

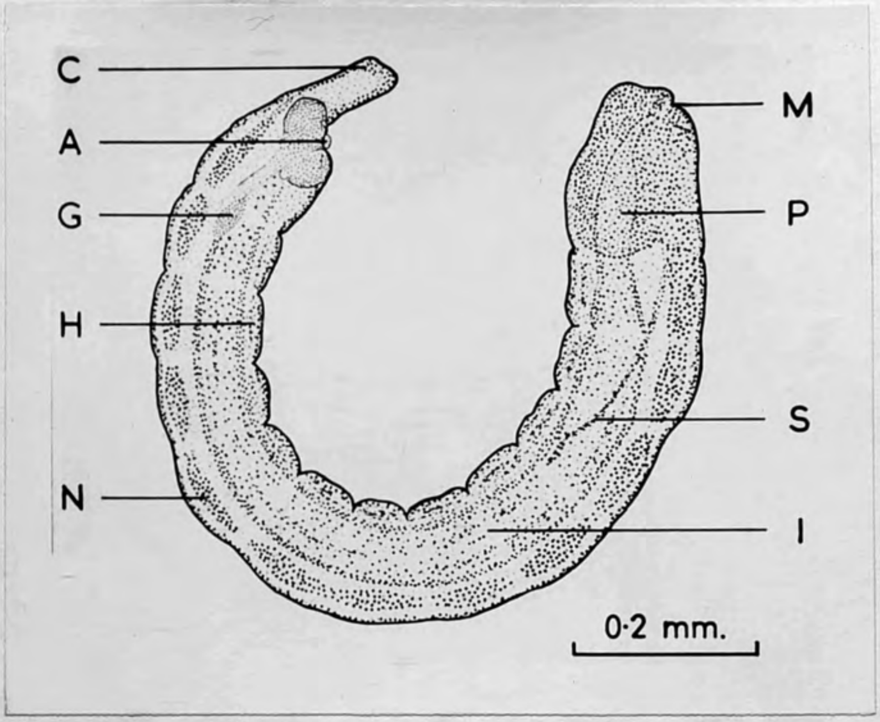
Fig. 4.19. Eggs of S. splendidus at various developmental stages.



All eggs taken from same host (B. terrestris forager) 26th May, 1967.

a : outline of smallest egg found.
b - e : intermediate stages.
f : largest and most advanced stage present.

Fig. 4.20. Some morphological details of 1st instar larva removed from embryonic membrane shortly before time of emergence.



A - Anus with rudiments of vesicle protruding.
C - Caudal process.
G - Genital gland.
H - Dorsal heart.
I - Intestine.
M - Mouth.
N - Ventral nerve cord.
P - Pharynx.
S - Salivary (labial) gland.

position, the caudal process lies next to the embryonic membrane.

Larvae escape from the embryonic membrane head first. Initially, the larva straightens out anteriorly, causing the surrounding membrane to bulge (Fig. 4.21) and also flatten laterally. Several "eggs" were found in bumblebees in this condition which suggests that the larvae remain for a time exerting pressure against the membrane, before finally breaking through the wall and escaping. Eventually, the head breaks through the membrane (Fig. 4.22). The larva remains in this position for a short time and then with a sudden straightening of the body posteriorly, escapes from the surrounding membrane. Pieces of the embryonic membrane do not remain attached to the larva following its escape.

The observed distribution of parasites of various stages in the host suggests that following their emergence from the embryonic membrane, larvae in the head or thorax of the host migrate to the abdomen, where their further development takes place. Such a migration is known to take place in <u>M. vittatae</u> (Smith, 1952).

Feeding begins shortly after emergence, and small, whole, dissociated cells of the embryonic membrane may then be seen in the gut (Fig. 4.23). Data on the length of time spent in the various larval instars have not been obtained. As well as embryonic membrane cells, yellowish globules of fat also appear in the guts of developing larvae.

Fig. 4.21. Position adopted by larva shortly before rupture of embryonic membrane.

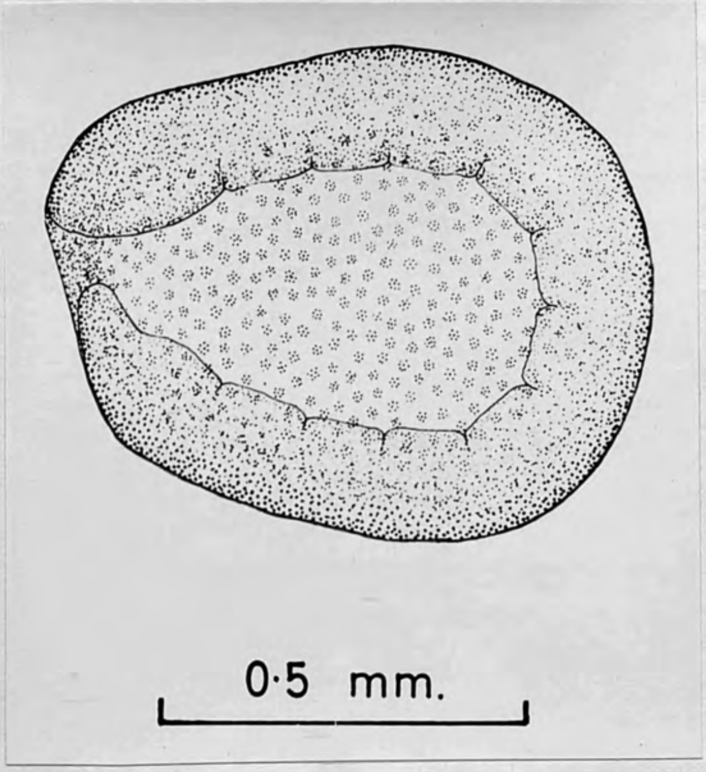


Fig. 4.22. Larva breaking through embryonic membrane.

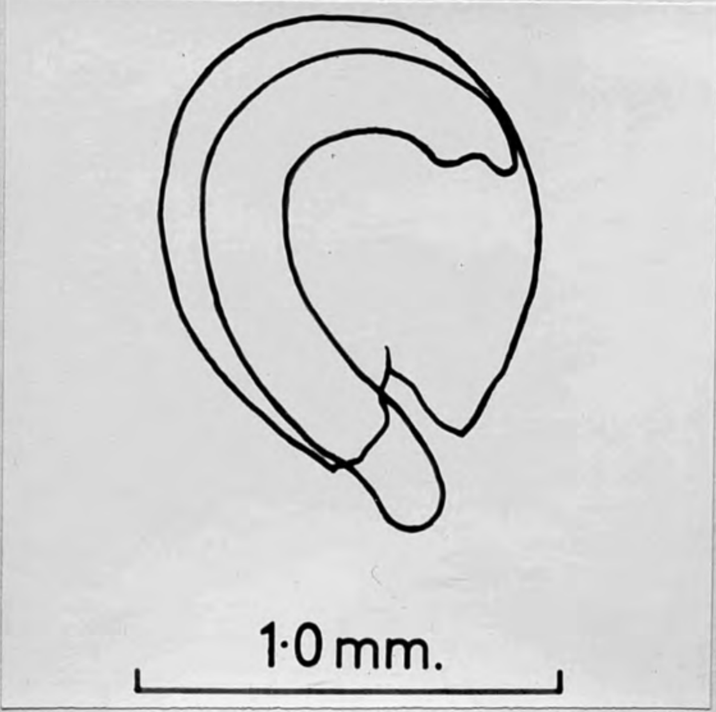
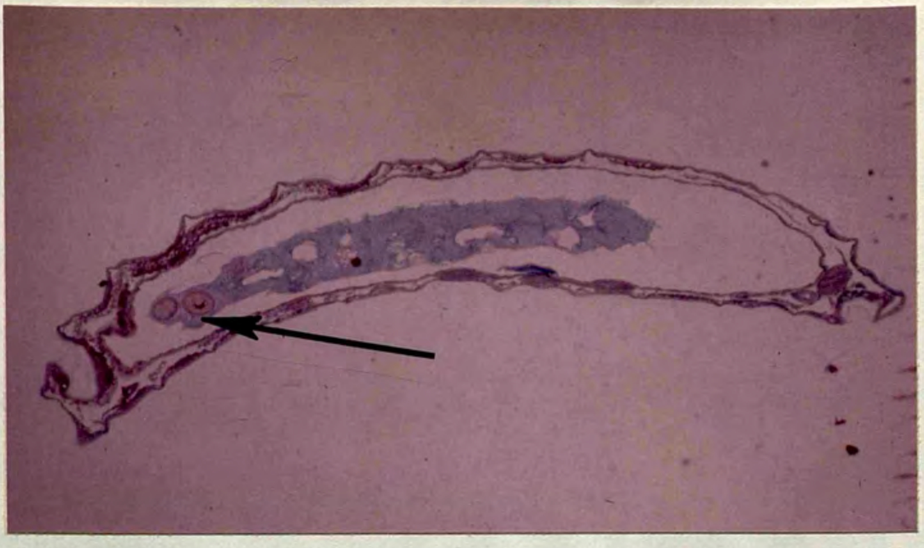


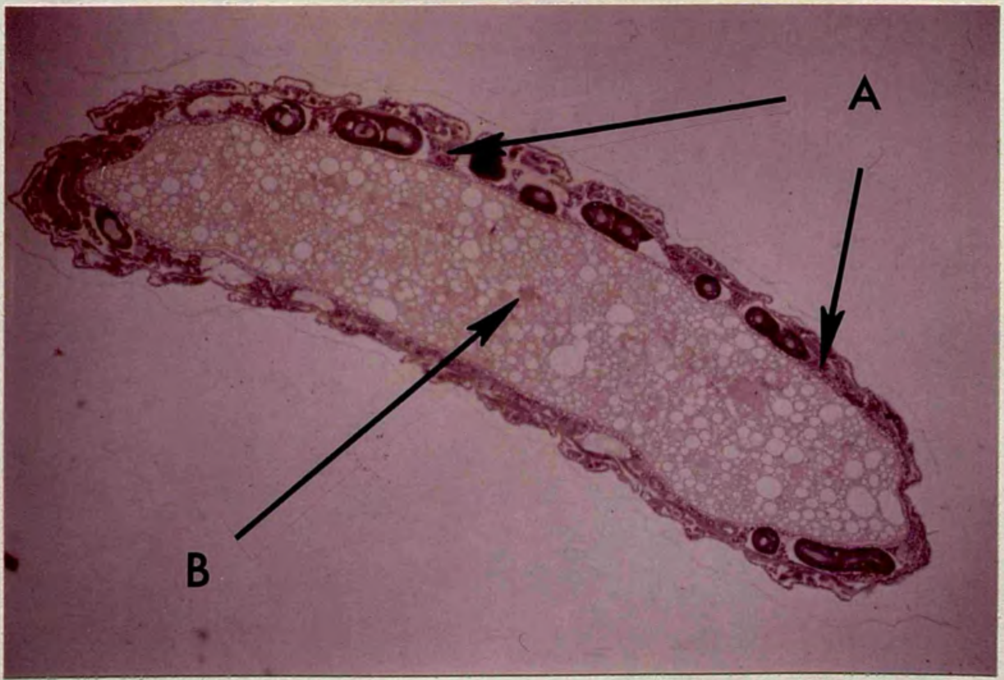
Fig. 4.23. Median longitudinal section of 1st instar larva shortly after feeding has begun, stained with Heidenhain's AZAN.



(x 50)

Embryonic membrane cells in gut arrowed.

Fig. 4.24. Median longitudinal section of fully grown 4th instar larva, stained with Best's Carmine.



(x 30)

A - Glycogen deposits. B - Gut packed with fat vacuoles.

Observations on living 2nd instar larvae showed that fat globules are broken down in size by muscular contractions of the intestinal wall which pass both anteriorly and posteriorly along the gut, forcing the contents through a small orifice at the centre of each constriction. The intestine of a fully grown larva is distended with small globules of fat (Fig. 4.24).

Larvae develop freely within the haemocoel of the host, although some tend to be entangled in the Malpighian tubules or squeezed between the abdominal fat body tissue and epidermis. No evidence was found to suggest that the larvae ever feed directly upon the host viscera.

By the time that the larvae are fully grown, there is no longer any sign of the hypertrophied embryonic membrane cells. Presumably all of them have been broken down and their contents ingested.

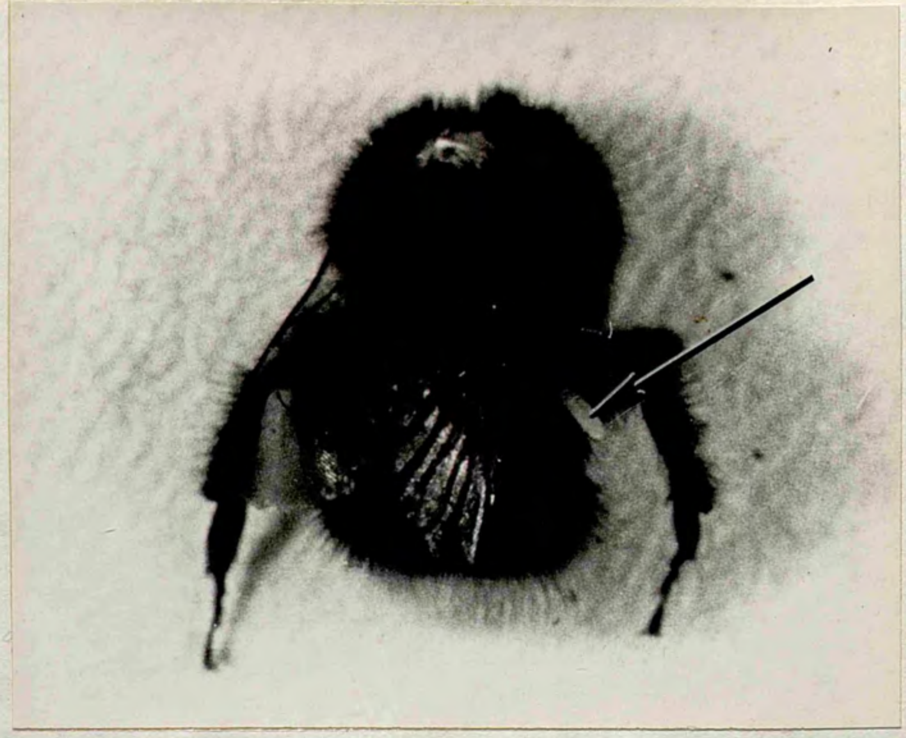
Following their ecdysis from the 4th to the 5th instar, larvae do not feed but escape from the host.

1) Emergence from the host and pupation.

Larvae escape from the host by tearing the intersegmental membrane between the 1st and 2nd (morphologically 2nd and 3rd) abdominal segments, usually to one side of the body (Fig. 4.25).

The emerged larvae are very active and progress with a "looping-rolling crawl" similar to that described by Smith (1952) for M. vittatae. The larvae travel backwards, and attempt to force their way beneath the surface of the ground.

Fig. 4.25. Parasite larva emerging from abdomen of host.



(x 3 approx.)

Once below the surface, each larva constructs a white, silken cocoon. The cocoons measure approximately 2.8 x 1.4 to 3.5 x 1.8 mm.. Those containing female individuals were the largest. Although soil particles are not intimately incorporated in the walls of the cocoons, some do adhere to and become slightly enmeshed in the outer strands of silk.

The outer layers of the cocoon are loosely spun and may be unwound without difficulty. Immediately surrounding the central chamber, however, is a greyish, semi-opaque, parchment-like layer. This forms a tough, protective envelope within which the pupa develops.
The larval meconium and exuviae are placed at the posterior end of the chamber.

In laboratory cultures, considerable mortality occurred following the emergence of larvae from the host (Table 17) and only about 40% of the larvae placed in rearing dishes succeeded in forming cocoons. Further mortality of larvae occurred within the cocoons before pupation.

Table 17 — Rearing successes of S. splendidus in laboratory cultures.

Year	No. of final instar larvae placed in rearing dishes	No. of cocoons formed	Dissected from cocoons			
			Pupae		Adults	
			m.	f.	m.	f.
1965	50	?	2	-	11	-
1966	70	30	-	-	11	1
1967	55	21	-	3 §	-	-

m. = male f. = female

§ = several cocoons not yet dissected (Sept. 1967)

m) Life cycle.

In Fig. 4.26, the stage of development of parasites, in various hosts, at different times of the year is given. The distribution indicates that S. splendidus is single brooded.

Unlike many Euphorine parasites which overwinter as 1st instar larvae within the host (Clausen, 1940), S. splendidus overwinters in the cocoon, either as a pupa or adult. Oviposition, therefore, occurs in the spring, probably extending from early May to the middle or end of June.

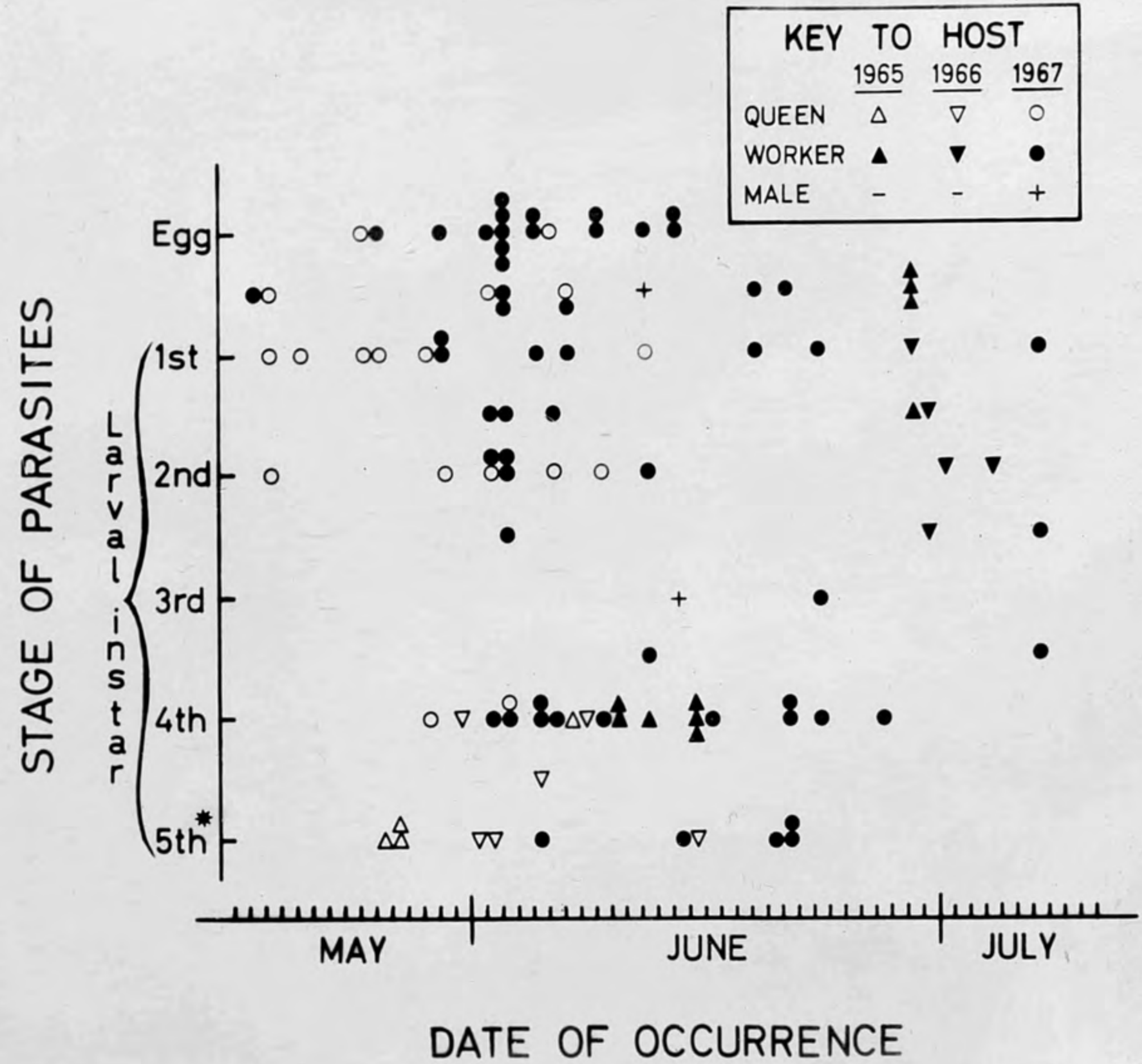
In 1967, eggs were found in bumblebees from the 18th May to the 21st June, and in 1965, as late as the 25th June. Since full-grown larvae emerged from their hosts from the end of May onwards, the time spent within the host is evidently quite short, probably lasting for a maximum of three or four weeks. Although in no season were full-grown larvae recorded after the 21st June, emergences of larvae from bumblebees are probably to be expected throughout July, when the young individuals present in bumblebees at the end of June and beginning of July complete their development.

n) Incidence of parasitism in bumblebees.

Dissections of several hundred bumblebees in March and Arpil during 1965, 1966 and 1967, failed to reveal a single individual parasitized by S. splendidus. Further, no parasitized bumblebees were found after the beginning of July.

In 1967, dissection of 352 foraging worker bumblebees

Fig. 4.26. Seasonal distribution of various parasitic stages of S. splendidus in bumblebees (1965 - 1967).



* - 5th instar larvae recorded under day of emergence from host.

during May, June and July, gave an incidence of parasitism of 14.5%. Although many foragers of B. hortorum were dissected (Table 18) none was parasitized, and if this species is omitted from the results, then the extent of Syntretus parasitism exceeds 17%.

Table 18 — Parasitization of foraging worker bumblebees by S. splendidus during May, June and July, 1967.

Species	No. dissected	Positive cases	% parasitized
B. terrestris	68x	16x	23.5
B. lucorum	137	15	10.9
B. pratorum	41	10	24.4
B. hortorum	58	0	-
B. agrorum	47	10	21.3
B. humilis	1	0	-

x :- excludes 4 parasitized individuals taken at nest entrance of Col. 140 (see text).

Samples of foraging queens taken in May and June, 1967, gave an incidence of Syntretus parasitism of 17.2% (Table 19). B. hortorum samples again were unparasitized and if excluded, then the extent of parasitism among foraging queens rises to nearly 31%.

With the exception of one B. pratorum queen (Col. 2), all foundress queens parasitized by S. splendidus (that is, queens collected with their colonies) were later-nesting species, and were attending colonies still in the incipient stages of development. Out of a total of 36 incipient colonies examined in 1965 and 1966, 8 (19.4%) were headed by *Syntretus*-infected queens. Seven of these (Cols. 4, 7, 17, 62, 65, 67 and 75) were *B. agrorum* colonies and the other (Col. 72) a *B. ruderarius* colony.

Table 19 — Parasitization of foraging spring queens by *S. splendidus* in 1967.

Species	No. dissected^{x}		Positive cases		% parasitized
	May	June	May	June	May + June
B. lapidarius	1	2	1	1	66.7
B. lucorum	2	-	-	-	-
B. pratorum	1	3	1	1	50.0
B. hortorum	29	15	-	-	-
B. agrorum	21	12	7	3	33.3
B. humilis	5	4	-	1	11.1
P. campestris	-	2	-	-	-
P. vestalis	1	1	-	1	50.0

x :- excludes queens parasitized by *Sphaerularia*.

The absence of *S. splendidus* from all samples of *B. hortorum* is unexplained. This species foraged, initiated colonies, and was common in the same areas as *B. agrorum* at the same time in the spring, and no differences in their forage preferences were observed. *B. hortorum* is a very long tongued species and since tongue length may be correlated with the length of time spent visiting a flower (see data summarized by Holm, 1966) it is possible that *B. hortorum* is less vulnerable to attack than other species because of its faster working speed when foraging.

The virtual absence of S. splendidus in queens of early-nesting bumblebee species (B. terrestris, B. lucorum and B. pratorum) is explained by the comparative lateness of the infective period of the parasite, since such queens will mostly have been superseded in their foraging duties by workers. Queens of later-nesting species, on the other hand, will still be actively foraging during the infective period since most will not have reared any workers, and thus they will be exposed to the dangers of infection.

o) Adult.

Marshall (1887) has described the adult of S. splendidus, under the name of Microctonus splendidus Marshall, from specimens collected at Nunton in Wiltshire. Unlike other described Syntretus species the first abdominal segment is aciculate. A male is illustrated in Fig. 4.27.

Descriptions of the genus Syntretus are given by Lyle (1927) and Muesebeck (1936). These authors also discuss its synonomy with related genera.

p) Sex ratio.

From material obtained in 1965, 1966 and 1967, a total of 24 male and 4 female pupae or adults were reared (Table 17). These figures are rather small, but suggest that males may be more common than females. Details of the sex ratio from individual hosts were not obtained because of the small number of parasite survivals.

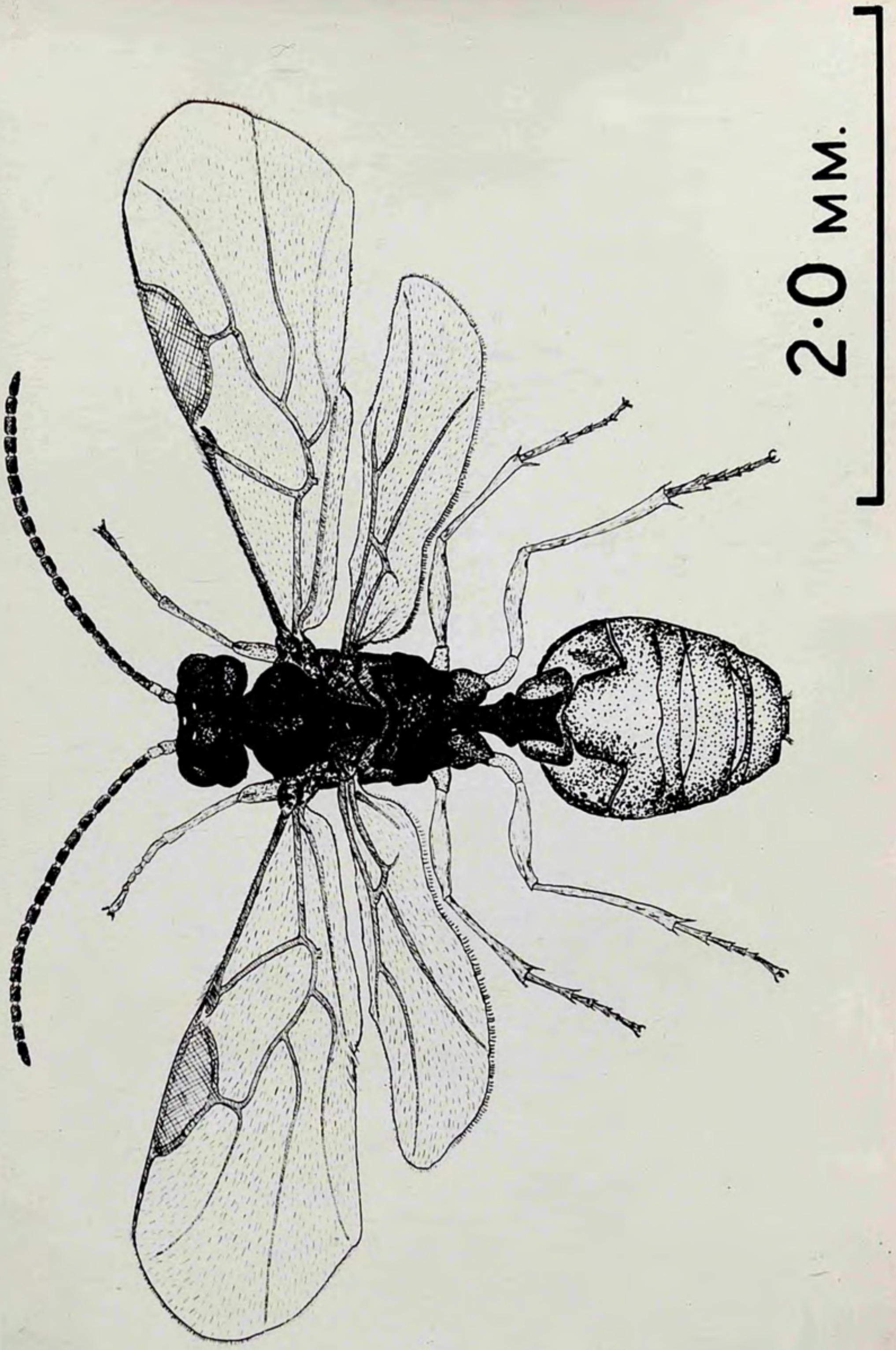


Fig. 4.27. Male of Syntretus splendidus (Marshall).

q) Effect of parasite on host.

Various writers have recorded the degeneration of the ovaries in female hosts infected by Euphorine parasites, and Legge (1937), in his description of the parasitized *B. terrestris* queen, mentions the poorly developed ovaries of the host.

All *S. splendidus*-infected queens dissected by the present writer, with the exception of those containing parasite eggs or 1st instar larvae, had degenerate or degenerating ovaries (Fig. 4.28). Further, since egg resorption occurs some time before the parasite larvae are full-grown, colonies headed by parasitized queens usually have no eggs or young larvae amongst the brood. Thus the presence of a *Syntretus*-infected foundress queen may be anticipated in those colonies which have pupal clumps but no egg cells or young larval clumps. The effect of parasitism upon the male gonads was not established.

Although the parasites probably do not feed directly upon the host's body tissue, the fat bodies of parasitized bumblebees are considerably reduced in bulk and are usually much distorted (see Fig. 2.11 page 95). This effect on the host is evident, even at an early stage of parasite development.

External distinctions between parasitized and healthy bumblebees cannot easily be made, except possibly from behavioural changes in the host shortly before the emergence of the full-grown parasite larvae.

Bumblebees infected by *S. splendidus* appear to carry

Fig. 4.28. Ovaries of foundress queens of B. agrorum to show effect of parasitization by S. splendidus.

(x 2)

1 - Ovaries of parasitized queen.

2 - Ovaries of healthy queen.

out their normal duties, until the final stages of infection, with little or no interference from the parasites. Parasitized workers were apparently able to forage normally and one B. agrorum queen was known to have successfully foraged for pollen, less than three days before full-grown parasite larvae emerged from her body. Parasitized queens continue to incubate their brood clumps and tend to the brood in the same way as healthy queens. Several hours before the emergence of parasites from their bodies, however, such queens ignore their brood. Those confined in nest boxes wander listlessly about and also remain stationary for long periods on the vertical walls of the boxes. Parasitized workers confined in the laboratory behave similarly.

A single field observation on hosts at a late stage of infection was obtained. In this instance, four workers of B. terrestris were found on the soil beneath an overhanging grass clump surrounding the entrance hole to their nest. These bees were sluggish and did not attempt to fly. Within 12 hrs. of their capture, larvae of S. splendidus emerged from their bodies.

Following the emergence of larvae from their bodies, bumblebees remain in a listless state and usually die some hours later. As mentioned earlier, the B. ruderarius queen parasitized by S. splendidus, died before the emergence of the parasites (Fig. 4.25). The unusual presence of full-grown larvae in the thorax and neck region of this queen (Fig. 4.2), probably brought about the premature death of the host in this instance.

In some cases the intersegmental membrane through which the parasites make their escape is considerably damaged and the tergites on either side are noticeably parted. In other instances, however, the site of emergence of the parasites is hardly visible. Apparently, bumblebees do not usually die as a result of the mechanical injury caused by the exodus of the parasites, but since they are too weak to feed, they die of starvation. All bumblebees which were examined after the emergence of the parasites, had empty, or virtually empty, honey stomachs.

According to Balduf (1926) Coccinellid adults parasitized by the solitary endoparasite Perilitus melanopus Ruthe are not killed by the feeding of the parasitic larvae or by the mechanical injury caused by their ultimate emergence from the body, but by starvation as the weakened hosts are each entangled in the cocoon which is constructed beneath the body. The beetle Olla abdominalis Say may be experimentally revived if freed from the parasite cocoon, and may eventually lay eggs. Further, if reinfected experimentally, a second mature parasite may be reared from the same host (Timberlake, 1916).

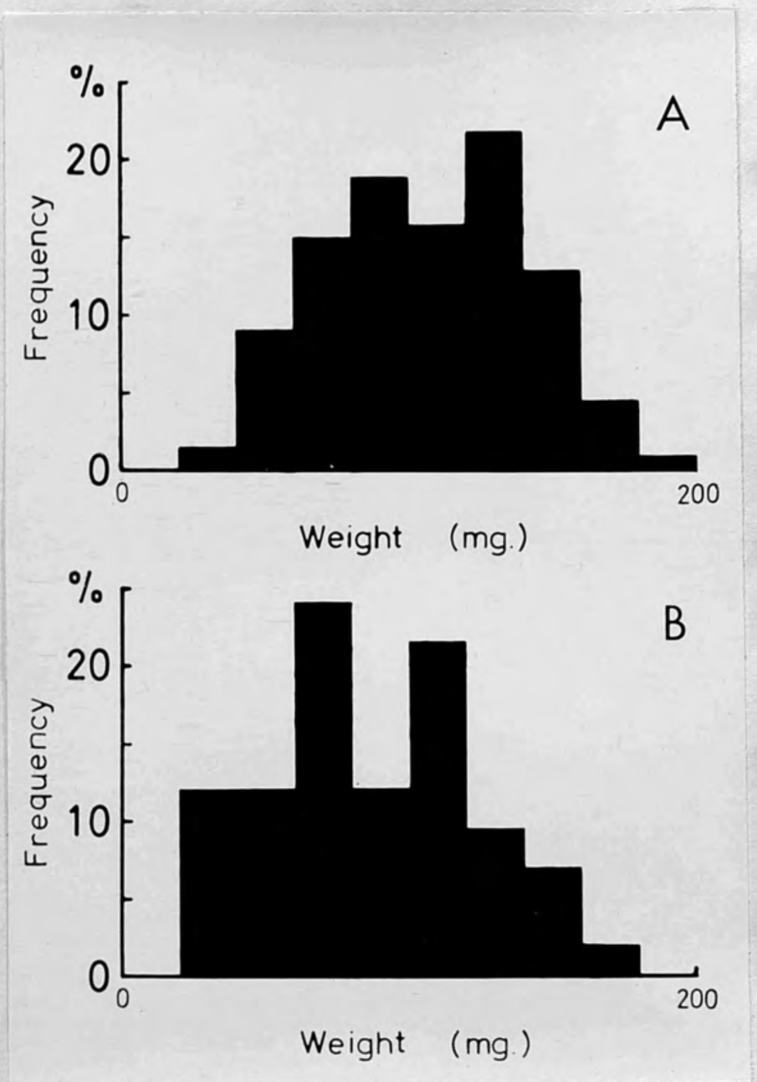
A few bumblebees survived for two days or more, following the emergence of parasite larvae, but such bees were never seen to feed. One B. agrorum queen became unusually active shortly after the emergence of the parasites, but died on the following day.

In order to obtain quantitative data on the possible deleterious effect of S. splendidus on its host, a comparison was made of the sizes of the 1st brood adults produced in colonies of both parasitized and healthy bumblebee queens. Several incipient colonies of B. agrorum, at the pupal stage, were collected in the springs of 1965 and 1966 from adjacent areas of rough grassland near the Zoology Department ("Alderhurst") of Royal Holloway College. Since all material was obtained from the same area any differences due to the foundress queens having foraged under different conditions or in areas with different forage potentials are reduced.

Comparisons were made on the adults produced from 30 incipient colonies of B. agrorum, of which 7 were headed by Syntretus-infected queens. Since the full-grown larval parasites emerged from the queens when the incipient clumps were in the pupal stage, or just after, much of the rearing of the brood by the queens must have been carried out during the time that they contained parasites. Although results are based on figures obtained for two seasons, they are summated since the ratio of parasitized to healthy queens was similar in each year and differences in the size of workers produced in clumps in each year were not significant. A more detailed analysis of the progeny of incipient colonies of B. agrorum has been given in Part III of the present thesis.

The weights of the 1st brood adults reared from colonies of the healthy and parasitized queens respectively are given as frequency histograms in Fig. 4.29. Data obtained from

Fig. 4.29. Size of 1st brood adults produced in incipient colonies of *B. agrorum*.



A - Progeny of healthy queens.

B - Progeny of queens parasitized by *S. splendidus*.

each colony as regards the number and size of individuals, the dates of their emergence, etc., are given in Appendix Table XXXI.

Adults from incipient colonies headed by healthy queens were heavier than those reared by parasitized queens ($p < 0.01$), although when weight was divided by wing length no difference was found. Since colonies may be initiated before the foundress queens become parasitized — this was certainly so in 1967 — queens are not necessarily affected for the whole period of brood rearing. Size differences between the progeny of healthy and parasitized queens may, therefore, tend to be reduced. Results are sufficient to indicate that the presence of the parasite has a deleterious effect on the brood rearing ability of queens, probably by reducing the overall amount of food which the queen can collect for the developing brood.

r) Economic importance.

Inspite of the high incidence of parasitism in certain bumblebee species, it is probable that S. splendidus is of little economic importance as a parasite of worker (forager) bumblebees since individuals continue to forage for the good of their colonies for most of the time during which they are parasitized. They may be less efficient foragers, but this will not necessarily affect the well-being of their colonies significantly. The forager force of a bumblebee colony is continually being replaced since foragers usually live no longer than three or four weeks (Brian, 1952). Death of individuals from Syntretus-parasitism is, therefore, of little importance.

As a parasite of spring bumblebee queens, however, S. splendidus may be of considerable economic importance. Field samples indicate that many bumblebee species serve as hosts for this parasite and that almost one third of all B. agrorum queens which escape parasitization by the nematode Sphaerularia, become infected by Syntretus. Figures of this magnitude indicate that this parasite is, at least in some areas, a potentially serious pest of certain bumblebee species and a causitive organism of the premature destruction of young colonies.

DISCUSSION

Both the entry into hibernation and the initiation of colonies in the following spring are the responsibility of individual bumblebee queens. The bumblebee population in succeeding seasons is, therefore, dependent not only on the number of young queens produced each year, but also on the ability of these queens to overwinter successfully and later to form colonies of their own. The availability of suitable hibernation and nesting sites has an important bearing upon bumblebee numbers as have many other extrinsic factors such as weather conditions, parasites, and the presence or absence of suitable forage (see review by Holm, 1966), since they strongly influence the number of queens that will succeed in establishing colonies in any one season.

Bols (1939) has referred to the numerous natural hazards faced by hibernating queens, but what percentage of queens entering hibernation actually survive is unknown and in natural populations would be difficult to measure. The relative merits of the different types of hibernation site selected by queens are also hard to assess. Basically all sites have similar ecological properties in that they are well drained and shaded from direct sunlight. Some species, as has been shown for <u>B. lucorum</u>, are somewhat adaptable in their choice of a hibernation site, but an element of preference within a species in usually found, which may be related to the normal time of appearance in the spring of hibernated queens of that species. There is apparently little or no relationship between the type of site chosen for hibernation and that selected for nesting since the species that form colonies deeper in the ground than others (for example the subterranean-nesting species B. terrestris) are often found hibernating in the more superficial types of site. Nor is there any relationship between the overwintering site selected and the main biological grouping of "pollen-storers" and "pocket-makers".

Little positive data have been accumulated on the hibernation habits of the surface-nesting species or "carder-bees" such as B. agrorum, B. humilis, B. ruderarius, but it is possible that these species may all hibernate in similar sites to those in which B. agrorum was found. Presumably carder-bees rarely, if ever, hibernate in banks, slopes, or under trees, since none was found hibernating in such sites in the present work and Bols (1937, 1939) recorded very few specimens from the area of his "summer camp". Observations made on queens parasitized by Sphaerularia support this view.

Sphaerularia bombi is one of the most important enemies of queen bumblebees, infecting queens some time during the hibernation period. The fact that special sites are frequented by hibernating queens and that infected queens are also attracted to such sites after their emergence from hibernation, is important in the life cycle of this parasite. What effect site selection has on their chances of becoming parasitized by

Sphaerularia is unknown. All the different types of hibernation site investigated in the field during the early summer, were frequented by greater or lesser numbers of nematode-infested queens, which indicated that the parasite was probably an established pest in all of these places and therefore in a position to attack many different host species. It is possible that where large numbers of queens regularly occur in hibernation sites of limited extent, the incidence of parasitism may be increased over that found in areas where queens frequent less well defined sites. This factor could explain why certain species tend to be more heavily parasitized than others. However, why, as found by Cumber (1949a) and also in the present work, B. lucorum should be more heavily parasitized than species such as B. terrestris and B. pratorum, when all tend to hibernate in similar sites, is not clear.

To judge from the reports of various workers (Palm, 1948; Cumber, 1949a; Medler, 1957, 1962; Fye, 1966; and others), the incidence of parasitism by Sphaerularia varies considerably from one geographical region to another. It is doubtful whether much reliance can be placed on the published extent of parasitism from samples of queens taken in the later part of the spring, especially among early-nesting species, because as healthy queens found colonies, they become more infrequently seen flying in the field. Cumber's (1949a) figure of 80%, erroneously cited by Holm (1966) as being the level of Sphaerularia infection among bumblebees in England, refers only to a sample of B. agrorum queens captured towards the end of June, when, as shown in Part III, colonies of this species are well under way. Healthy queens of this species are, therefore, rare in the field at this time of the year. In April and May, Cumber found that 3 out of 12 queens (25%) of this species were parasitized. Data on the percentage of queens infected by this parasite tend to be biased in favour of parasitized queens and in themselves unreliable, and need to be treated with caution.

Consideration of the number of parasites per individual queen recorded by different observers provides interesting data. Thus Cumber (1949a) found from 1 to 68 worms per host and Stein (1956c) observed a maximum of 30 and gives 4 to 6 as the usual number in his samples. The present writer observed from 1 to 42 worms per host, while Fye (1966) recorded only 1 or 2 worms per host in Wisconsin bumblebees. Medler (1957), also working in Canada, observed a maximum of 6 worms in any one individual. The percentage figures given by both Medler and Fye for the extent of parasitism are also rather low (up to 12%), inspite of the date of some samples, which indicates along with the small number of individual parasites per host, that Sphaerularia, although an important pest, is less extensive as a parasite of Canadian than of European bumblebees.

Up to the time of the production of the first workers in a bumblebee colony, the whole success of the future bumblebee population has rested with the young foundress queen. Analysis of the incipient stage of a bumblebee colony and the 1st brood adults produced gives an indication of the relative efficiency of the foundress queen. Thus, *B. agrorum* queens parasitized by *Syntretus splendidus* have been shown to produce smaller workers than healthy queens, and no doubt queens affected in other ways either as a result of intrinsic or extrinsic factors will produce inferior colonies. The effect of incipient colony development on the future success of the colony has not been estimated, but probably those queens producing the most efficient initial worker batches stand the best chance of producing successful colonies.

Although many investigators have succeeded in starting bumblebee colonies under laboratory conditions (see review by Holm, 1966) none has apparently attempted to find out how their results in the vital early stages compare with natural colonies. The present writer established several laboratory colonies of various species using spring queens but when results were compared with data from natural colonies they were always inferior. In laboratory initiated colonies of *B. hortorum* and *B. agrorum*, and probably in other species, fewer 1st brood adults were produced than the normal number in the wild. Individual workers were also smaller in size, although occasionally large specimens were reared. *B. pratorum* was the only captive species reared with apparent success but direct data from wild colonies were not available for comparison.

Data from incipient colonies of "pocket-makers" have shown that there is normally a range in the size of the 1st brood workers produced in any one colony. The significance of the size distribution of adults produced in incipient colonies has already been discussed. The size range of adults produced from any one larval group of more advanced colonies is greater in "pocket-makers" than in "pollen-storers" because of the different method of larval nutrition adopted in each case (Cumber, 1949b), but in the initial brood batch of "pollen-storers" the method of larval nutrition more closely approaches that of "pocket-makers" since some pollen provisioning occurs. It is possible that a more effective size range is achieved among 1st brood adults of "pollen-storers" by this alteration in the normal method of larval feeding. Observations to support this suggestion, however, have not been made.

Cumber (1949b) has pointed out that in "pocket-makers" the size difference between queens and workers is less obvious than in "pollen-storers", and that this difference is related to the method of larval nutrition. Although in some colonies there may be a complete range in the size of females, so that by size alone large workers and small queens cannot be distinguished (Frison, 1927; Plath, 1934; Cumber, 1949b), doubtful individuals can usually be distinguished by weighing them (Richards, 1946). Both Richards and Cumber have demonstrated that greater discontinuity is achieved when weight is divided by wing length.

The basic difference between a queen and a worker is a physiological one and as stated by Cumber (1949b) non-callows can readily be distinguished by dissection on account of the differences in their fat bodies. Some queens, particularly in "pocket-makers", are very small and the reports of workers overwintering or attempting to enter hibernation, as mentioned in Part I, are probably based on observations made on small queens.

Stein (1956a, 1956b) has reported that by confining queens (gynes) at temperatures of about 20°C. some individuals were induced to lay eggs and produce worker brood. Stein found, however, that their behaviour was worker-like in that the bees foraged and tended to the brood communally, unlike typical spring queens which found colonies individually. The present writer found that in some confined colonies undoubtedly queen-sized females occurred which appeared to behave more like workers than queens. Dissection showed that all such individuals had worker-like fat bodies and some had enlarged ovaries. It is probable that these individuals were destined to be queens, but that post-emergence factors affected their physiology so that they became worker-like. Such females probably cannot revert to being normal queens and will not enter hibernation. Their behaviour cannot, therefore, be considered as typical of gynes. It is possible that some, if not all, of the descriptions of gynes performing worker-like duties during the late summer and autumn, such as foraging for pollen, are the result of observations made on such anomalous queens. Physiological disturbances have also been noticed in confined spring queens. Such queens do not lay eggs but do accumulate fat reserves in their fat bodies. Medler (1962) who reported the occurrence of egg absorption in most of his confined spring queens suggested that the cause was the lack of a normal pattern of nesting and egg laying.

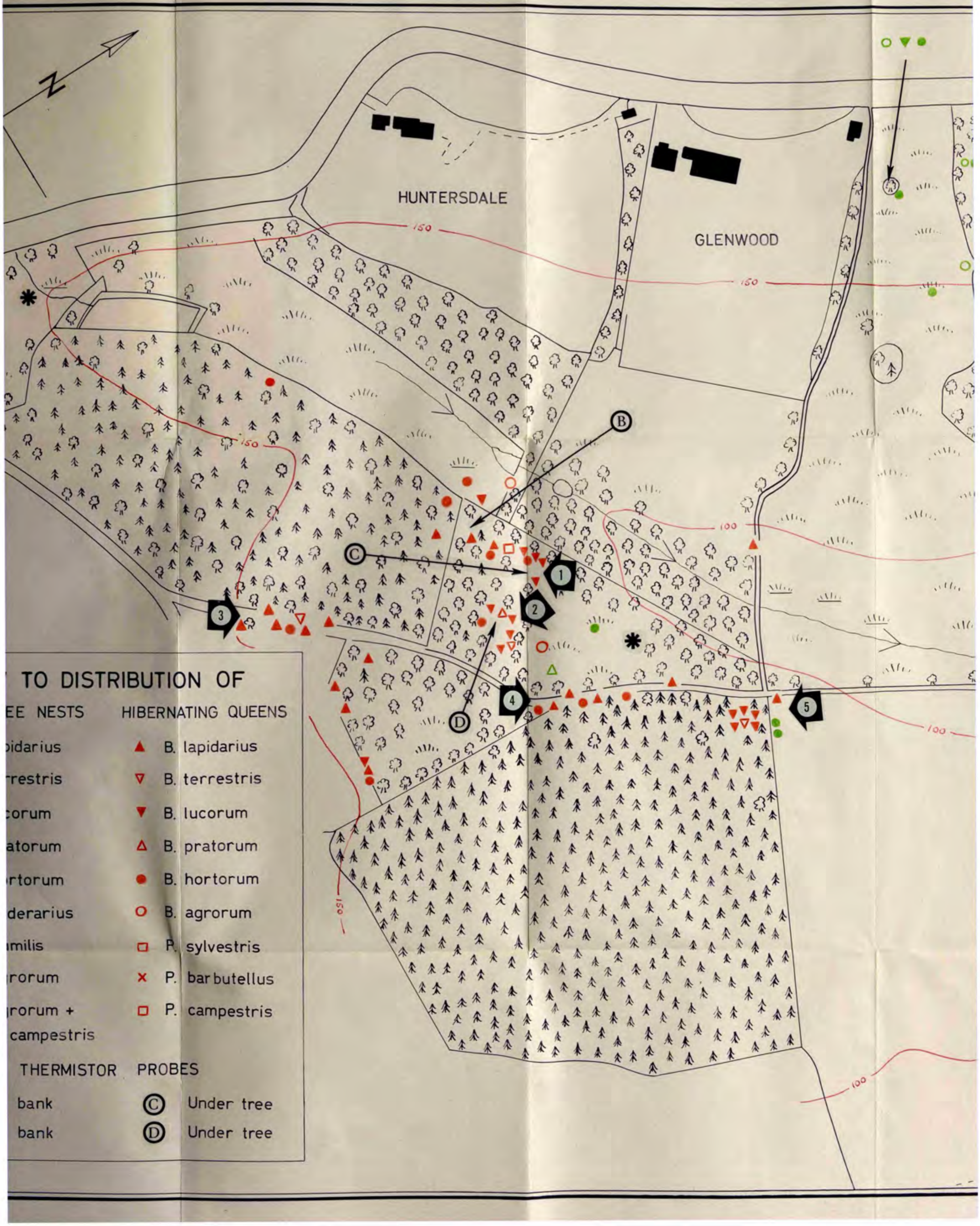
In view of the virtual absence of previous records of hymenopterous larvae in bumblebees, the extent of infection by _Syntretus splendidus_ found by the present writer is, at first glance, surprising. However, the present study of incipient colonies of bumblebees coincided with the major infective period of this parasite, since young foundress queens of certain species are major hosts. Studies on bumblebee colonies, including their parasites and commensals, have usually in the past been limited to the post-incipient stages, when, as shown in Part IV, _S. splendidus_ is unlikely to be found. The lack of records of parasitized foraging bumblebees is probably as much an indication of the scarcity of field observations as the rarity or limited distribution of the parasite.

Appendix Map 'A'

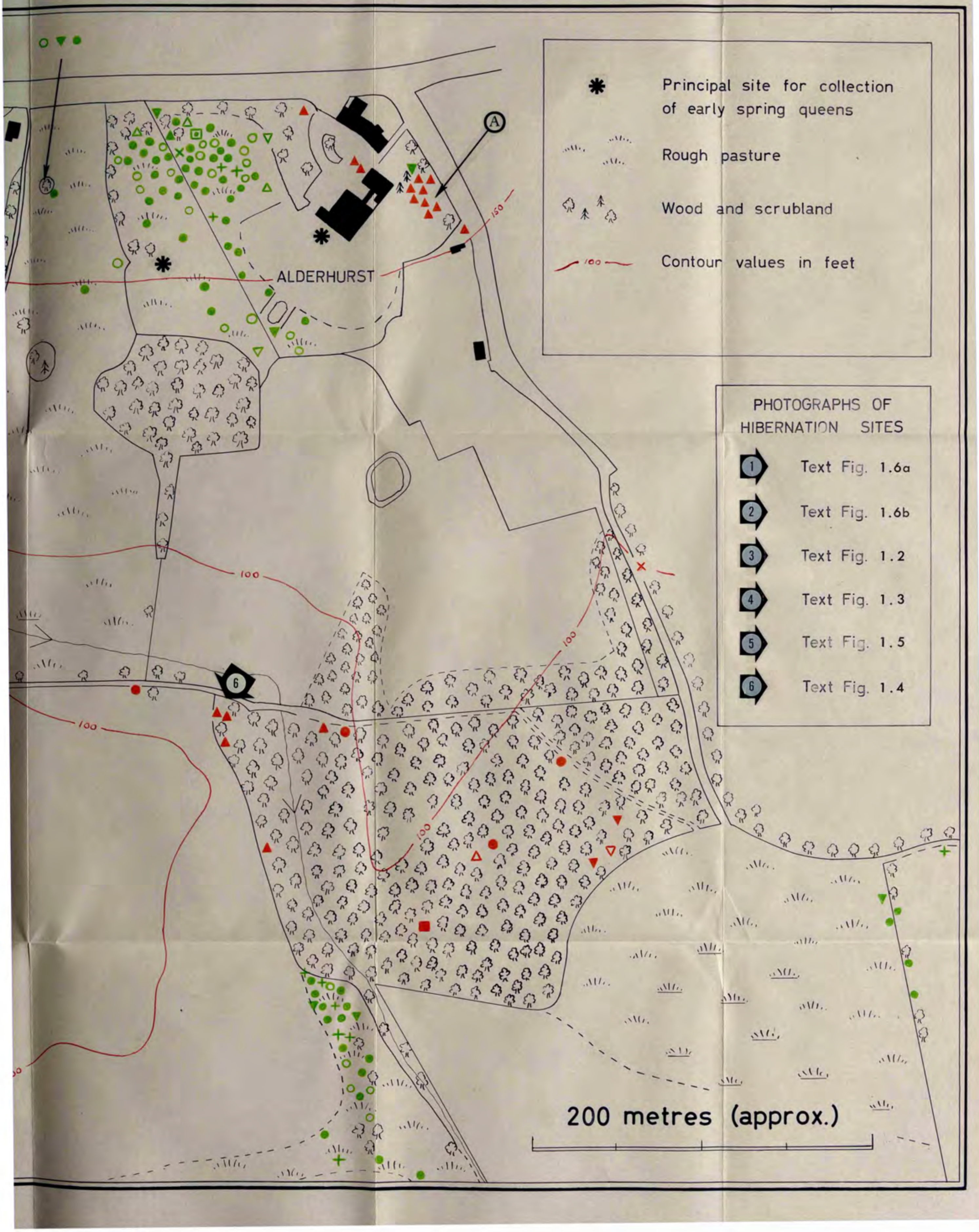
Map reference for "Alderhurst" :- SU 996698


N
KEY TO D
BUMBLEBEE NES
▲ B. lapidarius
▽ B. terrestris
▼ B. lucorum
△ B. pratorum
○ B. hortorum
× B. ruderariu
+ B. humilis
● B. agrorum
▣ B. agrorum
P. campes
THERM
Ⓐ In bank
Ⓑ In bank

SKETCH MAP OF PRINCIPAL FIELD STUD



STUDY AREA (1965 – 1967)



Appendix Table I

Nests found in field during 1965, 1966 and 1967.

(+ ..... within area of Appendix Map 'A')
(§ ..... with bee(s) parasitized by Syntretus)
(- ..... not in Englefield Green - Egham district: details at end of Table)

Colony No.	Date found	Species	Developmental stage
1+	19.v.65	B. agrorum	Incipient: larval
2+§	20.v.65	B. pratorum	Worker brood
3+	"	B. agrorum	Incipient: pupal
4+§	"	"	" "
5+	21.v.65	"	" "
6+	"	"	" "
7+§	"	"	" "
8+	"	"	" "
9+	24.v.65	"	" "
10+	25.v.65	"	" "
11+	31.v.65	"	" "
12+	2.vi.65	"	" "
13+	3.vi.65	"	" +1st adults
14+	"	B. hortorum	Worker brood
15+	4.vi.65	"	Incipient: +1st adults
16+	"	B. agrorum	" pupal
17+§	"	"	" +1st adults
18+	"	B. lucorum	Worker brood
19+§	"	B. lapidarius	"
20+	10.vi.65	B. pratorum	Queen, worker & male brood
21+	11.vi.65	B. hortorum	Male and worker brood
22§	28.vi.65	B. lucorum	Queen and male brood
23+	7.vii.65	B. hortorum	Male and worker brood
24+	"	B. terrestris	Queen, worker & male brood
25+	2.viii.65	B. lucorum	Male brood

Table continued overleaf

Appendix Table I continued:

Colony No.	Date found	Species	Developmental stage
26+	3.viii.65	B. agrorum	Male and worker brood
27	8.viii.65	B. lucorum	Male brood
28+	10.viii.65	B. agrorum	Queen, worker & male brood
29+	11.viii.65	"	"
30+	12.viii.65	"	"
31+	"	B. humilis	Worker brood
32+	"	B. agrorum	"
33+	"	"	Male brood
34-	14.viii.65	B. lapidarius	Queen and male brood (?)
35+	18.viii.65	B. agrorum	Male brood
36+	"	"	No brood
37+	"	"	Queen and male brood
51+	15.v.66	"	Incipient: egg
52	17.v.66	"	" pre-brood
53+	24.v.66	"	" pupal
54+	"	"	" "
55+	26.v.66	"	" "
56+	"	"	" "
57+	"	"	" "
58+	"	"	" pupal +1st adults
59+	"	"	" pupal
60+	"	"	" "
61+	"	B. hortorum	" "
62+§	29.v.66	B. agrorum	" "
63+	"	B. hortorum	" "
64+	30.v.66	"	" pupal +1st adults
65+§	"	B. agrorum	" pupal
66+	"	"	" "
67+§	"	"	" "
68+	"	B. hortorum	" pupal +1st adults
69+	"	"	" +1st adults

Table continued overleaf

Appendix Table I continued:

Colony No.	Date found	Species	Developmental stage
70+	31.v.66	B. agrorum	Incipient: pupal
71+	2.vi.66	"	" +1st adults
72+§	"	B. ruderarius	" pupal +1st adults
73+	6.vi.66	B. agrorum	" pupal
74+	"	B. hortorum	" "
75+§	8.vi.66	B. agrorum	" "
76+	"	"	" +1st adults
77+	"	"	Worker brood
78+	"	"	Incipient: +1st adults
79+	"	B. lucorum	Worker brood
80	19.vi.66	B. hortorum	Queen, worker & male brood
81	20.vi.66	B. ruderarius	"
82	21.vi.66	B. agrorum	Worker brood
83	22.vi.66	B. terrestris	Queen, worker & male brood
84	"	B. agrorum	Worker brood
85	"	B. pratorum	Queen and male brood
86	24.vi.66	B. lucorum	Queen, worker & male brood
87	"	B. hortorum	Male brood
88	"	"	No brood
89	25.vi.66	B. pratorum	"
90	27.vi.66	B. agrorum	Worker brood
91	29.vi.66	"	No brood
92	"	B. ruderarius	Male and worker brood
93	"	B. lucorum	Queen and male brood
94	5.vii.66	B. hortorum	"
95	7.vii.66	B. pratorum	"
96	"	B. lucorum	Queen, worker & male brood
97	18.vii.66	B. hortorum	Queen and male brood
98	"	B. lucorum	"
99	22.vii.66	B. agrorum	No brood
100+	28.vii.66	"	No brood

Table continued overleaf

Appendix Table I continued:

Colony No.	Date found	Species	Developmental stage
101+	2.viii.66	B. agrorum	No brood
102+	"	"	"
103+	"	B. humilis	Worker brood
104+	"	B. agrorum	Worker and male brood
105+	5.viii.66	B. humilis	No brood
106+	"	B. lucorum	Queen and male brood
107+	8.viii.66	B. agrorum	Queen, worker & male brood
108+	"	"	Worker brood
109+	"	"	No brood
110+	"	"	"
111+	9.viii.66	B. humilis	Queen, worker & male brood
112-	23.viii.66	B. terrestris	Queen and male brood
113-	22.iv.67	B. pratorum	Incipient: larval
114+	26.iv.67	"	" egg
115+	28.iv.67	B. agrorum	" egg
116+	"	B. humilis	" pupal +1st adults
117+	2.v.67	B. agrorum	" egg
118+	"	B. hortorum	" pre-brood
119+	"	B. agrorum	" larval
120+	3.v.67	B. hortorum	" egg
121+	"	B. agrorum	" "
122+	"	"	" "
123+	8.v.67	B. humilis	" "
124+	9.v.67	B. agrorum	" "
125-	10.v.67	"	" larval
126+	11.v.67	B. lucorum	Worker brood
127+	12.v.67	B. agrorum	Incipient: egg
128+	"	"	" "
129+	"	"	" "
130+	16.v.67	"	" larval
131+	"	B. hortorum	" egg/larval

Table continued overleaf

Appendix Table I continued:

Colony No.	Date found	Species	Developmental stage	
132+	16.v.67	B. hortorum	Incipient:	egg
133+	"	"	"	egg/larval
134+	19.v.67	"	"	egg
135+	"	"	"	"
136+	20.v.67	"	"	"
137+	21.v.67	"	"	"
138+	"	"	"	larval
139+§	"	B. lucorum	Worker	
140+§	23.v.67	B. terrestris	Worker	
141+	"	B. hortorum	Incipient:	larval
142+	24.v.67	"	"	"
143+	26.v.67	"	"	"
144+	"	B. humilis	"	egg
145+	"	"	"	larval
146+	"	B. lucorum	Worker	
147+§	"	B. pratorum	Worker	
148+	"	B. hortorum	Incipient:	larval
149+	31.v.67	B. humilis	"	egg
150+	"	"	"	pupal
151+	"	B. hortorum	"	egg
152+	"	B. humilis	"	pupal

Localities for colonies not in Englefield Green - Egham district:-

34	Tring, Herts.
112	Wye, Kent
113	Barnham, Sussex
125	Dorking, Surrey

Appendix Table II

Depths at which bumblebee queens were found hibernating and details of ground conditions.

Species	Depth (cm.)	Ground cover	Type of soil
B. lapidarius	20	Litter	Sand
"	8	"	Intermediate[x]
"	10	Clear	Sand
"	9	"	"
"	10 (2)	"	Intermediate
"	9	"	"
"	8 (3)	"	"
"	7	"	"
"	6	"	"
"	4	"	"
"	10	"	Chalk
"	8	"	"
"	7 (3)	"	"
"	6 (5)	"	"
"	5 (2)	"	"
"	4 (4)	"	"
"	3 (2)	"	"
"	9	Moss	Sand
"	8 (4)	"	"
"	7	"	Intermediate[x]
"	6 (3)	"	"
"	10	"	"
"	9	"	"
"	8 (4)	"	"
"	5	"	Chalk
"	4	"	"
"	3 (2)	"	"
B. hortorum	7	Litter	Intermediate[x]
"	10	Clear	"
"	8 (2)	"	"
"	5 (2)	"	"
"	4	"	"
"	3	"	"
"	4	"	Chalk
"	3	"	"
"	2	Moss	"
"	10	"	Sand
"	10 (2)	"	Intermediate
"	9	"	"
"	5 (2)	"	"
"	4	"	"

Table continued overleaf

Appendix Table II continued:

Species	Depth (cm.)	Ground cover	Type of soil
B. lucorum	4	Moss	Intermediate^{x}
"	10	Clear	"
"	5	"	Sand
"	3 (2)	Litter	(queen at soil-litter interface)
"	4 (2)	"	"
"	5	"	"
"	8 (7)	"	"
"	10	"	"
B. terrestris	3	"	"
"	4	"	"
"	6 (2)	"	"
"	8	Moss	Intermediate^{x}
"	2	"	Chalk
B. pratorum	3	Litter	(queen at soil-litter interface)
"	6	"	"
B. agrorum	8	"	"
P. campestris	3	Clear	Chalk
"	5	"	"
"	15	"	**Sand**
"	4	"	Intermediate^{x}
P. sylvestris	10	"	Chalk
"	4	Moss	"
"	11	"	Intermediate^{x}
P. barbutellus	8	Clear	"
P. vestalis	7	"	Sand

x = Soil heavier than sand but lighter than chalk.

Number in parenthesis refers to number of queens when more than one.

Appendix Table III

Weight of honey in crop of queens sampled from natural hibernacula at various times during hibernation.

Species	Month	Total live wt. (mg.)	Honey (mg.)	Honey, % live wt.
B. lapidarius	September	733	185	25
"	"	838	170	20
"	"	794	160	22
"	"	862	194	23
"	"	831	220	27
"	"	811	168	21
"	"	793	150	20
"	"	840	200	24
"	"	824	209	25
"	"	705	228	32
"	"	830	223	27
B. lucorum	"	760	165	22
B. terrestris	"	883	154	18
P. vestalis	"	612	88	14
P. campestris	"	556	74	13
P. barbutellus	"	580	64	11
B. lapidarius	December	524	45	9
B. lucorum	"	593	98	17
"	"	645	127	20
B. terrestris	"	605	77	13
B. pratorum	"	313	19	6
B. hortorum	"	474	22	5
"	"	551	22	4
"	"	424	78	18
"	"	397	8	2
"	"	461	15	3
"	"	423	57	13
"	"	437	31	7
P. sylvestris	"	490	34	7
B. hortorum	March	424	28	7
"	"	249	13	5

(Wt. of crop ignored)

Appendix Table IV

Size of trophocytes of queens expressed as a percentage of the total number of cells measured for each category.

Maximum Ø	Species:	B. terrestris				B. pratorum				B. lapidarius	
of cells	(n):	3	4	6	1	2	7	3	2	6	4
	Category:	A	B	C	D	A	B	C	E	B	C
0 - 1.9		-	-	-	-	-	-	-	-	-	-
2 - 3.9		-	-	-	-	-	-	-	-	-	-
4 - 5.9		-	-	4	-	-	-	4	5	-	-
6 - 7.9		4	-	7	10	13	-	16	28	-	4
8 - 9.9		8	-	15	25	18	8	40	32	-	24
10 - 11.9		15	7	33	55	36	15	32	24	4	28
12 - 13.9		23	7	19	10	22	19	8	5	4	20
14 - 15.9		23	17	11	-	11	19	-	5	12	12
16 - 17.9		19	12	7	-	7	15	-	-	12	8
18 - 19.9		8	15	4	-	3	12	-	-	12	4
20 - 21.9		-	28	-	-	-	8	-	-	20	-
22 - 23.9		-	11	-	-	-	4	-	-	12	-
24 - 25.9		-	2	-	-	-	-	-	-	12	-
26 - 27.9		-	1	-	-	-	-	-	-	8	-
28 - 29.9		-	-	-	-	-	-	-	-	4	-
30 - 31.9		-	-	-	-	-	-	-	-	-	-

Maximum Ø	Species:	B. lucorum				B. hortorum		B. agrorum				P. campestris		
of cells	(n):	1	7	5	2	8	3	3	2	1	4	1	6	1
	Category:	A	B	C	D	B	C	B	C	D	E	B	C	D
0 - 1.9		-	-	-	-	-	-	-	-	-	-	-	-	-
2 - 3.9		-	-	-	-	-	-	-	-	-	-	-	-	-
4 - 5.9		-	-	-	-	-	-	-	-	-	4	-	-	-
6 - 7.9		5	-	7	17	-	6	2	15	23	22	-	5	6
8 - 9.9		5	1	19	29	5	11	5	31	18	33	-	22	11
10 - 11.9		5	1	48	37	8	22	6	31	36	30	4	32	11
12 - 13.9		40	5	19	13	6	22	12	15	23	7	-	22	39
14 - 15.9		20	8	7	4	13	11	24	8	-	4	13	9	11
16 - 17.9		15	17	-	-	15	11	15	-	-	-	4	5	22
18 - 19.9		5	23	-	-	20	6	16	-	-	-	25	5	-
20 - 21.9		-	23	-	-	13	6	10	-	-	-	13	-	-
22 - 23.9		5	14	-	-	9	5	4	-	-	-	25	-	-
24 - 25.9		-	7	-	-	6	-	2	-	-	-	8	-	-
26 - 27.9		-	1	-	-	3	-	2	-	-	-	4	-	-
28 - 29.9		-	-	-	-	2	-	2	-	-	-	4	-	-
30 - 31.9		-	-	-	-	-	-	-	-	-	-	-	-	-

(n).... Number of bees sampled.
A .... Callows.
B .... Gynes.
C .... Early spring queens (unparasitized).
D .... Early spring queens (+ *Sphaerularia*).
E .... Foundress queens.

N.B. Appendix Tables IV / IX:- A minimum of 20 trophocytes and/or 10 oenocytes were measured in each bumblebee sampled. Measurements were made with the aid of a linear eye piece on stained sections, and are given in arbitrary units.
Trophocyte scale: 1 unit = 9.09µ.
Oenocyte scale : 1 unit = 2.33µ.
No comparison between species is intended.

Appendix Table V

Size of oenocytes of queens expressed as a percentage of the total number of cells measured for each category.

Maximum Ø	Species:	B. lucorum				B. agrorum				B. pratorum			
of cells	(n):	2	5	6	4	3	2	4	5	2	4	1	4
	Category:	A	B	C	D	A	B	C	E	A	B	C	E
7 - 7.9		-	-	-	-	-	-	-	-	-	-	-	-
8 - 8.9		-	-	-	-	13	-	-	-	-	-	-	-
9 - 9.9		20	-	2	-	30	-	2	-	10	-	20	-
10 - 10.9		55	-	24	-	40	25	28	-	25	-	40	-
11 - 11.9		25	42	37	-	17	65	53	-	45	5	30	-
12 - 12.9		-	14	29	-	-	10	10	2	20	22	10	-
13 - 13.9		-	12	8	13	-	-	7	10	-	35	-	5
14 - 14.9		-	8	-	15	-	-	-	20	-	23	-	12
15 - 15.9		-	14	-	23	-	-	-	34	-	10	-	10
16 - 16.9		-	8	-	20	-	-	-	16	-	5	-	17
17 - 17.9		-	2	-	25	-	-	-	10	-	-	-	8
18 - 18.9		-	-	-	2	-	-	-	8	-	-	-	8
19 - 19.9		-	-	-	2	-	-	-	-	-	-	-	35
20 - 20.9		-	-	-	-	-	-	-	-	-	-	-	5
21 - 21.9		-	-	-	-	-	-	-	-	-	-	-	-

(n).... Number of bees examined.
A .... Callows.
B .... Gynes.
C .... Early spring queens.
D .... Late spring queens.
E .... Foundress queens.

Appendix Table VI

Size of trophocytes of workers expressed as a percentage of the total number of cells measured for each category.

Maximum Ø	Species:	B. terrestris				B. lucorum		
of cells	(n):	3	3	1	1	10	3	11
	Category:	A	B	C	D	B	C	D
0 - 1.9		-	-	-	-	-	-	-
2 - 3.9		-	-	-	-	-	-	-
4 - 5.9		3	5	-	-	14	11	4
6 - 7.9		24	40	15	22	36	51	23
8 - 9.9		39	45	55	67	36	28	36
10 - 11.9		24	5	30	11	9	5	23
12 - 13.9		7	5	-	-	5	5	9
14 - 15.9		3	-	-	-	-	-	4
16 - 17.9		-	-	-	-	-	-	-

(n).... Number of bees examined.
A .... Callows.
B .... House bees.
C .... Laying workers.
D .... Foragers.

Appendix Table VII

Size of oenocytes of workers expressed as a percentage of the total number of cells measured for each category.

Maximum Ø	Species:	B. terrestris				B. lucorum		
of cells	(n):	3	3	1	2	7	2	7
	Category:	A	B	C	D	B	C	D
7 - 7.9		-	-	-	-	-	-	-
8 - 8.9		3	-	-	-	-	-	-
9 - 9.9		20	-	-	-	-	-	-
10 - 10.9		17	-	-	-	-	-	-
11 - 11.9		3	-	-	-	-	-	2
12 - 12.9		40	-	-	5	7	-	3
13 - 13.9		10	-	-	30	4	-	14
14 - 14.9		7	13	-	15	13	-	21
15 - 15.9		-	14	-	-	12	-	24
16 - 16.9		-	30	30	20	27	-	17
17 - 17.9		-	23	20	15	14	-	12
18 - 18.9		-	10	40	-	7	10	7
19 - 19.9		-	10	10	-	4	5	-
20 - 20.9		-	-	-	-	7	25	-
21 - 21.9		-	-	-	-	3	20	-
22 - 22.9		-	-	-	-	2	15	-
23 - 23.9		-	-	-	-	-	10	-
24 - 24.9		-	-	-	-	-	10	-
25 - 25.9		-	-	-	-	-	5	-
26 - 26.9		-	-	-	-	-	-	-

(n).... Number of bees examined.
A .... Callows.
B .... House bees.
C .... Laying workers.
D .... Foragers.

Appendix Table VIII

Size of trophocytes of males expressed as a percentage of the total number of cells measured for each category.

Maximum Ø of cells	Species:	B. lucorum		B. pratorum	
	(n):	1	5	1	5
	Category:	Callow	Mature	Callow	Mature
0 - 1.9		-	-	-	-
2 - 3.9		-	-	-	-
4 - 5.9		50	-	60	5
6 - 7.9		50	9	40	18
8 - 9.9		-	29	-	35
10 - 11.9		-	39	-	28
12 - 13.9		-	14	-	14
14 - 15.9		-	9	-	-
16 - 17.9		-	-	-	-

(n) ... Number of bees examined.

Appendix Table IX

Size of oenocytes of males expressed as a percentage of the total number of cells measured for each category.

Maximum Ø of cells	Species:	B. lucorum		B. pratorum	
	(n):	3	5	1	5
	Category:	Callow	Mature	Callow	Mature
7 - 7.9		-	-	-	-
8 - 8.9		10	-	40	-
9 - 9.9		17	-	30	-
10 - 10.9		43	8	30	-
11 - 11.9		17	12	-	-
12 - 12.9		10	20	-	8
13 - 13.9		3	34	-	10
14 - 14.9		-	12	-	10
15 - 15.9		-	12	-	40
16 - 16.9		-	2	-	16
17 - 17.9		-	-	-	12
18 - 18.9		-	-	-	-
19 - 19.9		-	-	-	2
20 - 20.9		-	-	-	2
21 - 21.9		-	-	-	-

(n) ... Number of bees examined.

Appendix Table X

Fat content of newly emerged workers.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	43.8	2.5	5.8
"	26.9	1.1	4.1
"	73.9	2.0	2.7
"	52.2	5.6	10.7
"	45.8	6.5	14.2
"	55.8	5.5	9.9
"	70.8	6.4	9.0
"	57.5	4.4	7.7
"	67.5	7.6	11.3
B. agrorum	41.3	5.1	12.3
"	12.4	0.9	7.3
"	22.8	0.8	3.5
"	26.2	1.7	6.5
"	40.4	2.6	6.4
"	37.4	0.8	2.1
B. humilis	24.1	1.4	5.5

Appendix Table XI

Fat content of non-callow workers.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	107.5	2.9	2.7
"	70.6	3.1	4.4
"	76.1	3.5	4.6
"	183.5	12.7	6.9
"	151.6	9.4	6.2
"	190.5	7.5	3.9
"	171.7	8.0	4.7
"	195.9	13.9	7.5
"	181.0	15.3	8.5
"	138.3	9.4	6.9
"	153.2	5.4	3.5
"	37.4	2.2	5.9
"	185.9	7.0	3.8
B. hortorum	96.5	3.8	3.9
"	125.8	9.7	7.7
B. agrorum	36.1	0.9	2.5
"	78.2	3.5	4.5

Appendix Table XII

Fat content of non-callow worker ABDOMENS.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	7.3	0.5	6.8
"	21.4	0.9	4.2
"	21.3	0.8	3.2
"	58.1	7.6	13.1
"	25.3	1.1	4.4
"	17.2	0.6	3.5
"	19.5	1.0	5.1
"	18.0	0.4	2.2
"	15.2	0.6	3.9
"	14.6	0.5	3.4
"	16.7	0.5	3.0
"	13.9	0.8	5.9
"	20.2	1.8	8.9
"	18.4	1.0	5.4
"	17.7	0.4	2.3
"	18.2	0.6	3.3
"	13.6	0.6	4.4
"	10.6	0.4	3.8
"	14.4	0.9	6.3
"	13.8	0.5	3.6
"	16.9	0.8	4.7
"	11.3	0.5	4.4
"	20.4	0.6	2.9
"	13.5	0.4	3.0
"	13.2	0.7	5.3
"	56.8	8.9	15.7
"	47.5	5.7	12.0
"	63.1	4.8	7.6
"	54.0	5.3	9.8
"	63.5	10.5	16.5
"	63.0	9.3	14.8
"	44.6	5.1	11.4
"	44.0	3.0	6.8
"	10.3	1.1	10.7
"	58.4	4.2	7.2
"	33.3	2.3	6.9
B. lucorum	17.0	0.6	3.5
"	12.9	0.4	3.1
"	17.0	0.6	3.5
"	13.5	0.7	5.2
"	19.1	0.9	4.7
"	12.9	0.4	3.1
"	14.6	0.3	2.1
"	17.8	1.1	6.2
"	41.5	7.5	18.0

Table continued overleaf

Appendix Table XII continued:

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. lapidarius	7.4	0.6	8.1
"	7.4	0.8	10.8
"	10.9	0.4	3.7
"	19.3	1.7	8.8
B. hortorum	14.5	0.8	5.5
"	27.4	1.5	5.5
"	38.0	5.4	14.2
B. ruderarius	24.0	3.5	14.6
"	9.2	1.0	10.8
"	9.4	0.5	5.3
B. agrorum	20.7	1.7	8.2
"	8.3	0.9	10.8
"	14.2	0.7	5.0
"	12.3	1.1	8.9
"	9.5	0.5	5.3
"	16.5	1.1	6.8

Appendix Table XIII

Fat content of newly emerged males.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	64.3	11.2	17.4
"	71.6	16.3	22.8
"	67.0	14.8	22.1
B. lucorum	58.0	12.0	20.7
"	60.7	13.7	22.6
"	63.0	15.5	24.6
"	46.7	12.0	25.7
"	44.5	8.9	20.0
"	47.2	9.5	20.1
"	64.0	13.5	21.1
"	51.5	9.0	17.5
"	56.0	11.5	20.5
"	55.0	10.8	19.6
B. hortorum	17.0	1.6	9.4
"	42.9	4.6	10.7
B. agrorum	30.0	3.5	11.7
"	33.0	1.6	4.8
"	33.5	2.8	8.4
B. humilis	27.2	1.9	7.0
"	30.5	3.1	10.2

Appendix Table XIV

Fat content of mature males.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	64.7	5.3	8.2
"	72.1	3.4	4.7
"	51.0	7.5	14.7
"	67.3	7.1	10.5
"	87.1	5.3	6.1
B. lucorum	67.7	10.4	15.4
"	96.1	10.2	10.6
"	78.7	5.7	7.2
"	57.7	10.5	18.2
"	68.8	15.1	21.9
"	51.8	9.0	17.4
"	67.0	16.4	24.5
"	52.4	6.3	12.0
"	54.4	3.8	7.0
"	74.1	8.2	11.1
"	74.5	5.8	7.8
"	67.3	6.8	10.1
"	52.9	7.0	13.2
"	79.9	9.1	11.8
"	71.4	8.8	12.3
"	83.7	9.5	8.9
B. hortorum	42.0	1.1	2.6
"	63.8	4.0	6.3
"	47.0	4.0	8.5
"	50.3	8.6	17.1
"	42.4	5.2	12.3
"	43.3	4.7	10.8
"	45.0	0.5	1.1
"	47.3	6.3	11.2
B. agrorum	56.9	2.2	4.0
"	37.5	3.3	8.8
"	68.4	3.9	5.7
"	62.4	4.9	7.9
"	81.8	3.7	4.5
"	33.5	6.2	4.4
"	36.5	4.9	4.4
"	40.2	1.2	3.0
"	34.5	1.7	4.9
"	36.8	1.0	2.7

Appendix Table XV

Fat content of newly emerged gynes.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	208.4	39.9	19.1
"	163.2	33.4	20.5
"	172.2	34.3	19.9
"	168.2	27.8	16.5
"	159.5	26.3	16.5
"	165.0	28.4	17.2
"	186.5	32.0	17.2
"	182.1	39.7	21.8
B. lucorum	106.4	11.0	10.3
"	106.1	19.1	18.0
"	147.3	15.4	10.5
"	103.6	16.5	15.9
"	112.9	23.8	21.1
B. pratorum	57.6	7.8	13.5
"	68.9	13.8	20.0
"	66.8	14.9	22.3
B. hortorum	85.9	7.2	8.4
"	90.6	9.0	9.9
"	114.4	16.8	14.7
B. agrorum	62.9	11.1	17.6
B. humilis	69.1	11.3	16.4
"	74.2	12.3	16.6

Appendix Table XVI

Fat content of gynes with well developed fat bodies.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	335.9	163.5	48.7
"	334.2	146.6	43.9
"	368.3	116.0	31.5
"	229.5	67.8	29.5
"	275.9	88.3	32.0
"	245.5	74.3	30.3
"	256.6	68.4	26.7
B. lucorum	288.4	76.1	26.4
"	166.9	51.0	30.6
"	145.6	37.7	25.9
"	257.7	108.0	41.9
"	242.7	105.5	43.5
"	261.0	80.9	31.0
"	245.5	82.3	33.5
"	228.0	90.7	39.8
B. lapidarius	387.8	94.2	24.3
"	340.1	111.2	32.7
"	287.0	84.6	29.5
"	347.5	85.6	24.6
"	349.9	107.9	30.8
"	238.9	74.0	31.0
"	231.0	82.0	35.5
B. pratorum	86.2	28.8	33.4
"	91.8	24.2	26.4
"	115.0	31.7	27.6
"	130.4	48.1	36.9
"	137.1	46.5	33.9
"	104.0	36.3	34.9
"	130.0	56.0	43.1
"	109.0	45.0	41.3
"	130.0	57.0	43.8
"	133.0	66.0	49.6
B. hortorum	319.0	91.3	28.6
"	235.4	107.4	45.6
B. agrorum	167.0	58.0	34.7
"	158.0	53.0	33.5
"	138.0	37.0	26.8
"	199.0	62.0	31.2
"	140.0	49.0	35.0
"	78.4	30.0	38.3

Appendix Table XVII

Fat content of hibernating queens sampled from natural hibernacula.

Species	Date of sample	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. lucorum	5.xii.66	251.3	24.0	9.6
"	7.xii.66	300.7	36.8	12.2
B. lapidarius	7.xii.66	215.5	28.4	13.2
B. hortorum	5.xii.66	147.7	33.1	22.4
"	"	189.8	30.8	16.4
"	7.xii.66	184.8	30.0	16.2
"	"	185.6	30.2	16.3
"	1.iii.67	126.0	18.1	14.4
"	"	170.4	25.6	15.0

Appendix Table XVIII

Fat content of spring queens.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	180.2	21.2	11.8
"	231.6	30.1	13.0
"	206.2	16.2	7.9
"	229.0	17.2	7.5
"	196.8	13.7	7.0
"	166.1	8.0	4.8
"	191.0	15.0	7.9
"	166.0	11.0	6.6
"	195.0	15.0	7.7
"	200.0	24.0	12.0
"	180.0	8.0	4.4
"	157.0	7.0	3.8
"	181.0	9.0	4.4
"	201.0	9.0	4.5
"	239.5	12.6	5.3
"	301.7	10.9	3.6
B. lapidarius	162.0	4.3	2.7
"	157.1	4.5	2.9
"	153.8	8.4	5.5
"	144.2	4.7	3.3
"	139.9	8.6	6.1

Table continued overleaf

Appendix Table XVIII continued:

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. lucorum	153.0	18.8	12.3
"	154.4	14.5	9.4
"	173.3	11.3	6.5
"	138.5	12.7	9.2
"	141.6	10.9	6.5
"	128.8	8.0	6.2
"	161.3	22.6	14.0
"	119.1	11.2	9.4
"	119.8	1.9	1.6
B. pratorum	81.4	12.7	15.6
"	77.7	3.1	4.0
"	80.4	5.6	7.0
"	80.1	7.0	8.7
"	67.4	2.9	4.3
"	79.0	6.0	7.6
B. agrorum	77.5	1.5	1.9
"	88.1	4.3	4.9
"	88.8	3.3	3.7
"	93.5	9.2	9.8
"	93.9	7.6	8.1
"	83.0	5.0	6.0
P. sylvestris	150.3	7.9	5.3
"	101.6	9.1	9.0
"	71.9	4.4	6.1
"	129.4	13.9	10.7
"	94.9	1.9	2.0

Appendix Table XIX

Fat content of foundress queens.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	206.0	12.5	6.1
B. lucorum	129.0	10.3	8.0
"	153.8	10.0	6.5
B. pratorum	71.3	1.8	2.5
"	83.0	6.7	8.1
"	90.1	6.0	6.7
B. hortorum	148.2	9.2	8.0
"	107.7	8.0	7.4
B. agrorum	79.2	2.1	2.7
"	77.5	6.4	8.3
"	89.0	6.9	7.8
"	85.6	5.9	6.9
"	113.4	6.8	6.0
"	86.6	6.2	7.2
"	78.1	4.8	6.1
"	86.5	5.2	6.0

Appendix Table XX

Fat content of spring queens parasitized by *Sphaerularia*.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. terrestris	252.0	9.4	3.7
B. lucorum	155.4	11.6	7.5
"	134.4	6.6	4.9
B. lapidarius	128.5	6.9	4.6
B. hortorum	121.6	10.6	8.7

Appendix Table XXI

Fat content of anomalous 'foundress' queens.

Species	Dry weight (mg.)	Fat (mg.)	Fat, % dry weight
B. agrorum	101.4	31.0	30.6
"	85.4	16.0	18.7
"	84.3	8.9	10.6

Appendix Table XXII

Fat content of various parts of the body (weights in mg.).

Species	Autumn queens			Spring queens		
	Head	Thorax	Abdomen	Head	Thorax	Abdomen
B. terrestris	1.3	15.2	71.8	0.7	4.2	16.3
"	1.5	8.3	85.5	1.4	4.0	24.7
"	-	-	-	1.3	3.1	11.8
B. lucorum	1.3	8.6	78.8	1.0	2.9	14.9
"	1.5	8.4	66.2	0.7	2.8	11.0
"	1.6	7.5	74.2	0.8	1.5	9.0
"	1.4	9.9	96.7	0.9	1.9	9.9
"	1.7	10.8	93.0	0.5	0.7	4.0
"	1.1	7.9	71.9	-	-	-
"	1.3	8.2	72.8	-	-	-
B. lapidarius	1.5	9.4	83.3	0.8	3.9	3.7
"	1.7	15.5	94.0	0.3	0.7	3.7
"	1.8	11.4	71.4	0.1	1.7	2.5
"	1.8	13.3	70.5	0.7	0.9	2.9
"	1.7	11.9	94.3	0.5	2.7	5.4

Appendix Table XXIII

Mean fat content of *B. lucorum* gynes of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	5	499.4	115.3	98.1	17.2	15.2
1	5	580.3	137.5	115.7	21.8	15.9
2	5	573.2	166.5	123.6	42.9	25.6
3	5	553.1	175.5	131.7	43.8	24.5
4	3	542.3	188.2	131.7	56.5	29.4
5	1	537.7	168.6	119.4	49.2	29.2
6	3	578.9	213.5	134.4	79.1	38.3
8	3	541.0	176.6	130.0	46.6	25.5
10	3	536.9	178.8	125.3	53.5	28.9
14	2	491.9	177.2	105.0	72.2	40.7
21	2	542.2	190.6	111.2	79.4	41.6

(n) = number of observations.

Appendix Table XXIV

Mean fat content of *B. hortorum* gynes of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	3	491.8	97.0	86.0	11.0	11.0
1	3	541.7	126.8	108.3	18.5	14.6
2	3	467.1	116.6	95.8	20.8	18.1
3	2	557.7	145.4	114.1	31.3	19.8
4	3	460.2	141.4	104.2	37.2	25.2
6	1	526.1	180.0	111.9	68.1	37.8
7	2	586.7	249.4	195.8	53.6	24.5
8	2	522.9	167.5	115.7	51.8	31.1
10	2	552.8	155.8	117.3	38.5	24.8
14	2	660.9	191.3	128.8	62.5	32.6
21	2	653.2	262.4	205.4	57.0	22.6

(n) = number of observations.

Appendix Table XXV

Mean fat content of *B. agrorum* gynes of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	1	298.6	62.9	51.8	11.1	17.6
1	2	327.5	111.8	93.8	18.0	16.4
2	4	311.8	95.5	71.7	23.8	24.8
4	4	342.5	132.8	84.2	48.6	36.6
6	4	362.5	152.1	102.3	49.8	33.0
8	3	396.0	178.8	127.5	51.3	28.2
10	3	353.7	154.8	91.6	63.2	39.1

(n) = number of observations.

Appendix Table XXVI

Mean fat content of *B. humilis* gynes of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	2	335.5	71.7	59.9	11.8	16.5
1	2	352.7	76.2	64.2	12.0	15.7
2	2	334.2	95.9	72.2	23.7	24.8
3	2	366.8	119.3	82.9	36.4	30.1
4	3	339.2	118.7	71.4	47.3	39.5
5	3	359.5	119.5	71.6	47.9	39.8
6	3	347.4	129.1	74.2	54.9	41.2
7	2	358.3	124.9	80.3	44.6	36.2
8	1	399.7	130.4	78.9	51.5	39.5
10	3	420.1	137.2	89.6	47.6	35.0
12	3	372.6	111.2	70.5	40.7	36.3
14	2	387.4	158.1	109.7	48.4	31.2

(n) = number of observations.

Appendix Table XXVII

Mean fat content of B. humilis workers of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	1	146.2	25.4	24.0	1.4	5.5
1	2	136.3	29.7	28.2	1.5	5.2
2	2	157.5	38.5	34.7	3.8	8.6
3	2	185.2	43.9	39.6	4.3	9.6
4	1	105.5	25.6	24.3	1.3	5.1
5	2	139.8	33.0	36.3	1.7	5.2
6	1	101.0	26.2	25.1	1.1	4.2

(n) = number of observations.

Appendix Table XXVIII

Mean fat content of B. terrestris males of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	3	281.3	67.6	53.5	14.1	20.8
1	2	266.5	65.9	55.2	10.7	16.7
2	2	259.2	70.1	59.9	10.2	14.5
3	2	313.0	88.7	78.1	10.7	12.0
4	2	327.5	80.4	73.0	7.4	9.0
5	1	276.2	97.0	88.6	8.4	8.7
>5	4	275.5	83.2	73.1	10.1	12.0

(n) = number of observations.

Appendix Table XXIX

Mean fat content of B. lucorum males of various ages.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	6	239.4	53.4	41.4	11.9	22.3
1	7	246.4	62.0	50.4	11.6	18.8
2	3	286.0	84.6	69.2	15.5	17.9
3	3	271.5	75.7	62.0	13.7	18.2
4	3	264.8	60.0	52.0	8.0	12.8
5	2	334.3	75.8	66.9	8.9	11.6
>5	6	309.0	85.8	71.6	14.1	18.3

(n) = number of observations

Appendix Table XXX

Mean fat content of B. humilis males of various categories.

Age in Days	(n)	Live wt. (mg.)	Dry wt. (mg.)	Extracted wt. (mg.)	Fat (mg.)	Fat, % dry wt.
0	2	142.7	28.9	26.4	2.5	8.6
1	2	161.6	36.0	32.9	3.1	8.4
2	2	160.5	37.3	32.1	5.2	14.0
3	2	135.6	40.3	36.4	3.9	9.7
4	2	143.4	37.4	31.9	5.6	14.7
5	2	161.0	41.8	36.9	4.9	11.7
>5	2	178.2	51.6	47.1	4.5	8.6

(n) = number of observations

Appendix Table XXXI

Details of 1st brood workers from incipient colonies of B. agrorum.

(Plan type refers to Text Fig. 3.9).
c = central bee
p = peripheral bee

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
3	(a) (f) (d) (b) (g) (e) (c) (h) type A	1.vi.65	d	9	98	10.9	c
		"	e	9	118	13.1	c
		2.vi.65	h	7	50	7.1	p
		3.vi.65	g	9	97	10.8	c
		"	b	7	75	10.7	c
		4.vi.65	f	7.5	80	10.7	p
		6.vi.65	c	6	43	7.2	p
			a	failed to emerge			
4	(a) (f) (d) (b) (g) (e) (c) (h) type A	27.v.65	d	9	122	13.6	c
		"	e	10	131	13.1	c
		29.v.65	b	10	128	12.8	c
		"	c	8	60	7.5	p
		"	g	10.5	145	13.8	c
		30.v.65	a	9	76	8.4	p
		"	f	9.5	102	10.7	p
		"	h	8	69	8.6	p
5	(a) (g) (b) (d) (h) (c) (f) (e) type B	22.v.65	d	11	149	13.5	c
		23.v.65	f	10.5	150	14.3	c
		24.v.65	c	10	149	14.9	c
		"	h	10	140	14.0	p
		25.v.65	b	8.5	78	9.2	p
		"	g	11	162	14.7	p
		26.v.65	a	8	93	11.6	p
		28.v.65	e	6.5	44	6.8	p

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
6		22.v.65	b	10	117	11.7	c
	(c)	24.v.65	a	10	113	11.3	c
	(a)(b)(d)	25.v.65	d	10	124	12.4	c
	(e)	26.v.65	e	9.5	97	10.2	p
type V		28.v.65	c (male)	9	101	11.2	p
7		4/5.vi.65	-	8.5	91	10.7	-
		"	-	7.5	65	8.7	-
type O		"	-	6.5	50	7.8	-
		"	-	5.5	34	6.2	-
8		30.v.65	c	10	148	14.8	c
	(a)(c)(e)	"	d	10	134	13.4	c
	(b)(d)(f)	31.v.65	b	10	137	13.7	?
	(g)	"	f	10	163	16.3	c
type F		1.vi.65	a	8	101	12.6	?
		2.vi.65	e	8	92	11.5	p
		"	g	8	85	10.6	p
9		27.v.65	e	10	146	14.6	c
	(a) (g)	28.v.65	d	9	120	13.3	c
	(e) (b) (h)	"	f	9	117	13.0	c
	(d)(f) (c) (i)	29.v.65	a	10	147	14.7	p
		"	g	10.5	133	12.7	p
type G		"	h	8.5	83	9.8	p
		31.v.65	b	7	55	7.9	p
		2.vi.65	c	7	47	6.7	p
		"	i	8	68	8.5	p

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
10		6.vi.65	d	9	122	13.6	c
	(a)(e)	8.vi.65	a	9	117	13.0	?
	(b)(d)(f)	"	e	10	139	13.9	?
	(c)(g)	10.vi.65	b	9	115	12.8	?
type E		"	f	9.5	123	12.9	?
		"	g	9	100	11.1	?
		12.vi.65	c	7.5	76	10.1	?
11		2.vi.65	e	9.5	127	13.4	c
	(a)(d)(f)	3.vi.65	d	9.5	125	13.2	c
	(b)(e)(g)	4.vi.65	b	10	123	12.3	c
	(c)(h)	"	c	10	125	12.5	p
		"	h	11	143	13.0	p
type A		5.vi.65	a	7.5	65	8.7	p
		"	f	9	95	10.6	p
		"	g	8	65	8.1	c
12		31.v.65	d	10	124	12.4	c
	(a)(d)(f)	"	e	10	133	13.3	c
	(b)(e)(g)	1.vi.65	b	9.5	116	12.2	c
	(c)(h)	2.vi.65	a	8.5	67	7.9	p
type A			cfgh	cells damaged			
13		Before	-	10.5	-	-	-
		3.vi.65	-	10	99	9.9	-
			-	9	85	9.4	-
type E			-	9	-	-	-
			-	8	57	7.1	-
			-	7	48	6.9	-
			-	7	46	6.6	-

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
16	(a) (f) (b) (c) (g) (d) (h) (e)	31.v.65	g	11	137	12.5	c
		1.vi.65	b	11	137	12.5	c
		"	d	10.5	184	17.5	c
		2.vi.65	c	10	117	11.7	c
		"	h	10	118	11.8	p
		3.vi.65	f	9.5	89	9.4	p
type C		4.vi.65	e	9	70	7.8	p
			a	failed to emerge			
17		Before	-	11	164	14.9	-
		4.vi.65	-	10.5	158	15.0	-
			-	10.5	-	-	-
			-	10	130	13.0	-
type D			-	8	67	8.4	-
			-	6.5	37	5.7	-
			-	6.5	-	-	-
			-	5.5	25	4.5	-
53	type Y	2.vi.66	-	10	151	15.1	c
55	(a) (e) (b) (c) (f) (d) (g)	1.vi.66	c	10	127	12.7	c
		"	d	9.5	118	12.4	c
		2.vi.66	b	10	136	13.6	c
		"	e	7.5	60	8.0	p
		"	f	9.5	114	12.0	c
type J		3.vi.66	g	8	63	7.9	p
		"	a	8	59	7.4	p

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
56		30.v.66	c	10.5	144	13.7	c
	(a)(c)(e)	"	d	10	127	12.7	c
	(b)(d)(f)	2.vi.66	f	7.5	74	9.9	?
	type U		abc	cells damaged			
57		2.vi.66	c	9	119	13.2	c
	(a)(d)	4.vi.66	a	8.5	83	9.8	c
	(b)(c)(e)	"	b	7	53	7.6	p
	type W	"	d	8.5	91	10.7	c
		"	e	8	73	9.1	p
58		Before	a	8	82	10.3	p
	(b)(e)	25.v.66	b or e	10.5	165	15.7	c
	(a) (d) (g)		c or f	9.5	143	15.1	c
	(c)(f)		d	11	176	16.0	c
			e or b	10	150	15.0	c
	type M		f or c	9	126	14.0	c
		25.v.66	g	8	80	10.0	p
59	(a)	4.vi.66	c	10	165	16.5	c
	(b)(c)	5.vi.66	b	9.5	109	11.5	c
	type X	6.vi.66	a	8.5	75	8.8	?
60		29.v.66	c	10	129	12.9	c
	(a)(c)(e)	"	d	10	149	14.9	c
	(b)(d)(f)	"	f	10	143	14.3	c
		30.v.66	e	10	128	12.8	p
	type T	31.v.66	a	8.5	79	9.3	p
		"	b	10	125	12.5	c

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
62		14.vi.66	a	6.5	53	8.2	p
	(a)(d)(f)	"	d	9.5	107	11.3	c
	(b)(e)	16.vi.66	e	8	76	9.5	c
	(c)	"	f	-	23	-	p
	type S		bc	failed to emerge			
65		1.vi.66	d	10.5	170	16.2	c
	(e) (c) (a) (f) (d) (b) (g) type K	2.vi.66	a	9.5	112	11.8	c
		"	c	8	72	9.0	c
		"	f	10.5	152	14.5	c
		3.vi.66	g	9	92	10.2	p
		4.vi.66	b	10.5	119	11.3	p
		"	e	8.5	75	8.8	p
66		5.vi.66	c	10	125	12.5	c
	(a)(c)	"	d	10	125	12.5	c
	(b)(d)(e)	6.vi.66	b	7.5	67	8.9	?
	(f)	"	e	6.5	27	4.2	p
	type Q	7.vi.66	a	8.5	97	11.4	?
		"	f	8	82	10.3	p
67		4.vi.66	d	9	105	11.7	c
		"	e	9	101	11.2	c
	(a) (f) (d) (b) (g) (e) (c) (h) type A	5.vi.66	b	9	97	10.8	c
		"	g	8	87	10.9	c
		6.vi.66	c	7	55	7.9	p
		"	f	6.5	40	6.2	p
		"	h	6	27	4.5	p
		7.vi.66	a	7.5	56	7.5	p

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
70		2.vi.66	b	9.5	124	13.7	c
	(b)(d)	3.vi.66	c	9	106	11.8	c
	(a)(e)	4.vi.66	e	9	81	9.0	c
	(c)(f)	"	a	8	73	9.1	c
		5.vi.66	d	7	47	6.7	p
	type O	7.vi.66	f	6	33	5.5	p
71		Before	bde or g	10	144	14.4	c
	(a)(d)(f)	1.vi.66	"	9.5	117	12.3	c
	(b)(e)(g)		acf or h	8.5	86	10.1	p
	(c)(h)		"	8.5	82	9.6	p
	type A		"	8.5	79	9.3	p
			"	8	67	8.4	p
73		1.vi.66	c	10.5	154	14.7	c
	(a)(c)(e)	2.vi.66	b	9.5	109	11.5	c
	(b)(d)(g)	"	d	10	141	14.1	c
	(f)	3.vi.66	f	10	112	11.2	c
		"	e	9	96	10.7	p
	type L	4.vi.66	a	8	65	8.1	p
		"	g	8.5	83	9.8	p
75		9.vi.66	a	10	108	10.8	c
	(c)	10.vi.66	d	8	84	10.5	c
	(a)(d)(f)	11.vi.66	b	9.5	106	11.2	c
	(b)(e)	"	e	9.5	106	11.2	c
		12.vi.66	f	8	68	8.5	p
	type P	13.vi.66	c	8	65	8.1	p

Table continued overleaf

Appendix Table XXXI continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio	
76		Before	bd or e	10.5	137	13.0	c
	(c)	7.vi.66	"	10.5	128	12.2	c
	(a)(d)(f)		ac or f	9	91	10.1	p
	(b)(e)		"	8	74	9.3	p
	type R						
78		Before	a or e	9	95	10.6	p
	(c)	7.vi.66	b or f	10	104	10.4	c
	(a)(d)(e)		c	7	48	6.9	p
	(b)(f)		d	11	177	16.1	c
			f or b	10.5	129	12.3	c
	type N		e or a	bee escaped			

77 = type H
80 = type A
82 = type A

} Not incipient colonies, but arrangement of cells of incipient pupal clump visible.

Appendix Table XXXII

Details of 1st brood workers from incipient colony of B. humilis.

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio
116		27.iv.67	c	10.0	128.0	12.8
		"	d	8.0	95.3	11.9
		28.iv.67	f	10.0	102.7	10.3
		29.iv.67	a	9.0	94.2	10.5
		30.iv.67	b	6.5	39.5	6.1
		"	e	6.5	34.0	5.2
		"	g	7.0	30.6	4.4

Appendix Table XXXIII

Details of 1st brood workers from incipient colonies of B. hortorum.

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio
15		Before	-	17.5	177	10.1
		4.vi.65	-	11.5	176	15.3
type A (Text Fig. 3.9)			-	10	132	13.2
			-	10	107	10.7
			-	9	112	12.4
			-	9	110	12.2
			-	8	89	11.1
			-	7	67	9.6

Table continued overleaf

Appendix Table XXXIII continued:

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio
61		3.vi.66	f	8	89	11.1
	(a) (g)	"	c	6.5	53	8.2
	(b) (d)(h)	5.vi.66	e	10	129	12.9
	(e)	"	b	9.5	119	12.5
	(c) (f)	7.vi.66	d	9	104	11.6
		"	h	7	46	6.6
		8.vi.66	a	7	55	7.9
			g	failed to emerge		
64		Before 30.v.66	e	9	146	16.2
		30.v.66	d	8.5	123	14.5
	(a)(d)(g)	"	g	8.5	99	11.6
	(b)(e)(h)	31.v.66	f	10.5	149	14.2
	(c)(f)(i)	"	h	10.5	147	14.0
		1.vi.66	b	8.5	96	11.3
		"	c	9	123	13.7
		"	i	10	136	13.6
		3.vi.66	a	10.5	147	14.0
68		29.v.66	f	8.5	103	12.1
	(a)	30.v.66	i	9	111	12.3
	(b) (d)	31.v.66	e	10	138	13.8
	(c) (e) (g)	"	h	10	127	12.7
	(f) (h)	1.vi.66	k	11	195	17.7
	(i) (j)	"	j	10	154	15.4
	(k)	3.vi.66	b	10.5	139	13.2
		4.vi.66	d	11	165	15.0
		6.vi.66	c	9	104	11.6
		9.vi.66	g	10	134	13.4
			a	failed to emerge		

Table continued overleaf

Appendix Table XXXIII continued;

Colony No.	Plan	Date of emergence	Cell	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio
69		Before 2.vi.66	-	10	177	17.7
			-	10	174	17.4
			-	8	101	12.6
			-	8	96	12.0
			-	11.5	199	17.3
			-	11	167	15.2
			-	11	165	15.0
			-	11.5	182	15.8
74		8.vi.66	a	8	85	10.6
		"	d	8	95	11.9
		9.vi.66	b	10	149	14.9
	(a)(d)	"	i	thrown out by queen		
	(b)(e)(h)	10.vi.66	f	10.5	149	14.2
	(c)(f)(i)(j)	"	h	10.5	131	12.5
	(g)(k)	12.vi.66	e	11.5	336	29.2
		13.vi.66	c	8.5	96	11.3
		"	g	11.5	187	16.3
		"	j	12	203	16.9
			k	failed to emerge		

Appendix Table XXXIV

Details of foundress queens from incipient colonies.

Colony No.	Species	Wing length (mm.)	Weight (mg.)	Wt./Wing length Ratio
53	B. agrorum	13.5	340	25.2
55	"	13.5	285	21.1
56	"	13.5	308	22.8
57	"	13	234	18.0
59	"	14	358	25.6
60	"	14	347	24.8
62§	"	14	342	24.4
65§	"	13	294	22.6
66	"	13.5	344	25.5
67§	"	14	468	33.4
70	"	13	234	18.0
71	"	14	358	25.6
73	"	13	271	20.8
75§	"	14	412	29.4
76	"	13.5	292	21.6
78	"	13.5	323	23.9
15	B. hortorum	16.5	456	27.6
64	"	13	304	23.4
68	"	14.5	348	24.0
69	"	16.5	531	32.2
74	"	15	424	28.3

§ = queen parasitized by *Syntretus*.

Appendix Table XXXV

Occurrence of *Syntretus splendidus* in bumblebees in 1965.

Date	Host		Stage of parasites or larval instar	Number of parasites in host §
26.v.65	B. agrorum:	queen	5th	32
27.v.65	B. pratorum:	"	5th	30
"	B. agrorum:	"	5th	46
7.vi.65	"	"	4th	32
10.vi.65	B. lucorum:	worker	4th	15
"	"	"	4th	10
12.vi.65	B. agrorum:	"	4th	15
15.vi.65	B. lapidarius:	"	4th	4
"	"	"	4th	6
"	"	"	4th	10
29.vi.65	B. lucorum:	"	1st & 2nd	13
"	"	"	Eggs & 1st	7
"	"	"	Eggs & 1st	11
"	"	"	Eggs & 1st	7

§ = All parasites in abdomen of host.

Appendix Table XXXVI

Occurrence of Syntretus splendidus in bumblebees in 1966.

Date	Host		Stage of parasites or larval instar	Number of parasites in Head	Thorax	Abdomen
31.v.66	B. pratorum:	queen	4th	-	-	12
1.vi.66	B. agrorum:	"	5th	-	-	19
2.vi.66	"	"	5th	-	-	27
5.vi.66	B. ruderarius:	"	4th & 5th	-	2	26
8.vi.66	B. agrorum;	"	4th	-	-	16
15.vi.66	"	"	5th	-	-	10
29.vi.66	B. pratorum;	worker	1st	-	-	6
30.vi.66	B. agrorum:	"	2nd & 3rd	-	-	11
"	"	"	1st & 2nd	-	-	10
1.vii.66	"	"	2nd	-	-	7
4.vii.66	"	"	2nd	-	1	15

Appendix Table XXXVII

Occurrence of *Syntretus splendidus* in bumblebees in 1967.

Date	Host	Stage of parasites or larval instar	Number of parasites in		
			Head	Thorax	Abdomen
18.v.67	B. pratorum: worker	Eggs & 1st	1	2	14
19.v.67	B. lapidarius: queen	2nd	-	-	46
"	B. humilis: "	Eggs & 1st	2	8	32
"	B. agrorum: "	1st	1	2	24
21.v.67	" "	1st	-	1	42
25.v.67	" "	Eggs	1	17	18
"	" "	1st	-	2	61
26.v.67	" "	1st	-	2	30
"	B. terrestris: worker	Eggs	1	10	26
29.v.67	B. agrorum: queen	4th	-	-	56
"	" "	1st	2	14	11
30.v.67	B. pratorum: worker	Eggs	2	2	28
"	B. lucorum: "	1st	-	1	26
"	B. agrorum: "	1st	-	-	20
"	B. pratorum: queen	2nd	-	-	33
2.vi.67	B. agrorum: "	Eggs & 1st	-	1	6
"	P. vestalis: "	2nd	-	-	77
"	B. agrorum: worker	1st & 2nd	-	5	21
"	B. pratorum: "	Eggs	-	11	13
"	B. lucorum: "	4th	-	-	48
"	" "	2nd	-	-	35
3.vi.67	" "	Eggs & 1st	-	8	13
"	" "	1st & 2nd	-	1	11
"	" "	Eggs	1	7	8
"	" "	2nd	-	-	31
"	B. agrorum: "	4th	-	-	10
"	" "	Eggs	-	10	12
"	" "	Eggs & 1st	-	4	13
"	B. pratorum: "	2nd	-	-	11
"	B. terrestris: "	Eggs	1	6	12
"	" "	Eggs	-	12	8
"	" "	Eggs	1	23	27
"	" "	2nd & 3rd	-	-	24
"	B. agrorum: queen	4th	-	-	17
5.vi.67	B. terrestris: worker	4th	-	-	50
"	" "	4th	-	-	38
"	" "	Eggs	-	2	15
"	" "	Eggs	-	5	14
"	" "	5th	-	-	29
"	B. lucorum: "	1st	-	2	8

Table continued overleaf

Appendix Table XXXVII continued:

Date	Host	Stage of parasites or larval instar	Number of parasites in		
			Head	Thorax	Abdomen
6.vi.67	B. agrorum: worker	1st & 2nd	1	-	18
"	B. terrestris: "	4th	-	-	37
"	B. agrorum: queen	Eggs	-	1	4
"	" "	2nd	-	-	28
7.vi.67	" worker	1st	-	2	20
"	B. terrestris: "	Eggs & 1st	-	1	14
"	B. humilis: queen	Eggs & 1st	1	1	26
9.vi.67	B. pratorum: "	2nd	-	1	46
"	" worker	Eggs	-	-	7
"	B. terrestris: "	Eggs	-	3	12
"	" "	4th	-	-	18
12.vi.67	" "	3rd & 4th	-	-	26
"	B. lucorum: "	Eggs	-	3	22
"	" male	Eggs & 1st	-	-	7
"	B. agrorum: worker	2nd	-	-	18
"	B. lapidarius: queen	1st	1	-	62
14.vi.67	B. lucorum: male	3rd	-	-	26
"	" worker	Eggs	-	2	10
"	B. pratorum: "	Eggs	-	2	14
"	" "	5th	-	-	4
16.vi.67	B. terrestris: "	4th	-	-	22
19.vi.67	" "	1st	-	1	26
"	B. lucorum: "	Eggs & 1st	1	2	20
20.vi.67	B. pratorum: "	5th	-	-	12
21.vi.67	" "	5th	-	-	12
"	" "	5th	-	-	10
"	B. lucorum: "	Eggs & 1st	-	6	16
"	" "	4th	-	-	23
"	B. agrorum: "	4th	-	-	15
23.vi.67	" "	3rd	-	-	12
"	B. lucorum: "	1st & 4th §	-	-	14
27.vi.67	" "	4th	-	-	43
7.vii.67	B. terrestris: "	1st	-	-	1
"	" "	2nd & 3rd	-	-	33
"	" "	3rd & 4th	-	1	23

§ ..... four 4th instar larvae & ten 1st instar larvae.

REFERENCES

Bailey, L. (1954). The filtration of particles by the proventriculi of various Aculeate Hymenoptera. Proc. R. ent. Soc. Lond. 29: 119-23.

Baker, J.R. (1958). Principles of biological microtechnique. Methuen: London.

Balduf, W.V. (1926). The bionomics of Dinocampus coccinellae Schrank. Ann. ent. Soc. Amer. 19: 465-98.

Bols, J.H. (1937). Observations on Bombus and Psithyrus, especially on their hibernation. Proc. R. ent. Soc. Lond. 12: 47-50.

—— (1939). Un remarquable terrain d'hivernation de Bombus et de Psithyrus près Louvain, à Lubbeek, en Belgique. Verh. VII Intern. Ent. Kongress 2: 1048-60.

Brian, A.D. (1951). Brood development in Bombus agrorum (Hym., Bombidae). Ent. mon. Mag. 87: 207-12.

—— (1952). Division of labour and foraging in Bombus agrorum Fabricius. J. Anim. Ecol. 21: 223-40.

Casselman, W.G.B. (1959). Histochemical technique. Methuen: London.

Clausen, C.P. (1940). Entomophagous Insects. Hafner: New York.

Cole, L.R. (1959). On a new species of Syntretus Förster (Hym., Braconidae) parasitic on an adult Ichneumonid, with a description of the larva and notes on its life history and that of its host, Phaeogenes invisor (Thunberg). Ent. mon. Mag. 95: 18-21.

Coupland, R.E. (1957). Observations on the normal histology and histochemistry of the fat body of the locust (Schistocerca gregaria). J. Exp. Biol. 34: 290-96.

Coville, F.V. (1890). Notes on bumblebees. Proc. ent. Soc. Wash. 1: 197-203.

Cumber, R.A. (1949a). Humble-bee parasites and commensals found within a thirty mile radius of London. Proc. R. ent. Soc. Lond. 24: 119-27.

—— (1949b). The biology of humble-bees with special reference to the production of the worker caste. Trans. R. ent. Soc. Lond. 100: 1-45.

—— (1953). Some aspects of the biology and ecology of humble-bees bearing upon the yields of red-clover seed in New Zealand. N.Z. J. Sci. Tech. B 34: 227-40.

—— (1954). The life-cycle of humble-bees in New Zealand. N.Z. J. Sci. Tech. B 36: 95-107.

El-Hariri, G. (1966). Studies of the physiology of hibernating Coccinellidae (Coleoptera); changes in the metabolic reserves and gonads. Proc. R. ent. Soc. Lond. 41: 133-44.

Fox-Wilson, G. (1946). Factors affecting populations of social wasp, Vespula species, in England (Hymenoptera). Proc. R. ent. Soc. Lond. 21: 17-27.

Free, J.B. (1955a). The collection of food by bumblebees. Insectes Soc. 2: 303-11.

—— (1955b). The division of labour within bumblebee colonies. Insectes Soc. 2: 195-212.

—— (1955c). Queen production in colonies of bumblebees. Proc. R. ent. Soc. Lond. 30: 19-25.

—— (1957). The effect of social facilitation on the ovary development of bumblebee workers. Proc. R. ent. Soc. Lond. 32: 182-4.

—— and Butler, C.G. (1959). Bumblebees. Collins: London.

Freudenstein, H. (1960). Einfluß der Pollennahrung auf das Bauvermögen, die Wachsdrüsen und den Fettkörper der Honigbiene (Apis mellifera L.). Zool. Jb. Physiol. 69: 95-124.

Freudenstein, K. (1924). Lage und Anordung des Fettkörpers der Honigbiene. Arch. f. Bienenk. 6: 49-82.

Frison, T.H. (1917). Notes on Bombidae, and on the life history of Bombus auricomus Robt.. Ann. ent. Soc. Amer. 11: 43-8.

—— (1926). Contribution to the knowledge of the inter-relations of the bumblebees of Illinois with their animate environment. Ann. ent. Soc. Amer. 19: 203-34.

—— (1927). The development of the castes of bumble-bees (Bremidae: Hym.). Ann. ent. Soc. Amer. 20: 156-80.

—— (1928). A contribution to the knowledge of the life history of Bremus bimaculatus (Cresson)(Hym.). Ent. Amer. (N.S.) 8: 159-223.

—— (1929). A contribution to the knowledge of the bionomics of *Bombus impatiens* (Cresson)(Hym.). *Bull. Brooklyn ent. Soc.* *24*: 261-85.

—— (1930). A contribution to the knowledge of the bionomics of *Bremus americanorum* (Fabr.)(Hymenoptera). *Ann. ent. Soc. Amer.* *23*: 644-65.

Fye, R.E. (1966). *Sphaerularia bombi* Duford parasitizing *Bombus* queens in Northwestern Ontario. *Canad. Ent.* *98*: 88-9.

Haydak, M.H. (1957). Changes with age in the appearance of some internal organs of the honeybee. *Bee World* *38*: 197-207.

Hoffer, E. (1882-3). *Die Hummeln Steiermarks, Lebensgeschichte und Beschreibug derselben*. Leuschner und Lubensky: Graz.

Holm, S.N. (1966). The utilization and management of bumble bees for red clover and alfalfa seed production. *Ann. Rev. Ent.* *11*: 155-82.

Huber, P. (1802). Observations on several species of the genus Apis, known by the name of humble-bees, and called Bombibatrices by Linnaeus. *Trans. Linn. Soc. Lond. Zool.* *6*: 214-98.

Hüsing, J.O. (1954). Vergleichend-histologische Beobachtungen am Fettkörper sozialer Hymenopteren (Gattung *Apis*, *Bombus*, *Vespa*, *Dolichovespula*). *Wiss. Ztschr. Univ. Halle, Math.-Naturw.* *4*: 319-30.

—— (1955). Vergleichende Beobachtungen am Aufbau des Fettkörpers staatenbildender Apiden und Vespiden. *Bericht über die 7. Wanderversammlung Deutscher Entomologen* 168-71.

Jackson, D.J. (1924). Insect parasite of the Pea-Weevil. *Nature* *113*: 353-4.

—— (1928). The biology of *Dinocampus (Perilitus) rutilus* Nees, a Braconid parasite of *Sitona lineata* L. Part I. *Proc. Zool. Soc. Lond.* *1928*: 597-630.

—— (1935). Giant cells in insects parasitized by hymenopterous larvae. *Nature* *135*: 1040-1.

Jordan, R. (1936). Beobachtungen der Arbeitsteilung im Hummelstaate (*B. muscorum*). *Arch. Bienenk.* *17*: 81-91.

Kilby, B.A. (1963). The biochemistry of the insect fat body. In *Advances in insect physiology*, I (ed. Beament, J.W.L., Treherne, J.E., and Wigglesworth, V.B.). Academic Press: London.

Kloet, G.S. and Hinkes, W.D. (1945). *A check list of British Insects*. Kloet and Hinkes: Stockport.

Köhler, A. (1921). Beobachtungen über Veränderungen am Fettkörper der Biene. *Schweiz. Bienen-Zeitung* *44*: 424-8.

Kramer, S. and Wigglesworth, V.B. (1950). The outer layers of the cuticle in the cockroach *Periplaneta americana* and the function of the oenocytes. *Quart. J. Micr. Sci*. *91*: 63-72.

Lees, A.D. (1955). *The physiology of diapause in arthropods*. Cambridge University Press.

Legge, M.M. (1937). Parasitic larvae in *Bombus terrestris* (L.). *Parasitology* *29*: 524-5.

Loan, C. and Holdaway, F.G. (1961). *Microctonus aethiops* (Nees) auctt. and *Perilitus rutilus* (Nees)(Hymenoptera: Braconidae), European parasites of *Sitona* weevils (Coleoptera: Curculionidae). *Canad. Ent*. *93*: 1057-78.

Lotmar, R. (1939). Der Eiweiß-Stoffwechsel im Bienenvolk (*Apis mellifica*) während der Überwinterung. *Landw. Jb. Schweiz*. *53*: 34-71.

Lyle, G.T. (1927) Contributions to our knowledge of the British Braconidae. Genus 8. *Syntretus*, Först.. *Entomologist* *60*: 83-6.

Marshall, T.A. (1887). Monograph of British Braconidae. Part II. *Trans. ent. Soc. Lond*. *1887*: 51-131.

McColloch, J.W. (1918). Notes on false wireworms with especial reference to *Eleodes tricostata* Say. *J. econ. Ent*. *11*: 212-24.

McManus, J.F.A. and Mowry, R.W. (1960). *Staining methods histologic and histochemical*. Harper and Row: New York.

Medler, J.T. (1957). Bumblebee ecology in relation to the pollination of alfalfa and red clover. *Insectes Soc*. *4*: 245-52.

—— (1962). Development and absorption of eggs in bumblebees. *Canad. Ent*. *94*: 825-33.

Michener, C.D. and Michener, M.H. (1951). *American social insects*. Van Nostrand: New York.

Muesebeck, C.F.W. (1936). The genera of parasitic wasps of the Braconid subfamily Euphorinae, with a review of the Nearctic species. U.S. Dept. Agr. Misc. Pub. 241: 1-37.

—— (1963). Host relationship of the Euphorini (Hymenoptera: Braconidae). Proc. ent. Soc. Wash. 65: 306.

Nair, K.S.S. and George, J.C. (1964). A histological and histochemical study of the larval fat body of Anthrenus vorax Waterhouse (Dermestidae, Coleoptera). J. Ins. Physiol. 10: 509-517.

Palm, N.B. (1948). Normal and pathological histology of the ovaries in Bombus Latr. (Hymenopt.). Opusc. Ent. Suppl. 7: 1-101.

Pearse, A.G.E. (1960). Histochemistry, theoretical and applied. Churchill: London.

Piek, T. (1964). Synthesis of wax in the honeybee (Apis mellifera L.). J. Ins. Physiol. 10: 563-72.

Plath, O.E. (1927). Notes on the hibernation of several North American bumblebees. Ann. ent. Soc. Amer. 22: 181-92.

—— (1934). Bumblebees and their ways. MacMillan: New York.

Putnam, F.W. (1864). Notes on the habits of some species of humble bees. Proc. Essex Inst. Salem. Mass. 4: 98-105.

Rau, P. (1941). A note on the oviposition by the queen bumblebee Bombus americanorum. Canad. Ent. 73: 55-6.

Réaumur, M. de (1742). Histoire des Bourdons velus, dont les nids sont de mousse. Memoires pour servir a l'histoire des insectes. Premier Mémoire 6: 1-38.

Reimann, K. (1952). Neue Untersuchungen über die Wachsdrüse der Honigbiene. Zool. Jb. (Anat.) 72: 251-72.

Richards, O.W. (1927). The specific characters of the British humblebees (Hymenoptera). Trans. ent. Soc. Lond. 75: 233-68.

—— (1946). Observations on Bombus agrorum (Fabricius) (Hymen., Bombidae). Proc. R. ent. Soc. Lond. 21: 66-71.

—— (1953). The social insects. MacDonald: London.

Rösch, G.A. (1930). Untersuchungen über die Arbeitsteilung im Bienenstaat. Z. vergl. Physiol. 12: 1-77.

Schmiedeknecht, O. (1878). Monographie der in Thüringen vorkommenden Arten der Hymenopteren — Gattung Bombus. *Jenaische Zeitschr. Naturw.* **12**: 303-430.

Schmieder, R.G. (1928). Observations on the fat-body in Hymenoptera. *Journ. Morph.* **45**: 121-84.

Schnelle, H. (1924). Der feinere Bau des Fettkörpers der Honigbiene. *Arch. f. Bienenk.* **6**: 83-117.

Seitner, M. und Nötzl, P. (1925). *Pityophthorus henscheli* Seitner und sein Parasit *Cosmophorus henscheli* Ruschka. *Ztschr. f. Angew. Ent.* **11**: 187-96.

Short, J.R.T. (1952). The morphology of the head of larval Hymenoptera with special reference to the head of Ichneumonoidea, including a classification of the final instar larvae of the Braconidae. *Trans. R. ent. Soc. Lond.* **103**: 27-84.

Siivonen, L. (1942). Zur Phänologie des Frühjahrsauftretens der Hummel (Bombus, Hym., Apidae). *Ann. Ent. Fenn.* **8**: 83-102.

Sladen, F.W.L. (1896). Humble-Bees. *Brit. Bee J.* **24**: 37, 47-8.

——— (1900). Humblebees in winter. *Brit. Bee J.* **28**: 72-4.

——— (1912). *The Humble-bee, its life history and how to domesticate it.* MacMillan: London.

Smith, O.J. (1952). Biology and behavior of *Microctonus vittatae* Muesebeck (Braconidae) with descriptions of its immature stages. *Univ. Calif. Publ. Ent.* **9**: 315-44.

Snodgrass, R.E. (1956). *Anatomy of the honey bee.* Comstock: New York.

Soulié, J. (1957). Quelques notes sur l'hibernation chez la fourmi *Cremastogaster scutellaris* Ol. et chez une espèce voisine *Cremastogaster auberti* Em. (Hymenoptera — Formicoidea). *Insectes Soc.* **4**: 365-73.

Speyer, W. (1925). *Perilitus melanopus* Ruthe (Hym., Braconidae) als Imaginalparasit von *Ceutorrhynchus quadridens* Panz. Zugleich eine kurze Zusammenfassung unserer bisherigen Kenntnisse von Schlupfwespen als Parasiten der Käfer-Imagines. *Ztschr. f. Angew. Ent.* **11**: 132-46.

Stein, G. (1956a). Beiträge zur Biologie der Hummel (*B. terrestris* L., *B. lapidarius* L. u.a.). *Zool. Jb.* **84**: 439-62.

—— (1956b). Über das Verhalten von Hummelköniginnen bei experimentell verhindertem Winterschlaf. Verh. dtsch. zool. Ges. 1956: 106-11.

—— (1956c). Weitere Beiträge zur Biologie von Sphaerularia bombi Leon Dufour 1837. Z. Parasitenk. 17: 383-93.

Stobbart, R.H. and Shaw, J. (1964). Salt and water balamce: excretion. In The physiology of insecta, III (ed. Rockenstein, M.). Academic Press: London.

Timberlake, P.H. (1916). Note on an interesting case of two generations of a parasite reared from the same individual host. Canad. Ent. 48: 89-91.

Tkalcu, B. (1960). Sur l'hibernation des bourdons. Bull. Soc. ent. Mulhouse 1960: 96-7.

—— (1961). Deuxième contribution sur l'hibernation des Bourdons. Bull. Soc. ent. Mulhouse 1961: 105-6.

Tower, D.G. (1915). Biology of Apanteles militaris. Jour. Agr. Res. 5: 495-506.

Townsend, L.H. (1951). The hibernation of Bombus impatiens Cresson (Hymenoptera: Bombidae). Ent. News 62: 115-6.

Wagner, W. (1907). Psycho-biologische Untersuchungen an Hummeln mit Bezugnahme auf die Frage der Geselligkeit im Tierreiche. Zoologica Stuttgart 19: 1-239.

Wigglesworth, V.B. (1942). The storage of protein, fat, glycogen and uric acid in the fat body and other tissues of mosquito larvae. J. Exp. Biol. 19: 56-77.

—— (1965). The principles of insect physiology. Methuen: London.

Wilde, J. de (1953). Provisional analysis of the imaginal diapause in an insect (Leptinotarsa decemlineata Say). Acta physiol. pharm. néerl. 3: 141-3.