DORMANCY IN LARVAE OF THE ANT MYRMICA

by

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I N T R O D U C T I O N

This thesis reports original work of the present author undertaken between the years 1951-1954, during the tenure of a Carnegie Research Scholarship as a Research Student in the Department of Zoology, in the University of Glasgow.

It comprises three separate papers reporting aspects of the sociology of myrmecine ants and discussing these in relation to certain problems of general insect physiology. In discussion in the final paper, a mechanism of social control of dormancy in larvae of Myrmica, is postulated.

The first of these papers (Polyethal worker conditions in Myrmica) deals with worker activity and behaviour. Appropriate acknowledgement is made in the text of this paper to the work of Ehrhardt (1931). Other investigations reported in this paper form part of the original work of the present author. In discussion of these results, comparison has been made with the work of Rosch (1925) on the honey-bee, and a similarity of the dynamic mechanisms of worker sociology in these two insects (Myrmica and Apis) has been noted. The results described in this paper have been utilised in experiments described in the other papers.

The second paper (Queen oviposition in Myrmica) describes an experimental investigation of queen oviposition in Myrmica.

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The existence of periodic egg production has been noted in colonies of this genus by previous authors. This paper represents the first full experimental investigation of this problem in any ant, although preliminary work on a limited aspect of this problem had been carried out by Mr. M.V. Brian, to whom the author is indebted for access to the unpublished results. These latter results are not quoted in this thesis.

The third paper (Sociology, growth and dormancy in early larvae of Myrmica) in this thesis reports an investi-:gation of the factors controlling growth and development in early larvae of Myrmica. Again, acknowledgement is made in the text to the work of Mr. M.V. Brian, and, at one point, the result of part of one of his experiments is briefly enumerated for discussion. With this exception, the paper reports original work of the present author.

Five appendices are included in this thesis. These are:-

1) Definitions of the usage of certain eco-physiological terms.

2) Definition of the usage of "a critical stage of larval development".

3) Summary and synoptic discussion of a yet unpublished study, by the present author, of the retrocerebral endocrine system in larvae of Myrmica.

4)/

4) Summary and synoptic discussion of a similar study of the structure and function of the alimentary canal in larvae of Myrmica.

5) Summary of the results of a similar study of structure and development of the antennal bud in larvae of Myrmica.

The results and conclusions enunciated in the three latter appendices are referred to in the text of the three papers comprising this thesis.

I wish to place on record my appreciation of the financial support and facilities afforded for this research by the Carnegie Trust for the Universities of Scotland and in the Department of Zoology, University of Glasgow, by Professor C.M. Yonge, C.B.E., F.R.S.

The research was suggested by, and discussed at all stages with Mr. M.V. Brian (now of the Nature Conservancy, Furzebrook, Dorset) for whose help I am most grateful.

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The papers forming this thesis have been read in MS and discussed with Mr. M.V. Brian, Dr. J.W.H. Lawson, Dr. W. Russell Hunter and Mr. A. Fraser, whose help and criticism are sincerely appreciated.

POLYETHISM IN WORKERS OF THE ANT *MYRMICA*

C O N T E N T S

1. INTRODUCTION

This work was undertaken in the course of an investigation into the factors controlling dormancy in female larvae of the ant Myrmica, carried out between the years 1951 and 1954 in the Zoology Department of the University of Glasgow. The material used in this section of the investigation comprised colonies of the following species:- Myrmica laevinodis Nyl., Myrmica scabrinodis Nyl., and Myrmica rubra (L) as divided into M. rubra microgyna and M. rubra macrogyna by Brian and Brian (1949).

All colonies of ants used were collected in the West of Scotland.

The significance of this section of the investigation and its relationship to other work which has been completed is as follows. Experiments on the induction of dormancy in female myrmicine larvae [carried out initially by Brian (unpublished) and subsequently by the present author] have shown that the physiological "condition" of the workers is important. Worker "condition" has been defined by Brian (1954) who has separated workers into three sequential seasonal categories (vernal, aestival and serotinal) which can be compared with a similar series of physiological "conditions" observed in the laboratory. The present author (Paper II, p.42) has used the term/

term prevernal to describe another seasonal worker condition. For larval growth, the 26 week summer in the West of Scotland is equivalent to a 13 week season at 25°C in the laboratory (Brian, 1954). The four terms used can therefore be defined as follows:-

Prevernal - Workers collected in the field in March and early April. Workers during the first three weeks of incubation at 25°C after hibernation at 10°C.

Vernal - Workers in the field in May, and workers between four and six weeks after the start of incubation at 25°C.

Aestival - Workers in the field in July, and workers between seven and nine weeks after the start of incubation at 25°C.

Serotinal - Workers in the field in September, and workers between ten and twelve weeks after the start of incubation at 25°C.

The present work attempts to survey the origin of these differences of "condition", and the mechanisms by which they affect the brood rearing capacity of workers at different seasons.

Erhardt/

Ehrhardt (1931), working on Myrmica laevinodis, showed that the behaviour of individual workers differed and that there was an apparent change in the behaviour of individual workers with age. The ethological changes she describes show that the young workers are associated for long periods with the brood mass but, as they become older, they show increasingly a tendency to stand near the brood mass, not, apparently, doing anything in particular. Finally the workers become foragers and spend little time with the brood mass. These observations showing ageing accompanied by worker polyethism in a monomorphic species of ant have not hitherto been pursued. In addition to the classical examples of polyethism described in highly polymorphic genera (examples are quoted in Wheeler, 1910), differences in worker behaviour within the same caste have been demonstrated by Chen (1937). Investigation of these conditions in colonies of characteristically monomorphic genera, as Wilson (1954) considers Myrmica, is desirable. In parti-:cular, both differential worker behaviour and the possible polymorphism associated with workers of differing ethal types (workers showing behaviour differences) require study. Both these problems have been investigated and the results are described in the present paper.*

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* The experimental results described in the present paper form part of the original work of the present author, although this line of investigation was prompted in part by the results of certain unpublished experiments by Mr. M.V. Brian on larval growth, and in part by other experi-:ments of the present author which are reported in paper II of this thesis.

2. PRELIMINARY EXPERIMENTS WITH COLONY FRAGMENTS

It appeared that the foraging activity of older workers might result in differential oxygen consumption between colony fragments of varying seasonal ages. This would be the case if the changes in worker behaviour and activity with senescence were solely responsible for the different brood rearing capa-:cities of worker groups of varying seasonal ages.

Experiment 1 was therefore undertaken as follows. The oxygen consumption of two series of colony fragments of M. rubra macrogyna was measured with modified Barcroft respirometers. One series of colony fragments was from a vernal colony, and the other series from a colony in a serotinal condition. This experiment failed because of erratic variation in the results attributable to the spasmodic locomotor activity of one or two workers in each fragment. No significant dif-:ferences were found between the oxygen consumption of the two seasonal groups. If the workers in these groups showed different oxygen consumption while on the brood mass, this was masked by the very high oxygen intake of individual active workers. Not more than 5% of the total number of workers was active at one time. Respirometry was therefore abandoned.

Experiment 2 was an attempt to measure locomotor activity by means of an actograph (Chauvin, 1949). An actograph was designed

designed and incorporated in a beam balance, and electrical contacts were used to measure displacement. The apparatus worked, but the results were of little value. In addition to the unavoidable exposure of the insects to the dangers of desiccation, starvation, unnatural stimulation, and the like, no significant statistical differences could be detected in the results between the activity of serotinal and vernal colony fragments. Individual workers were responsible for most of the activity recorded, and these few, highly active, workers masked any differential activity of the residual workers tending the brood. These experiments were also abandoned.

It was apparent from the results of these two experiments (1 and 2), that it was necessary to measure the differences in locomotor activity between the individual workers of separate colony fragments, and attempt assessment of their cause, and their effect on brood rearing.

3. CONDITIONS IN A COLONY OF MYRMICA SCABRINODIS

A Worker Segregation

The workers of a medium sized colony of M. scabrinodis wre separated into activity samples in the following way. The colony (which was collected in the field in early October, ad was therefore in a late serotinal condition) was cultured fr one week at 25°C without food. It is possible that the olony was short of food as a result. (Some workers may be aid to have been hungry?). The glass cover of the plaster rst (Brian, 1951a) was then removed from the food chambers ad the workers were collected as they emerged. The food ambers are furthest from the brood chamber. Samples were moved every ten minutes, but the number of workers in each mple could not be regulated accurately. The time required fr the removal of a sample of fifteen to twenty workers was, t first, four minutes, but rapidly increased. Similarly the mple size decreased. In order to keep the time of sampling, he time for the removal of the sample, and the size of the mple as constant as possible, the nest was stimulated by lowing. Blowing gently into the dry chamber of the nests nce every thirty seconds facilitated the separation of ctivity samples 6 to 9 .

After/

After the removal of sample 9 it was necessary to increase the amount of stimulation of the workers in order to maintain a constant sample size and time for removal. The nest was stimulated by blowing gently into it once every ten seconds. The necessity for stimulation may not have introduced a completely new factor into the worker separation, since it is impossible to say to what extent the workers were stimulated initially by vibration of the nest when the glass cover was removed or air displacements, etc, at that time. The levels of stimulation to which the colony was subjected during its separation have not been assessed relatively.

All two hundred and ninety one surviving workers were included in fifteen activity samples, the last of which, totalling about forty workers, comprised the residual workers and larvae from the brood chamber. During the removal of the eleventh sample, workers carrying larvae were first detected. The results of the separation are shown as histograms in figs.1.VII & VIII. In fig.1.VII, the black histogram shows the worker numbers in each activity sample, and the white histogram the approximate larval numbers. In fig.1.VIII the broken line shows the time in minutes for the removal of each sample, and the continuous line shows the points of increased stimulation. (S_1 = one puff every 30 seconds; S_2 = one puff every 10 seconds).

It/

It should be noted that at least three separate factors have been used to achieve this worker segregation. These are:-

A. Hunger, with possible resultant high locomotor activity in search of food.

B. Inherent high locomotor activity as opposed to inherent low locomotor activity.

C. High reactivity (sensitivity to stimulation) as opposed to low reactivity.

If the initial separation is by a hunger stimulus, ideally one should collect over a period of days those individuals which become hungry, if the basis of separation is to be strictly uniform throughout. The time taken to achieve such a separation would defeat the purposes of the investigation, so the process is speeded up by applying stimulation. But the situation is further complicated. It is convenient, at this point, to consider the separation as one of varying grades of locomotor activity. The assumption that hunger is partially responsible for the worker separation implies that "foragers" exist among the workers. The use of the term "forager" may be descriptive and imply ethological purposefulness accompanied by high locomotor activity. It is convenient to avoid the use of the term forager in this particular sense, since all workers with high locomotor activity need not, in theory, be foragers.

The/

The association of high locomotor activity with a tendency on the part of the worker to wander away from the brood mass would increase automatically the chance probability of the worker meeting suitable food. It is nevertheless convenient to use the term "forager" for workers with high locomotor activity. It should, however, be clearly understood that no purposefulness is implied by this phrase. This question is considered subsequently

The application of stimuli which speed up the process of separation, introduces another factor. It may be assumed that after the first stimulus (S_1) has been applied to the nest (if indeed the nest has not already been accidentally stimulated by previous removals of worker samples), worker separation is largely on the basis of reactivity or sensitivity. The degree of homology between this separation and that based on activity is unknown, and has not been estimated.

A further separation of the fifteen samples was then undertaken. Each sample was subdivided, after careful examination of the individual workers, into six melanic* groups designated I, II, III, IV, V and VI. Photomicrographs of/

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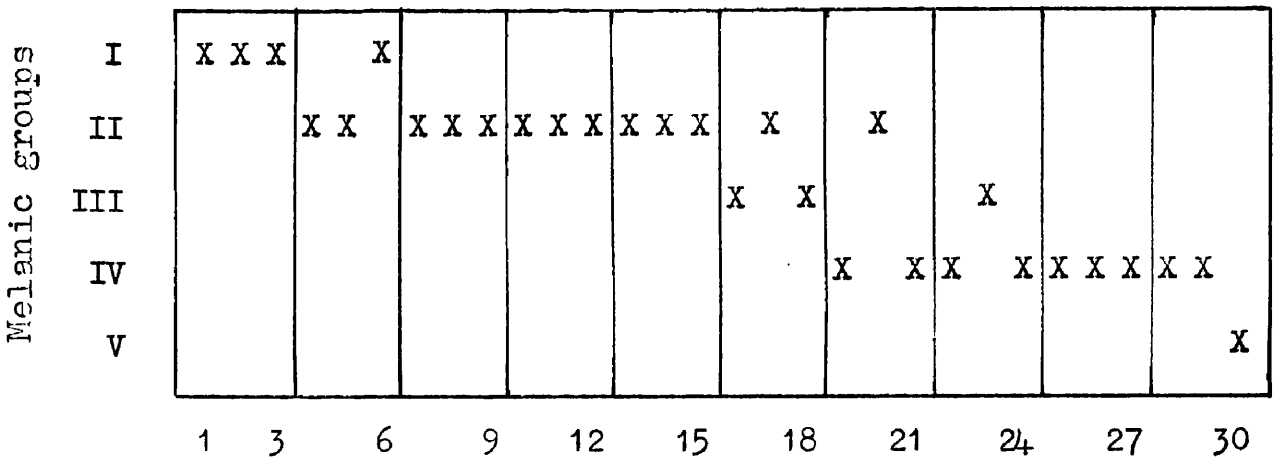
* The use of the word melanic denotes only a darkening of the cuticle. No biochemical tests have been made to determine whether this change is due to the pigment melanin as defined by Lison (1936). Subsequent research on this pigment has been reviewed by Fox (1953).

of the six melanic groups are shown in figure 2A. The percentage composition of each sample belonging to the appropriate melanic group is shown in figure 1. The most highly melanised individuals were those of group I, and the most lightly melanised those of group VI. From figure 1, it can be seen that the most highly melanised individuals, i.e. groups I and II, were confined to activity sample 1. Group III reached a maximum in sample 2, group IV in sample 4, and group V in sample 9. The most lightly melanised individuals (group VI) reached a maximum in sample 15. This segregation on the basis of melanisation shows that a series of visibly distinguishable activity groups has been separated. The melanic differences between adjacent groups were small. The separation was only accomplished by the comparison of individual workers under a spotlight. Relative differences are shown in figure 2B. It is apparent that there are three distinct major melanic types (groups I+II; III+IV; and V+VI), each of which has been subdivided by less obvious melanic differences to form six melanic groups. Only the separation of workers in groups I and II was difficult and, on a solely melanic basis, of doubtful validity. The small numbers of workers of groups I, II and III facilitated experimentation on this point. Those workers of groups I, II, III, IV and V, which had been removed in activity sample 1, were cultured together for a week at room temperature (22°C to 18°C) on a full food diet. The

separation/

separation of the workers in these groups was then repeated, without stimulation, and the individual workers were placed in numbered tubes as they emerged from the nest. This was repeated three times on three successive days. The results showed a high degree of correspondence, but the form of the experiment was not amenable to statistical analysis. A typical result is therefore shown in table I.

TABLE I



There appeared to be sufficient differential activity to justify the separation of these highly locomotor workers into the groups I, II and III. It is clear then that the difference between groups I and II and between groups III and IV, though small, is nevertheless real.

The/

The composition of the above nest is shown in table II.

TABLE II

MELANIC GROUP	TOTAL NUMBER OF WORKERS	% OF TOTAL POPULATION	
I	4	1.3%	
II	13	4.4%	10.5%
III	14	4.8%	
IV	62	21.3%	
V	133	45.7%	89.3%
VI	65	22.3%	

From table II it is apparent that melanic group V is the largest and that the adjacent groups IV and VI are next in size to it. Together these three groups represent 89.3% of the population. The highly active workers represent only 10.5% of the total population, which is therefore numerically dominated by groups associated with the brood mass.

It was apparent, from observation, that melanic group VI was composed of workers/

workers produced from eggs laid during the preceding summer, (i.e. from non-dormant larvae). It was equally apparent that melanic group V was composed of workers which had been produced early in the year from overwintered larvae. The implication of a progressive melanisation occurring throughout the life of the worker was noted. Melanic groups I to IV were therefore all composed of workers which had been adult for more than one year, and further, by implication, those in melanic groups I and II were considerably older.

The following experiment (3) was then carried out. Fifteen workers of each of the three melanic groups IV, V and VI, were cultured with five larvae at 25°C on a full food diet. After two months the surviving workers were examined. After removal of the new workers produced from the larvae in each culture, it was still possible to distinguish between the workers of the three original melanic types. There were few survivors from culture IV, but these were darker than other members of melanic group IV which had not been cultured at 25°C but kept at 10°C for some time previously. Similarly it was possible to differentiate between workers of the same original melanic groups (either V or VI) which had been reared, some at 25°C and some at 10°C. It can be concluded that melanisation occurs more rapidly at 25°C than at 10°C, and that the differences between the melanic groups are partially

attributable/

attributable to different ages. While this is true of the difference between groups V and VI (which represent respectively the overwintered brood and non-dormant brood of one season), the age relationships of the other melanic groups are obscure. This is discussed subsequently.

Visible differentiation of workers of melanic groups II, IV and VI was possible, even in dim light, and these groups were therefore used in subsequent experiments and observations. As previously stated, the colony was collected in early October. The subsequent separation, which has been described in detail, was carried out over a period of eight days, during which time the colony was kept at room temperature (18°C - 23°C). When the separation was complete, the six melanic components of each activity sample had been separated from each other and were being cultured in isolation on a full diet. The cultures were then left for one more week at room temperature and then transferred to a thermostatically controlled cold room at 10°C. Here they remained for a period of twenty-one days. They were then removed after this comparatively short chilling, and incubated at 25°C on full diet. The course of events can, as a result, be shown diagrammatically as in table III.

TABLE III/

TABLE III

Weeks	1	2	3	4	5	6	7	8
Temperatures	25°C	18-23°C	18-23°C	10°C	10°C	10°C	25°C	25°C
	Colony Separation		Isolated worker Components					

Such a perfunctory hibernation period has been successful for the overwintering of workers and queens. For the successful vernalisation of larvae this period may however be quite inadequate (Brian, 1954).

3.B. Worker Activity and Duty Preference.

The results contained in this section are derived from one experiment (4) carried out on the post-hibernated M. scabrinodis nest. Other observations on workers from this nest have confirmed the results enumerated below.

Experiment 4 began six weeks after the workers had been segregated, and was designed as follows. Eight plaster of paris nests (Brian, 1951a) were used, each with a full food supply. Conditions were standardised as far as possible. Each nest contained six workers and ten larvae. The larval growth is considered in Section C below. The worker composition of each colony fragment is shown in table IV, below.

Such an experimental design will reveal both lasting differences of activity within and between melanic groups, and also the effects of numerical variation of worker number.

At each observation, careful removal of the cover from the nest revealed both the position and occupation of the workers. During each census fifteen observations were made on each nest. The first activity census lasted six days, and the remaining two each lasted three days. Only some of the resulting data are described, namely those from which interesting sociological conclusions can be drawn or from which statistically significant differences have been obtained after F tests of the partitioning of/

TABLE IV

Worker Composition of Synthetic Colony Fragments.

	FRAGMENT 1			FRAGMENT 3			FRAGMENT 5			FRAGMENT 7		
Workers of melanic group VI from activity samples 14 or 15.	a	b	c	a	b	c	a	b	c	a	b	c
	VI	6	15	VI	4	15	VI	4	14 & 15	VI	4	14 & 15
				IV	2	15				IV	1	15
							II	2	1	II	1	1
	FRAGMENT 2			FRAGMENT 4			FRAGMENT 6			FRAGMENT 8		
Workers of melanic group VI from activity samples 11 or 12.	a	b	c	a	b	c	a	b	c	a	b	c
	VI	6	11 & 12	VI	4	11	VI	4	11 & 12	VI	4	11
				IV	2	15				IV	1	15
							II	2	1	II	1	1

Where: a = Melanic group of workers concerned,

b = Number of workers belonging to that melanic group,

c. = Activity sample in which the individual workers were segregated.

of variance (Snedecor, 1946). Results on worker activity are considered in three sections:-

a. Total nest activity

b. Variation within melanic groups

c. Differences between melanic groups

a. Total nest activity.

These results are tabulated in tables V.A-F. These show the numbers of workers at each census engaged in three possible "occupations" or "duties" similar to those described by Ehrhardt (1931), namely:-

- Workers on the brood mass (i.e. workers in contact with a larva).
- Workers near the brood mass (i.e. workers which by head or antennal movement would come in contact with a larva, or a worker on a larva, or another worker which was in such a position).
- Workers which were not near the brood mass.

In this last category, it was possible to distinguish between workers in the food chambers and the dry chambers, and workers in/

in the wet chamber. Similarly, it was possible to distinguish a fourth category of behaviour, namely that of workers which, by pulling on the cotton wool or biting the plaster of paris, were attempting to build a nest. The incidence of this behaviour is shown in table V.F.

Statistical analyses of the results presented in tables V.A-F show that the differences between certain pairs of nests (1+2; 3+4; 5+6; 7+8) are significant.

i. The number of workers on top of the larvae in nests 5+6 is low (table V.A)

ii. The number of workers near the larvae shows no significant difference (table V.B)

iii. The total number of workers on and near the larvae (i + ii) shows striking variation (table V.C). Nests 1+2 and 3+4 have uniformly high values, but the values for nests 7+8 are lower. Values for nests 5+6 are lowest of all.

iv. Corresponding to the values noted in iii above, the number of workers away from the brood mass shows an inverse situation, where nests 5+6 have the highest values, and nests 1+2 and 3+4 the lowest (table V.D)

v. The total length of all worker trips varies between the nests as does iv above (table V.E).

vi. The occurrence of attempted nest building is confined to two nests.

It is apparent that variation of the melanic group composition of synthetic colonies causes differential activity and behaviour.

TABLES V.A-F

These six tables show the variation in activity and worker occupation in the eight nests during the three census periods, each of three days' duration. The subdivision of the table into four columns facilitates comparison of the results between colony fragments of differing melanic group composition. The difference between the members of each pair shown in any one column is the intra-melanic difference of activity sample (p.18). No statistical differences are attributable to this latter level of worker segregation in these six tables.

TABLE V.A

Total number of workers on the brood mass

	NESTS							
	1	2	3	4	5	6	7	8
Census 1	69	59	65	49	46	48	61	61
Census 2	74	64	70	73	53	54	68	70
Census 3	66	62	70	80	54	49	66	63

TABLE V.B

Total number of workers near brood mass

	NESTS							
	1	2	3	4	5	6	7	8
Census 1	18	24	15	11	20	12	11	20
Census 2	13	22	14	13	8	21	7	9
Census 3	17	23	13	5	10	23	9	19

TABLE V.C

Total numbers of workers on or near brood mass

	NESTS							
	1	2	3	4	5	6	7	8
Census 1	87	83	80	60	66	60	72	81
Census 2	87	86	84	86	61	75	75	79
Census 3	83	85	83	85	64	72	75	82

TABLE V.D

Total number of workers away from brood mass

	NESTS							
	1	2	3	4	5	6	7	8
Census 1	3	7	10	13	24	30	18	9
Census 2	3	4	6	1	29	15	14	11
Census 3	7	5	7	4	26	18	14	8

TABLE V.E

Total length of worker trips

	NESTS							
	1	2	3	4	5	6	7	8
Cenus 1	6.5	19.5	17.5	31.0	48.5	74.5	40.5	30.5
Census 2	8.0	6.5	11.5	5.0	107.5	35.5	44.5	34.5
Census 3	14.0	8.5	18.0	7.5	68.5	43.0	41.0	26.5

In table V.E distance from the brood mass is shown in arbitrary units, where for instance:-

5.0 = Worker in food chamber (maximal value)

2.5 = Worker at entrance to wet chamber.

TABLE V.F

Number of workers engaged in attempted nest construction

	NESTS							
	1	2	3	4	5	6	7	8
Census 1				7				
Census 2				3			1	
Census 3				1			1	

b. Variation within melanic groups.

The results discussed in this section are given in tables VI.A-H. These have been simplified for ease of comprehension and show only the average values concerned.

MELANIC GROUP VI

[1] Workers on the brood mass./

[1] Workers on the brood mass.

Analysis of the number of workers of group VI on the brood mass (table VI.A) shows significant differences between:-

i. the numerical nest compositions

ii. the original activity samples

iii. the three census periods

(i) Adjustment of worker numbers to a uniform value in this analysis shows (table VI.B) that nests 1+2 [which, before this adjustment have a significantly higher number of workers on the brood mass compared with the other nests] have, when adjusted, a significantly lower number of workers on the brood. It is unreasonable to suppose that six workers cannot get on top of ten larvae. Therefore, while more workers were standing on the larvae when six workers were present, this increase was not proportional to the higher number of workers. Among the possible interpretations of this result are:-

a. Workers of melanic group VI normally stand on the larvae when workers of other melanic groups are present, but will do otherwise if no other melanic groups are present. (Teleologically they have a preference for one occupation).

b. Workers of melanic group VI show a range of behaviour and by themselves may stand on the brood or do otherwise. When workers of other melanic/

melanic groups are present there is competition for duties, resulting in group VI workers being forced onto the brood mass.

These two possibilities are not mutually exclusive and both may be operative in different populations.

(ii) Workers of melanic group VI from activity samples 10 + 11 spend less time on the brood than workers of melanic group VI from activity samples 14 + 15. This is in agreement with the results derived from the initial segregation and it is concluded that differences within the melanic groups, between workers of relatively low activity as opposed to workers of relatively high activity, are permanent and not ephemeral.

(iii) The significance attached to the differences between censuses is obscure, but probably reflects changes in the size of the brood mass.

[2] Workers near the brood mass.

Differences exist in the numbers of workers of group VI near the brood mass (table VI.C, adjusted for worker number), and occur as follows:-

i. between four melanic group compositions

ii. between the two activity samples of melanic group VI.

(i)/

(i) The higher values in nests 1+2 (even when adjusted) correspond to the lower values for workers on the brood noted in (i) [p.25 above]. Thus, in the absence of workers of other melanic groups there is a significant increase in the numbers of workers of group VI standing near the brood.

(ii) Relatively high activity workers of melanic group VI from samples 10+11, differ ethologically from the relatively low activity group VI workers of samples 14+15, in the higher percentage of their time spent standing near the brood, compared with low activity group VI workers. If "standing around" is a "duty", then differences in duties occur within melanic group VI in this respect also.

The repeated formation under all circumstances of a group of workers standing near the brood suggests that this group has a real sociological significance and does not merely represent workers with nothing else to do, or workers resting etc..

[3] Worker locomotor activity.

The locomotor activity of the workers of melanic group VI is considered under three headings:-

i./

i. The average length of each trip.

ii. The total distance covered by the workers of each melanic group divided by the total number of workers in that group (i.e. the length per unit worker of all trips).

iii. The number of trips

(i) There is no variation in the average length of trips by workers of melanic group VI with either time (larval increase in bulk), different group compositions, or with workers from different activity samples. It then seems probable that workers of group VI segregated in activity samples 10+11, were there because of differential sensitivity to stimuli or differences of behaviour (e.g. a tendency to stand near the brood), and not because of significantly higher locomotor activity.

(ii) This also shows no significant variation with any of the factors mentioned in (i) above, and the same conclusions can be drawn.

(iii) This analysis must be considered with caution because the number of trips is small. Analysis, without adjustment for worker number differences, shows surprisingly that there are no significant differences, despite the higher numbers of workers of melanic group VI in nests 1+2. Failure, as in

(i)/

(i) and (ii) [above], of the analysis of the adjusted worker number values to reveal any difference between workers of the two activity levels sampled, leads again to the conclusion stated in (i) above. Differences in the locomotor activity of group VI workers are detectable at the three censuses. These workers show an "initial" level of activity. (The colony fragments were allowed to establish themselves in the nests for twenty-four hours before measurements began). This "initial" activity level diminishes rapidly in nests 3 to 8, but in nests 1+2 remains at about the same level throughout the experiment. It may be suggested that this "initial" activity is produced by the interaction of larval groups of constant size on workers of melanic group VI (characteristic-:ally of low locomotor activity), which have been isolated for several weeks from workers of other melanic groups. Where reintroduction of workers of other melanic groups occurs, the "initial" activity of group VI workers is rapidly lost. It is difficult to visualise worker competition for locomotor activity in a relatively large nest, such as was used in these experiments. Teleologically, therefore, it appears that workers of melanic group VI "prefer" to stay on the brood mass.

MELANIC GROUP IV/

MELANIC GROUP IV

Table VI.F show the adjusted number of workers of group IV on the brood mass. Comparison of the unadjusted and adjusted values, shows that while these workers are more often on the brood mass when two are present, the increase is not proportional, and worker number adjustment reduces the value signficantly when two workers are present. Therefore, when the number of workers of melanic group IV is increased, there is a change in the proportion of "duties" carried out.

No differences are detectable in the number of workers of group IV standing near the brood (when the numbers are adjusted).

No differences are detectable in the number of workers of group IV which show locomotor activity (when the numbers are adjusted). But the unadjusted values show that two workers (nests 3+4) make more trips than one (nests 7+8). The total distance covered by these workers is higher in nests 3+4 (table VI.G). Similarly, the average length of trips by these workers is higher in nests 3+4 (table VI.H).

MELANIC GROUP II

The only significant difference found in this analysis is that two workers of group II make twice as many trips as one. Therefore, in worker groups of these sizes and numerical relationships, the locomotor activity of individual workers of group II is independent of each other.

TABLE VI.A-H.

Statistical differences attributable to the three census times are of obscure significance. Accordingly, for ease of assimilation, these tables show only the average or total values for the three census times.

TABLE VI.A

Averaged numbers of workers of melanic group VI, on the brood mass, unadjusted to worker number.

		NESTS							
		1	2	3	4	5	6	7	8
Initial Worker Activity Samples	10-11		61.6		47.6		46.3		51.0
	14-15	66.3		52.3		50.3		51.0	

TABLE VI.B

Averaged number of workers of melanic group VI, on the brood mass, adjusted to worker number.

		NESTS							
		1	2	3	4	5	6	7	8
Initial Worker Activity Sample.	10-11		41.0		47.6		46.3		51.0
	14-15	46.3		52.3		50.3		51.0	

TABLE VI.C

Total number of workers of melanic group VI standing near the brood. Adjusted to worker number.

		NESTS							
		1	2	3	4	5	6	7	8
Initial Worker Activity Sample	10-11		46		20		26		26
	14-15	32		14		18		17	

TABLE VI.D.

Total number of trips by workers of melanic group VI.
Adjusted for worker number.

		NESTS							
		1	2	3	4	5	6	7	8
Initial Worker Activity Sample	10-11		10.4		9.0		17.0		1.0
	14-15	8.6		9.0		11.0		10.0	

TABLE VI.E

Average number of trips by workers of melanic group VI,
disregarding initial activity samples
and considering only the censuses

	NESTS			
	1 + 2	3 + 4	5 + 6	7 + 8
Census 1	6.6	12.0	20.0	5.0
Census 2	4.6	1.0	7.0	4.0
Census 3	7.8	5.0	1.0	2.0

TABLE VI.F

Total number of workers of melanic group IV on brood mass. Adjusted for worker number.

NEST			
3	4	7	8
24	29	40	34

TABLE VI.G

Total distance covered by workers of melanic group IV, divided by the total number of workers

NEST			
3	4	7	8
15.8	9.5	3.0	3.0

TABLE VI.H

Average length of trips by workers of melanic group IV

NEST			
3	4	7	8
2.0	2.9	1.5	1.5

c. Differences between melanic groups II, IV and VI.

Tables VII.A & B show in tabular form the results of this section of the analysis.

[1] The time spent by workers of the three melanic groups II, IV and VI on the three "duties" described.

These are shown in table VII.A. It is apparent that while groups IV and VI are comparatively similar (though the differences between these two groups are statistically quite definite), group II shows very different behaviour. This result confirms the differences between workers used in the original segregation, and shows that workers of group II are characteristic/

characteristic foragers. The separation of melanic groups IV and VI is less definite but equally correct.

[2] Effects of group interaction.

The effects on the behaviour of workers of melanic group VI of the presence or absence of workers of other melanic groups, is shown in table VII.B. The overall change in the distribution of duty is apparent. This may reflect equally the increased numbers of workers of group VI in some cultures as well as the presence of workers of other melanic groups in the other cultures.

Comparable numerical effects in groups II and IV have been demonstrated previously (pp.30,31).

TABLE VII.A/

TABLE VII.A

% Time spent by workers in each of three possible occupations

		"DUTY"		
		On Brood	Near Brood	Away From Brood
Worker Melanic Group	II	8.9	26.3	64.8
	IV	73.9	17.4	8.7
	VI	79.2	16.7	4.1

TABLE VII.B

% Time spent on "duties" by workers of melanic group VI, in the presence and absence of workers of other melanic groups

	"DUTY"		
	On Brood	Near Brood	Away From Brood
No other Melanic Groups Present	74.1	22.0	3.9
Other Melanic Groups Present	84.4	10.8	4.8

3.C Brood Rearing.

Larval growth during experiment 4 (described above) was measured in two ways:-

a. By the increase in weight of each brood mass at each census.

b. By the areal increase of each individual larva at each census as measured by projection of the lateral view.

The use of weight alone as a measure of brood rearing efficiency is here ineffective, since the weight of the larval group gives no indication of the distribution of larval weight gains among the individual larvae. The technique of areal projection of the individual larvae presents difficulties inasmuch as the relationship between area and weight is non-linear. However, a precise relationship can be established and relied upon within the limits of larval turgidity. In view of the close correlation between the measurements of the summed areal increases and the weight gains, only the areal increases are here described.

All larval groups contained, initially, ten larvae, the frequency distribution of larval areas for each group being the same in all eight nests. Four larvae were lost or died during the experiment. Direct statistical analysis of the data would therefore be unreliable. Arrangement of the incremental/

incremental data for each census in a series of size arrays facilitates estimation of which particular larva has died since the previous census. Some larvae show negative areal increases, which are statistically intractable. Twenty units have therefore been added to all individual areal increase values to invalidate any negative signs.

Possible errors from these sources have been further reduced by the subdivision of the original array of larval sizes in each group into three sections.

i. Two large larvae

ii. Four medium sized larvae

iii. Four small larvae

The incremental data for the larvae within each of these sub-groups has been averaged and is used in the following analysis. Censuses were taken every three days from the start of the experiment. The larval groups were randomised (by tables of random numbers) after the second census and redistributed, after measurement, to the nests. The larval groups were rerandomised after the third census.

This experimental design was defective in that it was not possible to use a Latin square and analyse the effects of both larval groups and worker nests. The use of a Latin square with eight larval censuses is not feasible within the time taken/

taken for some of the larvae to reach prepupation, if ethological observations are to be made on the undisturbed colony.

Analysis of the incremental areal data by the partitioning of variance is shown in table VIII, in which the four censuses, three larval sizes, and eight worker nest compositions are all utilised. All the main comparisons are statistically significant, or on the verge of significance, as are all first degree interactions. The situation is therefore complicated, the occurrence of significant interaction having biased the probability of significance of the original comparisons. Re-estimation of their significance is necessary, using weighted measurements. The biological implications (differential treatment of larvae by workers) of the inter-:actions are, however, of fundamental importance, and in this respect re-estimation is unnecessary.

Examination of these results shows:-

i. There is evidence of consistently "good" and consistently "bad" worker nests as measured by brood rearing success.

ii. Differing areal increases are achieved by larvae of varying initial size. Large larvae show greater areal gains than medium sized larvae, which, in turn, gain more than small larvae.

iii./

TABLE VIII

Analysis of variance of areal gains of larvae of Myrmica scabrinodis

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F	P (PROBABILITY OF OCCURRENCE)
Worker Groups	7	720	103	2.654	< 10% - > 5%
Censuses	3	3,838	1,279	32.913	< .1%
Larval Sizes	2	2,029	1,014	26.093	< .1%
Groups × Censuses	21	1,716	81.71	2.013	< 5% > 1%
Groups × Sizes	14	1,441	102.93	2.649	< 5% > 1%
Census × Sizes	6	1,249	208	5.352	< 5% > 1%
Error (Second degree interaction)	42	1,632	38.86		
Total	95	12,625			

iii. The total areal gains by larvae at the four censuses are significantly different and can be arranged as follows:-

Census number	4	1	2	3
Total Areal Increase	946	662	650	538

The larvae are dehydrated when removed from hibernation for the experiment, and rehydration is partly measured during the first three days, (i.e. during the first census). This causes large areal increases. Growth during the second and third censuses is generally slow since, by chance, larval groups are passed from "good" worker nests to "bad" worker nests and vice versa. During the fourth census, the larval groups show rapid growth and development, particularly in "good" worker nests. The detailed causes of these variations are unknown.

iv. Interaction between larval size and worker nests is shown graphically in figure 3. Larger differential increases are shown by large larvae in "good" nests as compared with "bad" nests, when an overall comparison is made with the corresponding increases for medium sized larvae. Comparison of medium sized and small larvae shows similar results.

v. Interaction between larval size and census variation is shown graphically in figure 4. When the total areal increase at a census is large, large larvae show relatively higher areal increases compared with large larvae at a census where the total areal increase is small, comparison throughout being made with the corresponding results for medium sized larvae. These in turn show similar effects relative to small larvae.

vi./

vi. Interaction between censuses and worker nests is shown in figure 5. Where the total areal gain at a census is high, "good" worker nests show large gains compared with "bad" worker nests, comparison being made throughout with similar groups at censuses where the overall areal increase was low.

It is probable from these results, that had the entire experiment been replicated, the error due to variance within groups would have shown that the second degree interaction was also significant. Such a result would imply that in the feeding of certain larvae at certain times, the degree of bias was greater than that which might be expected in a serial system, i.e. where the degree of bias would be proportional to the initial larval weight. Such differential bias may occur in certain cases, e.g. queen potential larvae (Brian, personal communication), and may be partially explicable in terms either of differences in worker reactions to larvae of varying sizes (Section 4.C below) or of the subdivision of the brood mass by the workers, into portions which are treated differently (e.g. as described by Weber in Wheeler (1937) on Atta).

The relationship of worker activity and larval growth remains to be considered. Comparison, (figure 6), shows that there is some relationship between the presence of foragers and good larval growth. But the increase in larval growth during any one census may reflect treatment during a previous census/

census in a different worker nest. The necessity for time sequence analysis is apparent, but replication on a larger scale, or more frequent censuses, would be essential to such an analysis. There is no direct correlation between worker activity and larval growth at any one census as measured by these methods.

The amount of locomotor activity observed in cultures containing workers of melanic group II greatly exceeds that which might be supposed necessary for normal larval growth as observed in these groups.

3.D. Nest Building by Workers.

The nest building capacities of the worker melanic groups were investigated in an experiment (5) comprising eight nests. The nests used were first designed by Brian (unpublished) and consist of two sheets of glass separated by glass tubing waxed in position round the edge. The dislike of workers for waxed surfaces and bright light can be used to "force" them into a shaded central portion of the nest completely filled with damp artificial soil, composed of krilium, kaolin, sand and water. When mixed in the correct proportions, these give a moist, friable, easily worked medium for nest construction.

Six workers were used in each nest, two nests containing workers of melanic group III; two, workers of melanic group IV; two, workers of melanic group V; and two, workers of group VI. The results of the experiment are tabulated as observations after four, twelve, twenty-four and forty-eight hours (table IX).

Different melanic groups have different nest constructional capacities. Workers of melanic group III constructed chambers in the open by building walls without excavation of chambers. Workers of melanic group VI showed characteristic aimless soil disturbance and built few chambers. Workers of groups IV and V showed a wide capacity for construction of all kinds of earthworks, chambers, tunnels, etc.. There appeared to be excessive/

(See over)

KEY TO TABLE IX

C = CHAMBERS in the soil

(1-C = one chamber)
(2-C = two chambers, etc.)

M = MASONRY or individual sand grains pressed into position on the walls of the chambers giving a smooth appearance.

SD = SOIL DISTURBANCE. Scattering aimlessly the soil from the central mass and pushing aimlessly through it.

(SD = Major disturbance)
(sd = minor distrubance)

T = TUNNELS in the soil

(T = Major tunnelling)
(t = Minor tunnelling)

W = WALLS built in open spaces, usually round brood.

(W = Major walls)
(w = Minor walls)

VOLUME of nest filled by earthworks is also shown,

e.g. 50% where volume occupied equalled half the total.

TABLE IX

Nest Construction

Time of Observation in Hours.	MELANIC GROUP III		IV		V		VI	
	Nest 1	Nest 2	Nest 3	Nest 4	Nest 5	Nest 6	Nest 7	Nest 8
4	w	-	1-C t	-	-	-	-	-
12	W	W	1-C M T	1-C T	1-C	2-C sd	SD	2-C sd
24	W	1-C M t W	4-C M T W	3-C M T w	1-C M w	2-C M sd	SD	2-C M SD
48	M t W	1-C M T W	6-C M T W	5-C M T W	1-C M W	2-C M SD	SD	2-C M SD
VOLUME	25%	30%	60%	50%	20%	25%	0%	20%

excessive nest construction by these workers, far beyond their requirements. Individual workers of these groups would spend hours excavating chambers in the soil, although several chambers were already in existence. In experiment 4 workers of these groups showed most cotton-wool pulling, etc., and this probably represents the same form of behaviour.

3.E. Worker Sizes.

All workers surviving the period of hibernation and subsequent experiments were measured. High mortality and low initial numbers in worker melanic groups I, II and III, reduced the significance of measurements in these groups. Information derived from the analysis of melanic groups IV, V and VI only, is described here.

Many varied measurements have been used in the study of polymorphism in ants. The six measurements made on the head and thorax of the M. scabrinodis workers may be expected to reveal any allometry in these regions (Wilson, 1954). The measurements (figure 7) are:-

1. head width (between the eyes),
2. head length,
3. head angulation,
4. maximal thoracic width,
5. thoracic length,
6. distance between tips of the epinotal spines.

Five of these measurements showed simple arithmetic linearity and correlation, but the sixth (epinotal spine width), showed certain irregularities and has, for present purposes, been disregarded. Figure 8 shows the summed values of measurements 1-5/

1-5 for each worker, plotted against head width. It is apparent that head width provides a reliable estimate of total size, as measured by the five summed values (1-5). No allometry has been detected between these measurements in any of the melanic groups IV, V or VI.

Examination of these results shows:-

a. There is detectable intra-nidal variation in worker size.

b. In the five cases here examined there is no detectable allometry.

c. There are differences between the frequency distributions of worker size in melanic groups IV, V and VI. Workers of melanic group VI are of smaller average size than those of melanic groups IV or V, which are of similar size ranges.

It is known from observation that workers of melanic group VI are derived from non-dormant larvae of approximately four months previously. It is also known that workers of group V are derived from the dormant larvae produced during the previous year and which pupated approximately six months previously. If the size differences between groups V and VI reflect consistent differences in the average size of workers produced from non-dormant and dormant larvae, then:-

i. Workers of melanic group IV are derived from the dormant larvae of two years previously,

ii. Workers of melanic group III may therefore represent the non-dormant brood of that year,

iii./

iii. The rate of melanisation of workers from these two groups must be different, those from non-dormant (III) larvae melanising faster than those from dormant larvae (IV).

iv. The rate of change of social function (duty preference) must be correspondingly different since workers of non-dormant larval origin (III) are, within two years, of higher locomotor activity than those of group IV (produced from dormant larvae of the same year).

The relationship of these size differences to the fifteen activity samples of the initial worker segregation is shown in figure 9. This shows the average, for each activity sample, of the total of the first five measurements (1-5 above) on each worker in that sample. Two hundred and thirteen workers were measured, but samples 1, 2 and 7 contained too few survivors for any reliable estimates of average worker size, and this is indicated on the graph. The low average worker size in samples 14 and 15 reflects the low locomotor activity of the small workers of melanic group VI. Seasonal cycles of average worker size may be expected among those workers of low activity groups if there are consistent size differences among workers produced from dormant and non-dormant larvae. The uniform distribution throughout activity samples 3 to 15 of the thirty largest workers of melanic group V was equally striking, and gave the appearance, in samples 14 and 15, that larvae were reared by numerous small, pale workers, among which were a few darker workers of much larger size.

3.F. Discussion of Section 3.A-E.

Experiments have shown that the bases of segregation (section 3.A) were justified. Division of labour has been correlated with age and physiology (melanisation) (sections 3.B,D). Older workers have a higher foraging potential than younger workers which tend to stay on or near the brood. These observations are in agreement with those of Ehrhardt (1931) on Myrmica laevinodis.

Variation in worker activity and behaviour within certain melanic groups has also been noted in experiment 4. Two possible explanations of this are discussed below.

1. Activity and behaviour may vary with the number of workers of each melanic group present in one colony fragment.
2. Worker-duty may be altered by the qualitative composition of the colony fragment through worker competition or other means.

Such effects as the change in locomotor activity by workers of group VI (p.29) may be interpreted on the basis of either explanation, as may the ethological changes shown by these workers (pp.25-27). Differences in the behaviour of workers of melanic group IV are difficult to explain completely except in terms of variation in worker numbers (p.30). It is also apparent that locomotor activity by workers of group II is dependent solely on the numbers of that group present. It appears/

appears that workers of group II will always show locomotor activity irrespective of the needs of the colony. Further, workers of group VI always show a tendency to get on top of the brood mass, again, apparently irrespective of the numbers already there. In the absence of workers of groups II and IV, however, workers of group VI can assume all three locational duties concerned, irrespective of their relative efficiency in these duties.

Such ethal plasticity may only be interpreted in terms of a system of dynamic worker=duty-preference. Any such system of dynamic worker-duty-preference is related, in some cases, to factors such as the total size of the brood mass, which may be limiting. This will inevitably result in the incidence of quantitative effects. Therefore the two possible causes of variation noted above are not mutually exclusive and may well be interdependent.

Duty preferences can be expressed as the percentage time spent in any "occupation", since all workers appear capable of participating in any "duty" at any time. Workers of melanic groups I, II and III, appear to have a preference for locomotor activity. These workers may be considered as "foragers" since high locomotor activity outside the brood chamber automatically increases the chances of contact with potential environmental food (p.9). Workers of groups IV and

V/

V (i.e. those shown to stand around the brood mass, and also to have a low degree of locomotor activity) show a preference for nest construction (experiment 5, section 3.D above). It should be noted that in experiment 4 there was no "outlet" for the energy of workers preferring to construct nests (p.20). Workers standing around the brood mass in laboratory nests may well represent an unemployed group which would prefer to build nests, and may be characteristic only of laboratory conditions. These groups may be designated "domestics".

Finally workers of melanic group VI have been demonstrated to have a preference for standing on the larval brood mass (experiment 4), and may be considered as "nurses". These conclusions are incorporated in table X.

It should be noted that workers of melanic group IV show, in excessive nest construction, the same occupational "exuberance" shown by workers of group II in excessive foraging, and workers of group VI in standing on top of the brood mass. It appears that the activity and behaviour of individuals of these worker groups when engaged in their preferential occupation is a factor of the total number present. In the absence of workers of certain other melanic groups ethological plasticity is apparent, but the differential behaviour shown may not then be dependent on worker number.

Finally/

TABLE X

Worker Melanic Groups

	MELANIC GROUPS VI and V	MELANIC GROUPS IV and III	MELANIC GROUPS II and I
Time of pupation on the basis of constant melanisation rates	Workers produced during current season Year (x)	Workers produced during previous season Year (x-1)	Workers possibly produced two seasons previously Year (x-2)
Preferential Duty	On brood mass (Nurses)	Nest Construction (Domestics)	Locomotor Activity (Foragers)

Group III is anomalous, since in the initial separation it shows the characteristics of foragers. This is discussed below (p.58), as is the problem of the time of origin of workers of groups I and II.

Finally, it may be suggested that the location in the laboratory of a proportion of workers of melanic group IV round the brood mass, indicates that this middle-aged group, potentially the most plastic ethological group in the nest, represents the mobile worker reserve of the colony which can be changed from one duty to another according to the needs of the colony. This provides some explanation of observations such as those of Eidmann(1927) on foraging recruitment in M. laevinodis. The presence in the colony of a small number of workers of permanent high locomotor activity which, on the detection of suitable food, stimulated large numbers of unemployed workers in the nest and enabled these latter to find their way to the food by a scent trail, would provide a very efficient mechanism of forager recruitment without excessive and wasteful energy expenditure.

Theoretical worker-duty-preferences have been utilised (Ribbands, 1953) to explain the time sequence of worker duty in Apis colonies (Rosch, 1925; Lindauer, 1952). Similar duty-sequence conditions have been reported in Polistes (Steiner, 1932), and in various ants by Forel (1874), Buckingham (1910) and Heyde (1924). The normal duty sequence in these ants follows that described by Ehrhardt (1931) in Myrmica laevinodis and reinvestigated by the present author in Myrmica scabrinodis (above) and in Myrmica rubra (section 4 below).

Such/

Such widespread occurrence of this social mechanism justifies usage of the word polyethism to describe, in characteristically monomorphic species, the condition of worker-duty-preference alteration with age, accompanied by the retention of a high degree of ethological plasticity.

The association of such systems of worker polyethism with monomorphism has been indicated by Ribbands (1953) and Schneirla (1953, in Roeder, 1953) among others. While the investigations described in section 3.E above show that M. scabrinodis is a characteristically monomorphic species, there are size differences between the melanic groups concerned. Noteworthy is the association of larger individuals with nest construction. The occurrence, in polymorphic species, of phragmotic majors is well known (Camponotus [Colobopsis] Wheeler, 1910; Forel, 1921; and Creighton, 1954), as is the association of large workers with nest construction (Oecophylla, Doflein, 1905).

The time of origin of melanic groups I and II is doubtful. Brian (1951b) considers maximal imaginal worker life to be two years in M. laevinodis. The problem of melanisational causality then arises. There are two possibilities. Does increased locomotor activity cause increased melanisation, or does melanic change accompany ethological change depending solely on worker age. Considering the latter possibility, if constant rates of melanisation/

melanisation of the M. scabrinodis workers are assumed (p.55), the time of origin of workers of melanic groups I and II may be three years previous to the time of experimentation (Table X). But if the evidence of the size analysis of workers (section 3.E, p.49 above) is accepted as showing that melanic group IV repre-:sents workers produced from the dormant larvae of the preceding year, then group III must represent workers derived from non--dormant larvae. Further, acceptance of this hypothesis implies that during a period of two years these two worker groups have reversed their original melanic differences as determined by the time of pupation, and that small workers derived from non--dormant larvae have a higher rate of cuticular melanisation compared with workers derived from non-dormant larvae. In addition, the rate of change of functional preference would have to be faster in non-dormant workers (group III). Such effects would be consistent with a continuation into imaginal life of differing rates of tissue differentiation established in the larva, since ethological change in the worker may well be connected with temporal changes in the nervous system.

If constant melanisation rates do occur, then workers of groups I and II may be three years old. But if individual activity is responsible, groups I and II may represent a few individual workers of any age group which, for unknown reasons, show inherent high locomotor/

locomotor activity, and so have become strongly melanised foragers. In this connection it is of interest to note that melanisation may be affected by cestode parasitisation (Muir, 1954). Again, however, high locomotor activity accompanied by strong melanisation may expose certain individuals to a greater risk of infection. It remains possible that melanisation reflects an ineffective disposal of residual metabolic waste, and may therefore result from a drain on anabolised material caused by such factors as continued oviposition, parasitisation or high locomotor activity. Since worker oviposition utilises fat body, it is conceivable that there exists in the imago physiological competition between organ systems, comparable to that which may be supposed to occur in the larva (i.e. between the imaginal rudiments and the larval fat body), where it is controlled to some extent by the retrocerebral endocrine system. Also, it seems probable that the imaginal cuticle acts as a dumping ground for waste material.

Finally, the significance of periodic worker recruitment must be considered. New workers are produced in two brood batches during the summer. The first consists of workers produced from dormant larvae, the second of workers from non-dormant larvae. Observation in other colony fragments of M. scabrinodis shows that both groups of recently emerged callow workers (three weeks old) show a strong preference for brood rearing/

rearing. (The first fourteen days of imaginal life are also spent on the brood mass but workers may not contribute actively to brood rearing during this period.) The possibility of seasonal cycles of size variation among nurses has been indicated. Further, the sudden influx of a group of workers into an occupation representing one end of a dynamic preferential system cannot fail to influence the numbers of workers engaged on the other occupations if some social mechanism like worker competition for duty preferences is present, i.e. an influx of nurses will cause a corresponding though smaller increase in the number of domestics, and this in turn may affect the numbers of foragers. Such effects on worker foraging would be independent of the foraging activity of melanic groups at the extreme end of the worker ethological scale (i.e. the confirmed and preferential foragers), but would increase the percentage time spent in foraging by plastic, middle-aged workers.

Thus social periodicity might be initiated by the temporal separation of brood masses. The causes of periodicity in the production of larvae are considered elsewhere (Paper II of this thesis). Cycles of queen oviposition, larval growth and foraging activity in sub-tropical conditions have been extensively described in Eciton by Schneirla (1953, and previous papers). The hypothesis outlined above supplies an interesting/

interesting comparison with the sub-tropical work of Schneirla, and shows how adverse microthermal conditions, necessitating the overwintering of larvae, may in this case initiate social periodicity.

Comparison of the activity measurements with the results of the brood rearing analyses are disappointing. It appears that much of the locomotor activity shown by the workers is excessive (p.44), compared with the growth shown by the appropriate larval groups. One experimental difficulty arises from the provision in all these nests of an abundance of environmental food, easily accessible even to workers of low locomotor activity. Results described elsewhere by the present author and Brian (personal communication) show that compared with conditions in the field there is an excessive food flux in these nests as measured by the quantitative development of the larval fat body and the gut. The effects of differential worker activity, probably important in nature, will tend to be obscured in the laboratory. Experiments on these lines of investigation using colonies of M. rubra microgyna are described in section 4 of this paper.

While abundant environmental food near the brood chamber has masked the differential effects on larval growth which might be expected in nature from nests containing these proportions of worker types, the importance of worker-laid

eggs/

eggs and worker glandular secretions as larval food must not be overlooked. While larval growth may be augmented by extra-sociological (environmental or allochthonous) food procured in certain cultures by workers of group II, it may be augmented to an equivalent extent by sociological (autochthonous) food (e.g. eggs or glandular secretions) if these are produced by workers of group VI. Such differences in egg production are described in section 4.E below. If such a dual origin of larval food causes differences in larval growth these may be qualitative, not quantitative, and will be affected by changes in the proportions of worker types present. There can be little doubt that the polyethal worker conditions here demonstrated have critical effects on larval growth and development in nature.

4. CONDITIONS IN COLONIES OF M. RUBRA MICROGYNA

A. Worker Segregation.

A typical segregation is described below. This form of segregation has been carried out, with minor variations, on numerous nests of M. rubra.

An early serotinal colony of M. rubra microgyna from the field was placed in a large container in the laboratory. Dormant larvae were being produced and were in the early third instar. The colony, containing about five hundred workers, was cultured with full food at 25°C for several days prior to segregation. It was not possible to distinguish the six melanic groups observed in the colony of M. scabrinodis (section 3.A above). The workers were therefore segregated by rapid removal with sucking tubes into the three ethological worker types noted above (section 3.F) and described by Ehrhardt (1931) in M.laevinodis. These are:-

1. Workers showing high locomotor activity (here designated F-type workers),

2. Workers standing near the brood mass (here designated D-type workers),

3. Workers standing on the brood mass (here designated N-type workers).

The/

The present section attempts to show that F-type workers are characteristic "foragers", that D-type workers are "domestics" and that N-type workers are "nurses", these three terms being used in a purely descriptive sense. Initially, these worker types are referred to as belonging to group F, D or N.

After the initial separation of the workers each group was reseparated after twenty-four hours, and only those in the same category at both separations were used experimentally, forty percent being discarded. For example, workers which (in terms of the results on M. scabrinodis) at the first separation "preferred" or were sociologically "forced" by worker competition to stand near the brood mass during the first separation, were placed by themselves, with ample larvae, and resegregated after twenty-four hours. Only those workers which were then standing round the brood mass were used experimentally, others, which had shown a change of duty, being discarded.

It is presumed (for the purpose of segregation) that a mechanism of dynamic worker-duty-preference comparable to that described in M. scabrinodis is operative in M. rubra microgyna. The functional plasticity implied by such a system is, in fact, observed among the forty percent of workers which are discarded. The/

The experimental results described below are concerned with this functional plasticity.

While this separation lacked the delicacy and completeness of that undertaken on M. scabrinodis it was, nevertheless, ethologically thorough. Melanic differences could be detected but the attempted subdivision of the three ethological types into discontinuous melanic groups was unsuccessful. Callow workers (i.e. workers produced during the current season) could be completely removed from the three ethological groups by their lighter cuticular melanisation, but older workers showed a continuous range of melanisation from dark brown to light brown. Removal of callow workers provided a series of callow ethological types (i.e. callow foragers, callow domestics and callow nurses), although very few callow foragers were encountered. The possible significance attributable to the differentiation of callow workers into ethal types is considered below (p.103). In order to render the ethological separation as efficient as possible, three arbitrary shades of brown were selected among workers from the previous season to represent the dominant shade of each ethal type [light brown for N-type workers, brown for D-type workers and dark brown for F-type workers], and any workers of strikingly different melanisation were discarded from the three types. About ten percent of the initial number of workers in the colony were discarded in this way.

The/

The implications of the methods of separation used on the colony of M. scabrinodis have been discussed previously (p.9). Reference should be made to this discussion, bearing in mind that the present segregation has been made on the basis of:-

1. High locomotor activity as opposed to low locomotor activity.
2. Ethal differences in the presence of larvae.
3. Segregation of arbitrary melanic groups.

4.B. Worker Brood Rearing and Areal Relationships of the Brood Mass.

In the investigation of differential growth and development of larvae in groups, measurement of the degree of discriminate biased feeding (Brian, 1951c) is of utmost importance (experiment 4, section 3.C above). This is considered further in experiment 6 below.

Experiment 6.

In each culture twenty-five workers of different ethal types, as described above (p.63), were cultured with forty-eight larvae. An ethological separation of both callow workers and overwintered workers was undertaken, the nest being in a serotinal condition. The experimental design is shown in table XI below where w = workers, and l = larvae:-

TABLE XI

	N-type Workers	D-type Workers	F-type Workers
Overwintered workers [Experimental designation = Series A]	25 w 48 l N	25 w 48 l D	25 w 48 l F
Overwintered workers [Experimental designation = Series B]	25 w 48 l N	25 w 48 l D	25 w 48 l F
Callow workers [Experimental designation = Series B]	25 w 48 l NN	25 w 48 l ND	25 w 48 l NF

Nests of special construction (figure 10) were used, these having been originally designed by the present author in another connection. These nests provided a perfectly flat surface for larval brood rearing. It is probable that the degree of biased feeding in a larval brood mass may be significantly influenced by nest structure and resultant brood piling by workers. Where ten larvae are used per colony fragment (experiment 4) the problem of brood piling may not arise, but where forty-eight larvae are present standardisation of nest conditions with regard to brood piling is essential. In this connection, the significance of the nest design is as follows. The use of lights above the nest (figure 10) for twenty-four hours after the larvae and workers are placed in the nest, allows central "fixation" of the workers and brood mass, the use of the lights then being discontinued. Experiments have shown that workers avoid:-

- Excessive light,
- Dry conditions,
- Waxed surfaces.

These are all combined in the nest to overcome the tendency for workers to place the larvae in the corners of normal plaster nests.

Central "fixation" was difficult in the case of D-type workers and centralisation was only achieved after twenty-four hours/

hours in nests containing overwintered workers. The use of numerous spotlights and other lamps failed to make the callow domestics assume a central position. After twenty-four hours continuous light treatment, combined with three changes of nest, this culture was abandoned as intractable. The experiment was carried out in a thermostatically controlled hot room at a temperature of 25°C. All cultures were supplied with a full food diet.

The frequency distribution in each nest of the lengths of all larvae used was recorded initially. The results of the brood rearing analysis are shown as histograms in figure 11. All larvae over thirty units in length are undergoing development and metamorphosis. It is apparent that all worker ethal types , even in serotinal condition, are capable of producing some non-dormant larvae. There is no single ethal worker type present in the nest at this time of year which is solely responsible for the induction of larval dormancy. There are, however, significant differences ($P = <5\% >1\%$) between ethal worker types with regard to relative brood rearing success. (Table XII).

TABLE XII/

TABLE XII

The number of larvae metamorphosing after sixteen days in experiment 6.

	N-type Workers	D-type Workers	F-type Workers
Series A	12	3	8
Series B	15	4	13
Callows	10	Discarded as Intractable	9

To verify the low results in D-type cultures, and confirm the initial random allocation of larvae, another experiment (7) was undertaken. Workers used in cultures of series B of experiment 6 were used again in similar nests, which were subjected to similar experiment treatment as in experiment 6. All larvae used in experiment 7 were taken from nests of experiment 6 and all were under twenty units in length (i.e. they had not started to develop). These larvae, largely derived from D-type cultures, were rerandomised, remeasured and the new frequency distribution of larval length recorded.

Smaller/

Smaller numbers of larvae (twenty larvae) and eighteen workers in each nest showed better growth than in experiment 6. Results after twelve days were as shown in table XIII.

TABLE XIII

Number of larvae metamorphosing after twelve days in experiment 7.

N-type Workers	D-type Workers	F-type Workers
10	2	9

These confirm the previous result, and show that D-type workers are particularly inefficient in brood rearing under these conditions. Also they show that no physiological diapause has been induced in these larvae during the previous fourteen days.

Further information can be derived from the frequency distribution of larval length after these experiments (figures 11 and 12). It can be seen that larvae in culture D of each series are concentrated in smaller size frequencies compared with

with larvae in other cultures which show a complete range from large to small, and an even distribution throughout this range. The latter type of frequency distribution, characteristic of the N and F cultures, shows that a large amount of biased feeding has occurred. This bias has been both strongly marked (in the largest larvae) and widespread (many larvae showing increased sizes). Bias in cultures of the D group is however restricted to a very few larvae, although in these larvae it is almost as strongly marked as in other cultures. Heavy larval mortality in all cultures due to serotinal workers and the exigencies of nest design is shown in table XIV.

TABLE XIV

% Larval mortality in experiments 6 and 7

	N-type Workers	D-type Workers	F-type Workers
Experiment 6			
Series A	52	42	44
Series B	48	50	42
Callows	62	-	44
Experiment 7	25	35	35

There is lower percentage mortality in experiment 7 due perhaps to the higher worker/larva ratio, but there is no differential mortality between worker groups. The biased larval growth has therefore not been affected by the absolute size of the larval group within the range occurring. Variation in the degree of biased feeding must then be due solely to the worker ethal types.

The mechanism controlling the administration of such varying degrees of bias to the larval groups has been further investigated as follows. Repeated observations were made on the nests of series A and B of experiment 6. When the position of the brood mass had been fixed, by light, on the plaster of paris, the brood mass was photographed on this relatively small surface. A cine-camera was arranged at predetermined focus and placed immediately above the nest. The camera was then started, the cover of the nest removed, and photographic lights switched on for a period of a few seconds. This was sufficient to record the area of the larval group and the area occupied by the workers which surrounded it. It was impossible to measure directly the focus during these experiments and slight differences in the height of the plaster of paris caused an appreciable decrease in the quality of the negative. The presence of a glass cover over the nests, and the high degree of enlargement required from the small sixteen millimetre negatives,/

negatives, precluded the possibility of making accurate measure-:ments of the number of workers on top of the brood mass. In certain nests under certain conditions workers were standing on top of each other over the brood mass. Measurement of anything except the areas of workers and larvae then became impossible.

The experimental distinction between dim light and darkness was achieved throughout these observations. The first was derived from the light of one sixty watt lamp at a distance of six feet above the nests, shining onto the waxed perimeter of the arena in which the ants were kept. The ants were shaded from the light by a circle of black paper about six inches in diameter which was placed over the centre of the circular cover of the nest. Only a very dim light penetrated to the ants. Secondly, darkness was achieved by placing large sealed boxes over all the nests undergoing this treatment. For photography, the room was completely darkened and, by red torchlight, the box was removed from each nest in turn, the camera placed over each nest at a pre-arranged distance, and the black cover, if present, also removed. The camera was then started and the photographic lights switched on. A series of exposures was made throughout the period of fifteen days that the experiment was in progress, and the particular series of nests undergoing each lighting treatment was varied systematically. The following factors could then be analysed statistically by the analysis/

analysis of variance (Snedecor, 1946).

1. The area occupied by workers of the three types F, D and N.

2. The area occupied by the workers under the two different lighting conditions.

3. The area occupied by the workers as individual larval growth increased the bulk of the larval group in the course of the experiment.

These three factors and their interactions are shown in the analysis in table XV. A similar analysis with respect to larval area is shown in table XVI.

From tables XV and XVI (see below) it is apparent that the ethal worker type exerts a significant effect on both worker and larval group areas. This effect is shown graphically in figures 13 and 14 which show the average values for both series at five successive times. The point P on the graph in figure 13 indicates that a number of workers escaped from their container at about this time, with the consequent areal reduction shown. These workers were replaced by others of the same type at point Q. In both cases the F-type area is significantly larger than the N and D areas. There is (as might be expected) a significant change in the larval area in the course of time, but there is, surprisingly, no significant interaction between worker type and time, on larval area. The effect/

TABLE XV

Worker areas - analysis of variance.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F	P (PROBABILITY OF OCCURRENCE)
Worker type	2	168,978	84,849	30.390	< .1%
Light	1	37,288	37,288	13.355	< 5% > 1%
Time	3	11,882	3,960	1.418	> 5%
Workers × Light	2	17,991	8,995	3.222	> 5%
Workers × Time	6	15,556	2,594	.929	> 5%
Light × Time	3	15,274	5,091	1.823	> 5%
Workers × Light × Time	6	16,755	2,792		
Total	23	283,724			

TABLE XVI

Larval areas - analysis of variance.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F	P (PROBABILITY OF OCCURRENCE)
Worker Type	2	1,054	527	8.405	< 5% > 1%
Light	1	267	267	4.258	> 5%
Time	3	1,256	414	6.602	< 5% > 1%
Workers × Light	2	368	184	2.934	> 5%
Workers × Time	6	752	125	1.993	> 5%
Light × Time	3	123	41	0.653	> 5%
Workers x Light x Time	6	376	62.7		
Total	23	4,196			

effect of light is significant only with regard to the worker area. The presence of a dim light significantly reduces the "spread" of workers over the plaster of paris and causes a build up in the numbers of workers actually on top of the brood mass, i.e. there is, in dim light, a significant increase in worker density on the brood mass.

While it is desirable to attempt some correlation of these areal relationships with differences in the distribution of larval bias, such correlation is not obvious. If there is no circulation of larvae in the brood mass, only those larvae lying on top will be fed. In such conditions, the extent of larval bias, i.e. the number of larvae showing growth, will be a factor of the surface area of the brood mass. While results from cultures of D-type and F-type workers are in accordance with what might be expected, the extent of larval bias is unduly large in cultures of N-type workers. Observation suggests that there is larval circulation in cultures of N-type workers.

Further experiments on the behaviour of these worker types while on the brood mass were unsuccessful. Observation, by mirrors, of the lower surface of the brood mass allowed accurate measurement of the degree of larval circulation in the brood mass. The light necessary for such observations, introduced the differential worker reactions noted above, with the result that differing worker densities were observed on top of the brood mass. Further experiments were abandoned.

4.C. Worker Reaction to Isolated Larvae.

The relative attractiveness of larvae of varying sizes to these workers was shown as follows (experiment 8). Nine larvae, three large, three medium and three small, were stuck with paraffin wax on to a sheet of filter paper, along the circumference of a circle of four inches diameter. Larvae were arranged in threes so that no two larvae of the same size were ever together. Ten workers of each ethal type from the same colony as the larvae were then released from a central container and the number "attracted" by the larvae during the following seventy-five seconds was counted. For each ethal type, three sets (each of ten workers) were used. All larvae remained alive throughout the experiment. Total results are shown in table XVII. Significant differences are attributable to the three larval sizes, and to F-type workers as opposed to N- and D-type workers. Surprisingly perhaps, workers which spend most time on the brood mass are least sensitive to the presence of larvae, while workers of high locomotor activity rapidly detect such larvae.

TABLE XVII

Total Number of Workers Attracted by the Larvae in Experiment 8.

	F-type Workers	D-type Workers	N-type Workers
Large Larvae	70	21	27
Medium Larvae	53	12	19
Small Larvae	21	6	0

4.D. Worker Foraging Potential and Survival.

Differences in the foraging potential of the three worker ethal types are probably connected with differences in their aggressiveness or "killing power", which have been investigated in nine experiments. These will not be described in detail.

In five of these experiments, special culture tubes were used which did not contain a wet cotton wool plug. The water supply was provided by a fine tube with a cotton wool plug in the end which siphoned water to the level of the tube. Workers could reach this plug and drink from it. Air circulation was also achieved by a fine capillary tube plugged with cotton wool. A fixed number of workers and larvae were placed in these tubes along with a fixed number of insect larvae of other kinds, and the tube sealed with a wax cork for several hours. The number of the other insect larvae killed was then found.

Results of these five experiments showed that all worker types could kill a high proportion of the Drosophila larvae administered. Only when excessive numbers of Drosophila were administered could any conclusive differences be established. F-type workers then killed many more Drosophila larvae than were necessary for food consumption.

The use of blowfly larvae (Lucilia and Calliphora) of different sizes did not result immediately in any differential killing./

killing. But if the blowfly larvae were removed after some hours in the ant nest and reared for several days, significant differential mortalities resulted (e.g. no blowfly larvae from F-type cultures survived more than twenty-four hours, while some blowfly larvae from D-type cultures and all blowfly larvae from N-type cultures survived apparently unharmed). Stings and bites on the blowfly larvae could be counted and these showed a significantly higher number of attacks by F-type workers.

The death rate of F-type workers was significantly high in these experiments, after culture for twenty-four hours with large blowfly larvae. This appeared to be due to "exhaustion" following repeated and prolonged attacks on the blowflies. This"worker exhaustion" condition was recognised by the workers being stationary for long periods, showing spasmodic twitching of legs, abdomen and head, and by what appeared to be a lack of muscular tone, in legs, thorax and abdomen.

The relative survival under adverse conditions of these three types of workers was examined in experiment 9. A colony containing about 500 workers in aestival condition was separated into types F, D and N. The D group was subdivided into two sections depending on whether they resembled type F [i.e. D(F)] or type N [i.e. D(N)]. These four worker groups were then cultured in plaster of paris nests (Brian, 1951a) at 25°C, without food, but with water, for thirty-four days.

Dead/

Dead bodies were removed each week. The original numbers in each culture, the number of survivors and the percentage survival is shown in table XVIII.

Table XVIII

Worker survival in experiment 9

	Worker Type			
	F	D(F)	D(N)	N
Initial number of Workers	83	99	120	185
Number of Survivors	5	6	53	165
% Survival	6%	6.1%	44.2%	89.2%

It is apparent from table XVIII that the expectation of life under these conditions is high in group N, and moderate in D(N), and very low in D(F) and F. Among the possible explanations of such a result are:-

1. The presence of a larger fat body in N-type workers.

2. Lower locomotor activity in N-type workers causing/

causing smaller consumption of the fat body compared with high energy utilisation of F-type workers (assuming comparable quantities of reserve food are present in both these types of worker).

It is noteworthy that this differential mortality has occurred even in the absence of insect prey, so "worker exhaustion" as described above cannot be held responsible. Observation on these nests showed that despite the higher worker numbers in the N-type culture, these workers were all grouped in the wet chamber, while F-type workers were distributed uniformly throughout the nest, and other cultures showed intermediate distributions.

Estimations of the total nitrogen content of these three worker types were made, using the micro-kjeldahl technique of Ma and Zuazaga (1942). While significant differences were found between workers of these groups with regard to the nitrogen content of the abdomen, the full implications of these differences can only be understood by examination of individual workers. This is at present incomplete. It appears, however, that some of the seasonal differences observed are related to the exhaustion of the worker fat body in early aestival condition, and the subsequent increase in the bulk of the fat body in serotinal workers. There is therefore in some workers a seasonal cycle of exhaustion and replenishment of the fat body.

A/

A cycle has as yet only been investigated in N-type workers. The results of the nitrogen analysis are shown in table XIX. Only the abdomen was analysed. Table XX shows the averaged percentage water and nitrogen compositions of six groups of workers each comprising four groups of ten workers. The result of the equivalent nitrogen estimation on twenty recently emerged workers (twenty days old at 25°C) is also shown. While percentage values are shown in table XX, the absolute values may have greater biological significance. Some of these are shown in table XIX. The present work shows that there are consistent chemical differences between certain of these worker groups. The biological importance of these observations will be considered elsewhere.

It should be realised that cuticular nitrogen is also estimated and may be the source of much of the nitrogen estimated. If cuticular nitrogen is a constant by comparison with the variation in nitrogen in internal organs, then the observed variation in nitrogen content may be more significant than appears here. Results as given here should however be treated with reserve since size differences between the ethal groups (as demonstrated in section 4.F below), must inevitably affect these results by differences in the quantity of cuticular nitrogen, and possibly by differences in the maximal bulk of the fat body.

Experiments/

TABLE XIX

The averaged values of the wet weight and dry weight of the abdomen, for ten workers of each type, in winter and summer

	AESTIVAL WORKERS		HIEMAL WORKERS	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight
F-type Workers	5.8	2.2	8.5	3.2
D-type Workers	6.7	2.2	8.3	3.1
N-type Workers	6.7	2.3	7.6	2.6
Callow Workers	7.5	2.1	-	-

TABLE XX

The % wet weight composed of water and the % dry weight composed of nitrogen, of the abdomen.

	AESTIVAL WORKERS		HEIMAL WORKERS	
	% Water	% Nitrogen	% Water	% Nitrogen
F-type Workers	63.2	10.0	62.1	9.4
D-type Workers	67.5	9.2	62.5	8.8
N-type Workers	66.0	8.9	65.4	9.8
Callow Workers	72.5	12.9		

Experiments have been undertaken to measure the food preferences of these three worker types throughout the year. These were unsuccessful. Accurate measurements were made over a period of days of the amount of sugar solution ingested in a series of vernal worker cultures. Feeding was sporadic, reaching a peak between the third and eighth days, and observa-:tion showed that it was largely due to individual workers in each culture. Dye marking of sugar showed that individual workers could retain and redistribute this sugar to other workers for periods of up to nine days after feeding (at 25°C). These "sugar reservoirs" may be supposed to have masked differences in the sugar consumption of the three worker types during the period of investigation.

Similar experiments on the measurement of protein consumption were still more markedly unsuccessful. Crushed blowfly (Calliphora)fat body was used to provide a uniform natural insect protein supply. This was largely refused by workers of all kinds, even under starvation conditions. Certain of these workers readily attacked and killed live blowfly larvae (Calliphora) when placed in the same containers. It seems that the crushing of the blowfly fat body rendered it much less attractive (or recognisable) to workers. It is possible that stimuli (possibly scent) from living or recently dead insects are necessary before workers will feed on them.

4.E. Worker Oviposition.

Brian (1953b) has investigated worker oviposition in Myrmica. He concludes that eggs are laid by all workers. The present experiments amplify Brian's observations in respect of worker polyethism and the utilisation of anaesthetics. Three experiments are described below of which 10 and 11 have been described more fully in paper II of this thesis (as experiments VI and IV respectively). In these two experiments, unlike those of Brian (1953b), anaesthetics were not employed at any time.

Experiment 10: This comprised three groups, each of thirty-five workers of types N, D and F. The results of weekly egg censuses (the egg input) during a period of five weeks is shown in figure 15. Type N has an egg input maximum during the second and third weeks. This maximum is greater than that of either type F or D, which achieve a maximum about the third week.

Experiment 11: This comprised four sets of cultures of each of the three worker types (F, D and N). The four consisted of two, five, ten and twenty workers. The egg input from all cultures during the first week was nil, but during the second week the egg input of cultures of N-type workers showed a regression on worker number. [$e = 3.515w - 1.014$ where (as in Brian, 1953b) e = number of eggs, and w = number of workers. Standard error of the regression coefficient being .014 eggs per worker]. Egg input during this week in cultures of types/

types F and D was sporadic. If 80% efficiency of separation of the worker types is claimed, oviposition in F and D cultures is explicable on the basis of the inclusion by chance of 20% N type workers in these cultures. If this is the case then the egg input of N-type cultures represents only 80% of the maximal theoretical value.

Experiment 12: This consisted of five groups, each of four cultures, each culture containing ten workers. The workers had been hibernated at 10°C immediately prior to the experiment. Of the four cultures in each group, one consisted of F-type workers, two of D-type workers and one of N-type workers. The weekly egg input of each culture during seven weeks is shown in tables XXI and XXII. Four of the five groups of cultures were treated each with one of four anaesthetics, the fifth acting as a control. Anaesthetics used were carbon dioxide, nitrogen, nitrous oxide and compressed air saturated with ether. The control cultures were subjected to a stream of compressed air for ten minutes every day. In the case of carbon dioxide, nitrogen, and nitrous oxide, each culture was anaesthetised for five minutes each day, from the moment that all the workers in the culture had been immobilised. In the case of ether, cultures were subjected to the gas mixture for only one minute after the immobilisation of the workers. This was sufficient to keep the workers narcotised or partially narcotised for twenty minutes after removal of the gas source.

TABLE XXI

Egg production in experiment 12.

	ANAESTHETIC USED															
	Carbon Dioxide				Nitrogen				Nitrous Oxide				Control Compressed Air			
Worker Type	F	D	D	N	F	D	D	N	F	D	D	N	F	D	D	N
Week 1										3	4	6				
2									4	4	8	10				
3							3	3	3	12	13	17		6	9	12
4					5	24	-*	29	2	14	12	27	25	32	33	33
5		17	13		7	23	--	20		21	17	34	13	27	23	30
6		9	16		6	13	--	21	5	9	14	30	17	16	9	21
7					2	****	-	5		7	15	12	14			6
Total egg Production		26	29		20	60	3	78	14	70	83	136	69	81	74	102
Worker Survival	8	6	4	3	3	-	-	5	7	5	9	6	9	7	7	9

* Culture accidentally destroyed.

** No workers alive after this week.

TABLE XXII

Egg production in experiment 12 disregarding anaesthetic used

	WORKER TYPE			
	F	D	D	N
Week 1	-	3	4	6
2	4	4	8	10
3	3	18	25	32
4	32	70	45	89
5	20	88	53	84
6	28	47	39	72
Week 7	16	7	15	23
Totals	103	237	189*	316

* One culture lost.

The results of experiment 12 show that in no case was worker oviposition and worker survival comparable on all grounds to that of the controls. The four groups present a graded series in respect of both percentage worker mortality after seven weeks (table XXIII) and total egg input during the same period (table XXIV).

TABLE XXIII

Worker mortality in experiment 12.

Anaesthetic	Ether	Nitrogen*	Carbon Dioxide	Nitrous Oxide	Control
% Worker Mortality	100%	73%*	47%	33%	20%

* One culture of type D was accidentally destroyed, but this has been allowed for.

TABLE XXIV

Total egg input during experiment 12

Anaesthetic	Ether	Carbon Dioxide	Nitrogen	Nitrous Oxide	Control
Total egg Egg	Nil	55	161	303	326

Separation of the workers into three types F, D and N, reduced the variability attributable to worker egg production. Workers of type F showed a consistently lower egg input compared with those of type D, which in turn was lower than that of type N. These differences could be observed in spite of the effects of the anaesthetics on both oviposition and worker survival (table XXII).

Anaesthetics magnified to some extent the differences in egg production between the three worker types. Earlier results on worker oviposition in Myrmica (Brian, 1953) may have been affected both by the lack of ethal segregation of the workers and by the use of anaesthetics in handling.

Much of the variability encountered by Brian is interpret-:able in terms of worker polyethism. For example, in Brian's results the delay in the onset of oviposition in some cultures suggests accidental partial separation of the workers into ethal groups. Similarly, the remarkable sequence of changes in the level of egg input, observed by Brian, can be explained in terms of two successive peaks of egg production. The first, derived from N-type workers merging into a second and less abrupt peak caused by F and D type workers. The greater rapidity of these changes in cultures of isolated worker types may be attributed to the use of a different subspecies M. rubra microgyna, as opposed to M. rubra macrogyna (or to the systematic/

systematic fragmentation in the present experiments of the integrated worker components of the colony).

Conclusions with regard to polyethism and worker oviposition are:-

a. All experiments show differences between the three worker types, nurses having higher egg inputs than domestics, which may have higher inputs than foragers.

b. Differences in the time of production of eggs by these worker types show a high initial egg input peak of the N-type workers, followed by the lower egg input peak of the other types.

c. None of the anaesthetics used is suitable for extensive use if workers are expected to remain healthy and normal.

4.F. Worker Sizes.

Measurements were made on workers of M. rubra microgyna from a colony in aestival condition. Workers were segregated into ethal types F, D and N. At the time of measurement, no workers had been produced from non-dormant larvae of that season. Callow workers produced in the spring from dormant brood were however present and could be segregated from over-:wintered workers. These callows were removed from each ethal group after separation and were measured separately. Complete segregation into ethal types of all workers being impossible, only a sample from each type was measured. Sample sizes are shownin table XXV.

TABLE XXV

Numbers of workers measured, and their designation

		TIME OF PRODUCTION	
		Present Season (Callows)	Previous Seasons
ETHAL TYPE	Nurses	NN - 50	N - 50
	Domestics	ND - 30	D - 40
	Foragers	NF - 50	F - 40

Total number = 260

The measurements made were those of head length and head width (1 and 2, as for Myrmica scabrinodis, section 3.E, p.49 above). It is probable, from the results of the measurements of M. scabrinodis that head width is an adequate measure of total worker size. These measurements are shown in figures 16 and 17.

The following conclusions can be drawn.

a. There is considerable intra-nidal size variation.

b. There is no detectable allometry.

c. There is considerable overlap of the frequency distributions of the overwintered workers, but differences are apparent.

d. In the overwintered worker groups, while nurses and domestics show a complete equivalence of frequency distribution, foragers are smaller than either of these groups.

e. Differences in the frequency distributions of the sizes of callow workers are also apparent. Therefore, it appears that within a few weeks of pupation, some degree of functional differentiation, correlated with size, has been achieved.

f. Among the callow workers, foragers alone include a complete size range with small individuals. Nurses include a higher percentage of large individuals, and domestics include only these large individuals.

4.G. Discussion of Section 4.A-F.

It is concluded, from the above observations on conditions in colonies of Myrmica rubra microgyna that the segregation of workers into three ethal types is justified. Diagnostic differences between these three types of Myrmica rubra microgyna (F, D and N) may be characterised as follows:-

F-type Workers. These show:

1. High locomotor activity in the original segregation.
2. Strong cuticular melanisation.
3. Large areal spread of groups round the brood mass when by themselves.
4. High efficiency in killing blowfly larvae.
5. High mortality after culture with blowfly larvae.
6. High mortality during starvation.
7. High efficiency in the detection of isolated larvae of all sizes.
8. Low egg production.

D-type Workers. These show:

1. Low locomotor activity, standing near the brood mass in the original segregation.
2. Moderate-strong cuticular melanisation.

3./

3. Small areal spread of groups round the brood mass when by themselves.

4. Moderate efficiency in killing blowfly larvae.

5. Low mortality after culture with blowfly larvae.

6. Moderate mortality during starvation.

7. Moderate efficiency in the detection of isolated larvae of large and medium sizes, but low in the detection of small larvae.

8. Moderate-low egg production.

N-type workers. These show:

1. Low locomotor activity, standing on the brood mass in the original segregation.

2. Moderate cuticular melanisation.

3. Small areal spread of groups round the brood mass when by themselves.

4. No efficiency in killing blowfly larvae.

5. No mortality after culture with blowfly larvae.

6. Low mortality during starvation.

7. Moderate efficiency in the detection of isolated larvae of large and medium sizes, but low in the detection of small larvae.

8. High egg production.

Comparison/

Comparison of these results shows that on the basis of these eight differences alone, the worker separation into types F, D and N is justified. By analogy with the colony of Myrmica scabrinodis described earlier, types F, D and N may be designated "foragers", "domestics" and "nurses" respectively. The relation-:ship of these groups to the melanic groups demonstrated in M. scabrinodis is discussed below (p.106).

In addition to the eight differing aspects of the worker types enumerated above, there are other diagnostic features. Differences in queen-rearing efficiency between these three worker types are described in paper II of this thesis (N-type workers can be distinguished from the other types on this basis). The implications of worker size differences, and worker brood rearing differences are discussed below (pp.106, 107). Differences have been shown to exist between these three types with regard to nitrogenous composition. The latter differences are not considered further in this section, since their biological implications are obscure.

Differences in the brood rearing success of these three worker types are examined in section 4.B, experiment 6, p.67 above. It is shown that differences in the extent and distribution of biased feeding may vary with the surface area of the brood mass. The larval area in F-type cultures is large since the larvae are not piled. Many larvae show growth increases./

increases. Thus the high foraging potential of F-type workers allied to their tendency to spread the brood mass under these conditions, may cause the widespread bias observed.

Low foraging potential allied to brood piling in D-type workers may cause the opposite effect. Larvae reared by D-type workers show bias restricted to a very few larvae.

The same condition apparently prevails in cultures of N-type workers which also show low foraging potential combined with brood piling tendencies. The extensive larval bias shown by this group (and comparable to that shown by F-type workers) can be explained by the following observations:-

1. In certain nests, circulation of the larvae in the brood mass by the workers is known to occur. The factors controlling brood circulation are unknown. Such circulation results in the incessant movement of larvae from one portion of the brood mass to another so no single larva may be exposed for excessively long periods on top of the brood mass. The distribution of bias is then not controlled by the surface area of the brood mass.

2. While workers of type N show low foraging potentials similar to those of type D, they have a much higher rate of egg production compared with D-type workers. There is therefore ample larval food available in N-type worker cultures, but it may have differing qualitative effects compared with the F-type larval food. These might not be detectable in this experiment.

The apparently surprising result (Section 4.C) that F-type workers were more "attracted" to individual larvae than workers of types D or N is comprehensible if the experimental conditions are considered. Workers were subjected to stimulation both by removal from a culture tube, liberation, and the presence of a bright light. Also, in the case of N and perhaps D-type workers, there was no nest scent in the new environment. F-type workers, with higher locomotor activity may well have been repeatedly exposed to these and other stimuli, and so might be partially conditioned to ignore them. Also it may be suggested that F-type workers, possibly three years old, may be more sensitive to the presence of individual larvae. Perhaps also, one of their normal duties is the recovery and transport of individual larvae in the nest. For example, after severe nest damage, they are active in removing larvae from regions exposed to day-:light.

The significance of size differences between workers of these three ethal types lies in the possibility that size may, to some extent, control the functional preference of the worker. Such a possibility was demonstrated in the colony of M. scabrinodis (Sections 3.E,F). The size analysis of samples of workers from a colony of M. rubra microgyna (Section 4.F) shows that F-type workers cover only part of the size range of N and D-type workers, the average size of F-type workers being smaller. Among the possible explanations of this result are:-

[1]/

[1] There has been experimental discrimination of small workers for measurement from the F-sample due to unknown reasons.

[2] This size range difference is realistic and occurs among all workers in the colony. If this is the case the possible explanations can be further analysed as follows:-

i) There is differential mortality of large workers prior to or during the time of foraging.

ii) No large workers become foragers.

iii) Assuming a change of duty preference with age, initial worker size differences are accompanied by differences in the rate of change of duty preference, and, in this species, the duration of worker life is such that no large workers (with slow changes of duty preference) live long enough to become foragers.

Further light is cast on this problem by the size measure-:ments of callow workers collected within these three ethal groups, during the initial segregation of a particular colony (pp. 63, 95).

This colony was in aestival condition when sampled. The "callow" workers present in the nest (i.e. those workers which were distinguishable by their pale cuticle and which had therefore/

therefore been produced during the current season) were derived entirely from overwintered (dormant) larvae, no non-dormant larvae having pupated at the time of collection. It should be remembered that in the colony of M. scabrinodis the "corresponding" brood group of workers (melanic group V) occurred throughout the complete range of activity samples, from sample 1 to sample 15. It is not then surprising to find that callow workers of this group, i.e. M. rubra microgyna) also show a range of activity or behaviour, and may be segregated in all three ethal worker types.

The analysis of the sizes of these callow worker types shows that some size differences do in fact appear. D-type callow workers show a higher proportion of large workers than either of the other groups, while only N-type callow workers show an even complete distribution of worker size throughout the possible range. Among the possible explanations are:-

1) Non-random experimental selection of workers for measurement, due to unknown causes.

2) The partial control of worker duty preference by size differences. This latter implies that even a few weeks after pupation, the order of preference for duties other than (and subsequent to) brood rearing has already been established. (All callow workers show a preference for brood rearing, although some may be separated among F-type workers. This is comparable to the occurrence in the M. scabrinodis separation, of workers of melanic group V in activity samples 1-3).

5. GENERAL DISCUSSION AND CONCLUSIONS OF SECTIONS 2, 3, AND 4.

The experimental work undertaken in these sections shows conclusively that there is some degree of division of worker labour in both Myrmica scabrinodis and Myrmica rubra microgyna. This system is best analysed on the basis of three worker occupations.

1. Nurses
   [Workers on the brood mass.]
   [N-type workers]

2. Domestics.
   [Workers standing near the brood mass.]
   [Workers preferring to build nests]
   [D-type workers]

3. Foragers.
   [Workers showing high locomotor activity]
   [F-type workers]

These are approximately the same categories as Ehrhardt (1931) distinguished in colonies of M. laevinodis.

Detailed comparison of the conditions observed in these three species (M. scabrinodis, M. rubra, and M. laevinodis) is difficult because of the different basis of worker separation employed in each species. The degree of worker segregation achieved in/

in Myrmica laevinodis (Ehrhardt, 1931) and in M. rubra microgyna (section 4 above) is approximately comparable, but many experimental consequences of this worker separation have been demonstrated in M. rubra microgyna. This contrasts with Erhardt's work which was based largely on the observation of individual workers over long periods of time.

Worker segregation into ethal types of an entire colony of this genus has not previously been achieved, and the segrega-:tion of the colony of M. scabrinodis (section 3 above) has allowed more detailed investigation of the worker age-function relationships than has hitherto been possible. The results of this analysis show the existence of M. scabrinodis of a dynamic worker-duty-preference mechanism comparable to that known to exist in Apis. The implications of this mechanism in M. scabrinodis have been fully discussed in section 3.F above.

There remains the difficulty of reconciliation of the results achieved in these two species. The occurrence of detectable melanic differences in M. scabrinodis facilitated both complete segregation and rapid visual differentiation, while neither was possible in M. rubra. This has resulted in a different experimental approach to these two species. Only in M. scabrinodis have dynamic worker duty preferences been experimentally confirmed. Nevertheless, the fact that it was possible to segregate M. rubra colonies shows that this mechanism, with/

with its plasticity of worker function exists also in M. rubra.

It is necessary therefore to extrapolate those conclusions which were derived from experiments on M. scabrinodis and apply them to M. rubra. It seems probable that the melanic-age--activity-behaviour separation carried out on M. scabrinodis cannot be applied to M. rubra largely because of the lesser degree of melanisation shown by workers of M. rubra. This shorter worker melanic range of M. rubra in the West of Scotland may be due to worker physiological differences, worker age differences, differences of field ecology, or relative worker activity, among other possibilities. The possibility remains of minor variations between the polyethal worker systems of these two species. In any case, the fundamentals of the dynamic worker-duty-preference system do not vary significantly between the two species.

In both species there is some evidence that worker size may affect worker-duty-preference. The variation in average worker size of the two annual brood batches may therefore result in seasonal changes of "bulk" worker-duty-preference, although the dynamic nature of the system may well entirely mask such effects.

The theoretical implications of this dynamic system on myrmicine sociology have been examined partially in section 3.F above, and will be considered elsewhere in greater detail.

The/

The primary aim of this investigation, which was to examine the variation in worker activity and the relationship of this variation to differences in worker brood rearing capacity, has only been partially successful. Experimental conditions masked the relevant effects in sections 3.B,C. Quantitative effects on the distribution of bias among groups of larvae were however forthcoming both from the results of section 3.C and from section 4.B. These show that D-type workers of M. rubra are consistently unsuccessful in brood rearing, and tend to produce a few large developing larvae from any one group, this effect being due perhaps to both low oviposition rates and brood piling without circulation (the latter possibly a laboratory artifact). Both F and D type workers produce a wide range of larvae of all sizes.

In M. scabrinodis the most obvious differences were due to the bad larval growth in cultures which contained only nurses. This may be interpreted in various ways, e.g. inadequate worker hibernation. It appears that the presence of a small proportion of workers of other melanic groups produces the best results.

In both species the brood rearing success of the colony will be affected by changes in the proportions of workers of these types in the colony, e.g. the incidence of nurse dilution by inefficient domestics in M. rubra colonies will reduce/

reduce brood rearing success. Such changes in colony composition may well be seasonal, and occur in nature. The variation in worker composition of colonies throughout the year has not been investigated.

6. SUMMARY

Variation in worker locomotor activity and worker behaviour in Myrmica scabrinodis and Myrmica rubra microgyna has been experimentally investigated.

MYRMICA SCABRINODIS

1. Three worker duties are recognised. Workers carrying out these duties have been designated nurses, domestics, and foragers. Differences in locomotor activity, behaviour, brood rearing, size, and nest-building capacity have been recorded.
2. Worker-duty-preference (worker polyethism) has been demonstrated forming a dynamic system.
3. Worker-duty-preference changes with age in the sequence nurse ⟶ domestic ⟶ forager.
4. Ethologically domestics are most plastic and are preferential nest builders.
5. Cuticular melanisation increases as ethal changes occur. The significance to be attached to the rate of cuticular melanisation and to size variation between melanic groups is discussed.

MYRMICA RUBRA MICROGYNA/

MYRMICA RUBRA MICROGYNA

1. Three ethal worker groups are recognised as in M. scabrinodis. Differences between these three groups in locomotor activity, behaviour, brood rearing, larval detection, killing power, survival, chemical constitution, oviposition, and possibly size, have been described.

2. Worker ethal plasticity has been demonstrated and aspects of a dynamic worker-duty-preference organisation are detectable, despite the absence of discontinuity of melanisation between worker age groups in this species.

The significance of these observations is discussed in relation to other work of the present author and earlier work.

FIGURE 1

This shows in parts I - VI, the percentage composition of each activity sample composed of workers of the appropriate melanic group (I - VI).

The abscissa shows activity samples.

H - high activity

L - low activity

The ordinate axis shows percentage composition.

Part VII shows the total sample size in black, and the occurrence of larvae in white, as plotted against activity sample (as before).

Part VIII shows the time of removal of each activity sample in minutes, and the points of artificial stimulation (first, S_1; second, S_2) both plotted against activity sample.

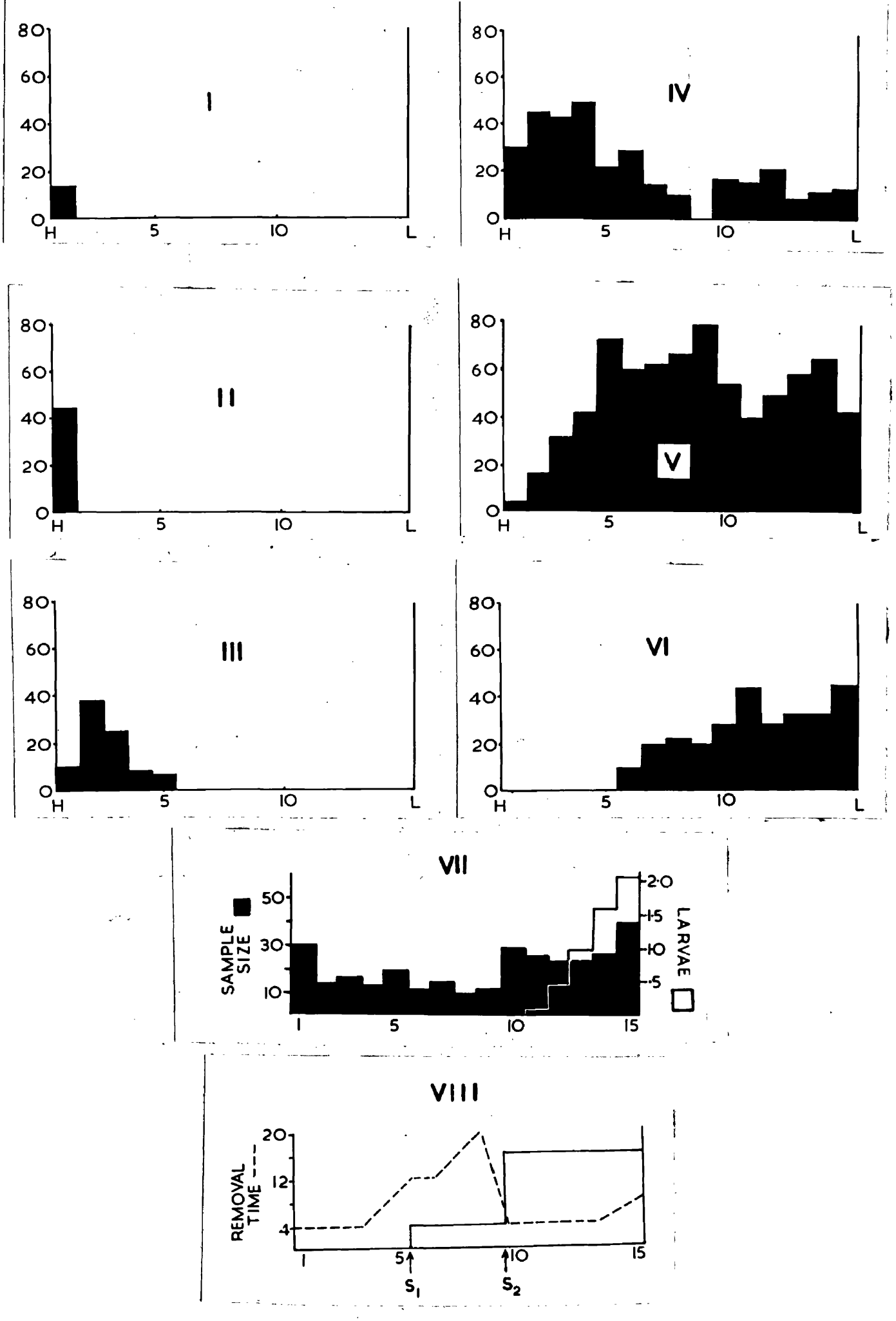
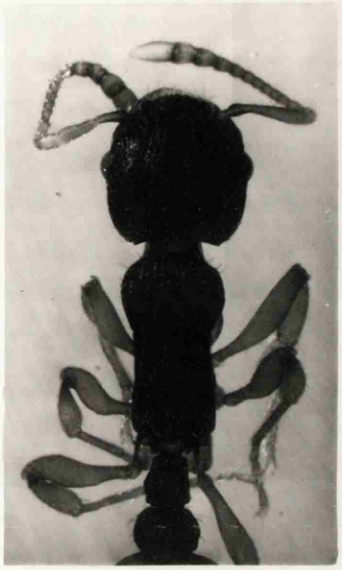

I
II
III
IV
V
VI
VII
VIII
80
60
40
20
0
H
5
10
L
SAMPLE SIZE
50
30
10
1
5
10
15
LARVAE
2·0
1·5
1·0
·5
REMOVAL TIME
20
12
4
S1
S2

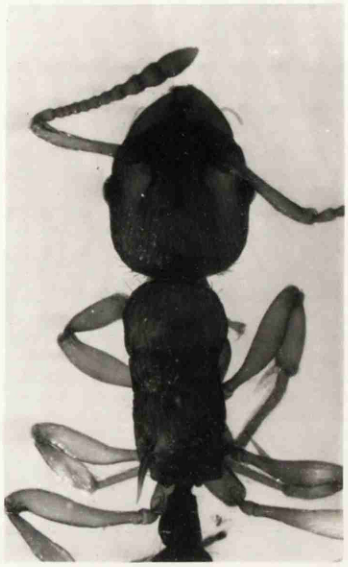
FIGURE 2 A & B

This shows the six melanic worker types. It should be noted that the antennae and legs do not show the range of cuticular melanisation shown by the cephalic and thoracic cuticle.

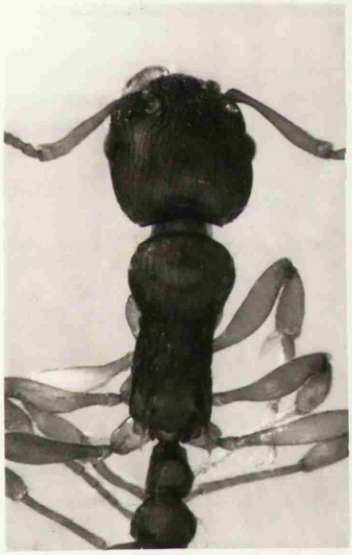


I

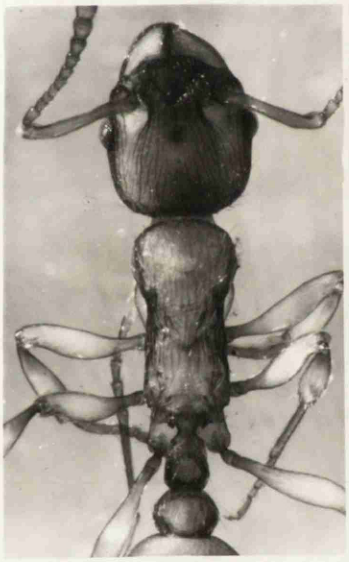
II



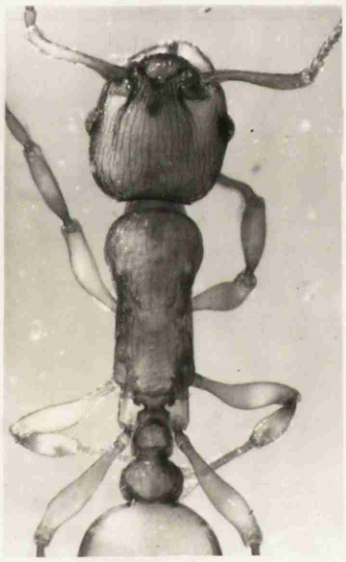
III



IV



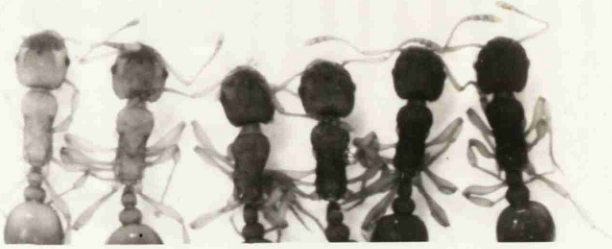
V



VI

A.

B



VI V IV III II I

Figures 3, 4 and 5, show diagrammatically the results of larval growth in experiment 4. Areal increments are given in arbitrary units.

FIGURE 3

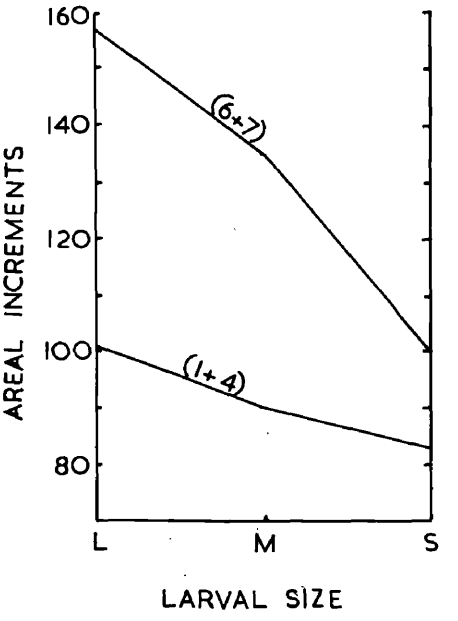
This shows the variation in areal growth increments of larvae between "good" worker nests (6+7) and "bad" worker nests (1+4) when plotted against larval size.

FIGURE 4

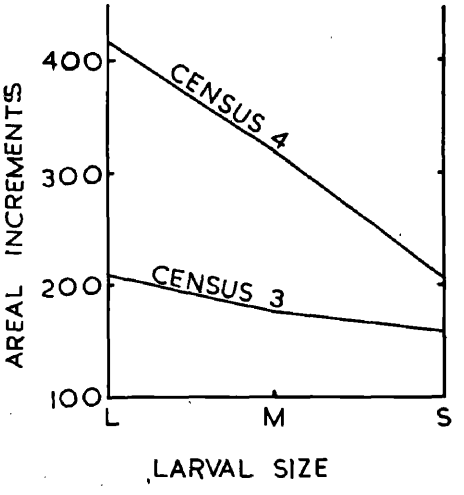
This shows the variation in areal growth increments of larvae between "good" censuses and "bad" censuses when plotted against larval size.

FIGURE 5

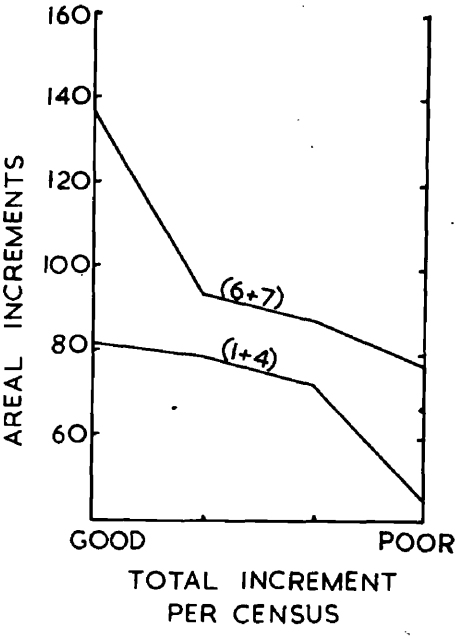
This shows the variation in areal growth increments of larvae between "good" worker nests (6+7) and "bad" worker nests (1+4) when plotted against "good" censuses and "bad" censuses.


160
140
120
100
80
AREAL INCREMENTS
(6+7)
(1+4)
L
M
S
LARVAL SIZE

3


400
300
200
100
AREAL INCREMENTS
CENSUS 4
CENSUS 3
L
M
S
LARVAL SIZE

4


160
140
120
100
80
60
AREAL INCREMENTS
(6+7)
(1+4)
GOOD
POOR
TOTAL INCREMENT
PER CENSUS

5

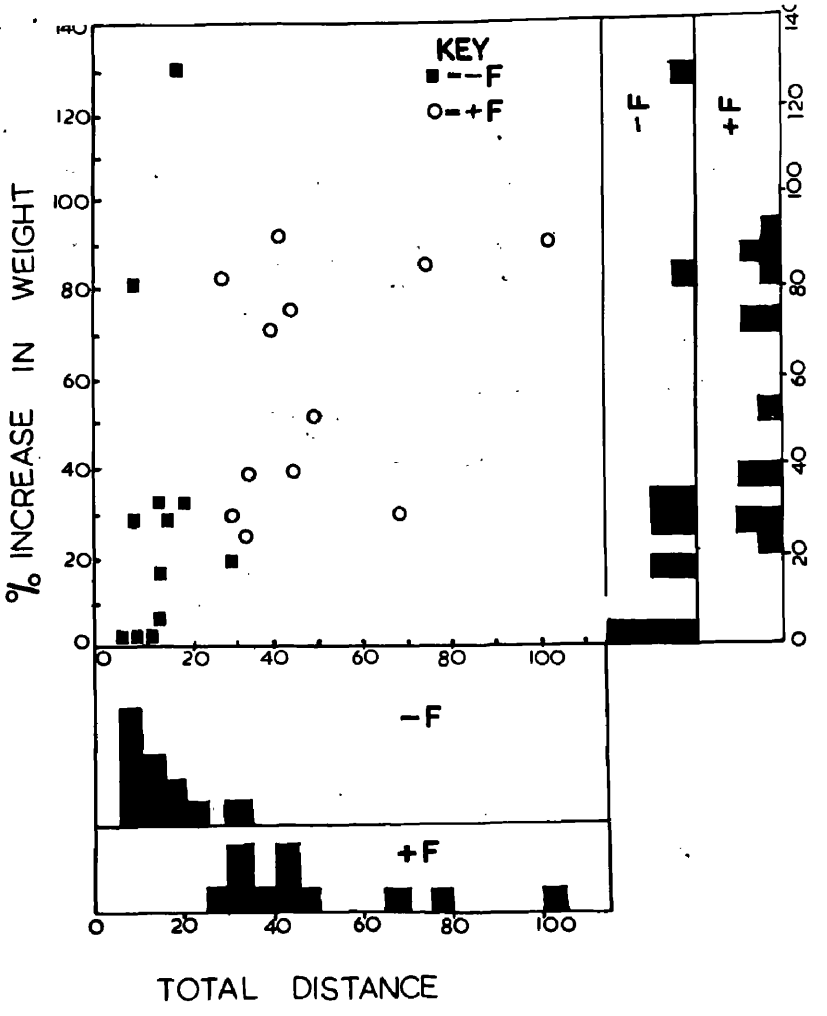
FIGURE 6

This shows the relationship of worker type to per-:centage increase in larval weight at each census of each brood mass. The worker type considered is the presence (+) or absence (-) of foragers (F). The appropriate values are also shown as histograms.

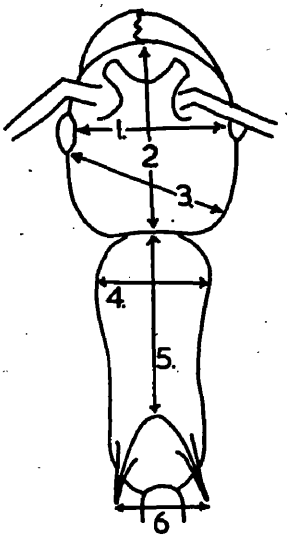
FIGURE 7

This shows the six measurements made on the head and thorax of workers of Myrmica scabrinodis:

1) Head width (between the eyes)
2) Head length
3) Head angulation
4) Maximal thoracic width
5) Thoracic length
6) Distance between the tips of the epinotal spines



6



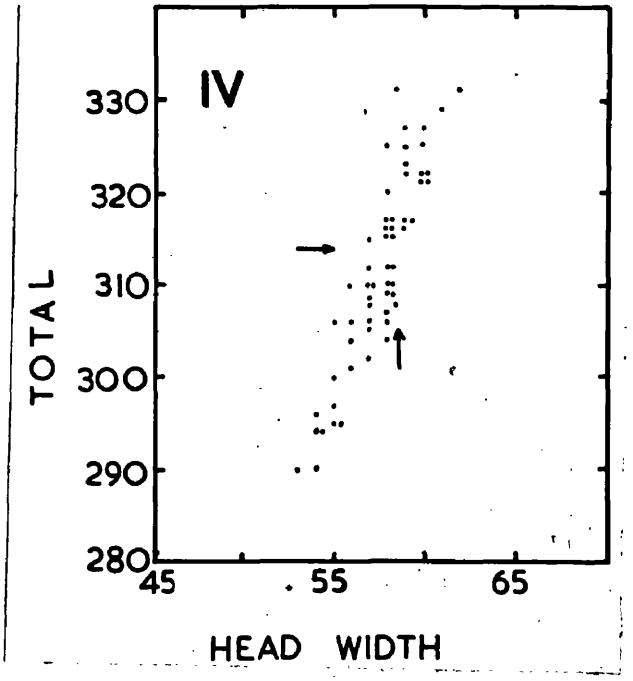
7

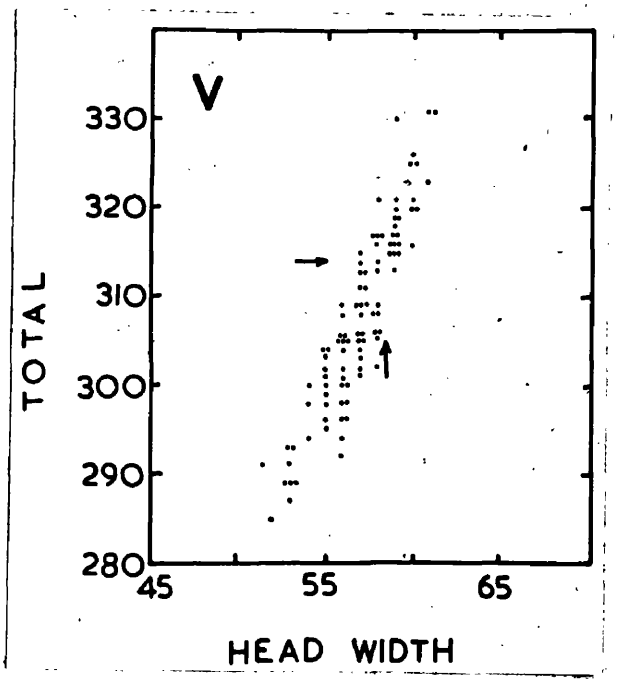
FIGURE 8.

This shows the relationship between head width and the sum of the five measurements utilised in this analysis.

1 unit = 0.0154 mm.

The three melanic groups IV, V and VI are considered separately. The arrows delineate that zone which includes 95% of the individuals of melanic group VI. It is apparent from the corresponding arrows on the other diagrams that melanic groups IV and V include many larger individuals.


IV
330
320
310
300
290
280
TOTAL
45
55
65
HEAD WIDTH


V
330
320
310
300
290
280
TOTAL
45
55
65
HEAD WIDTH

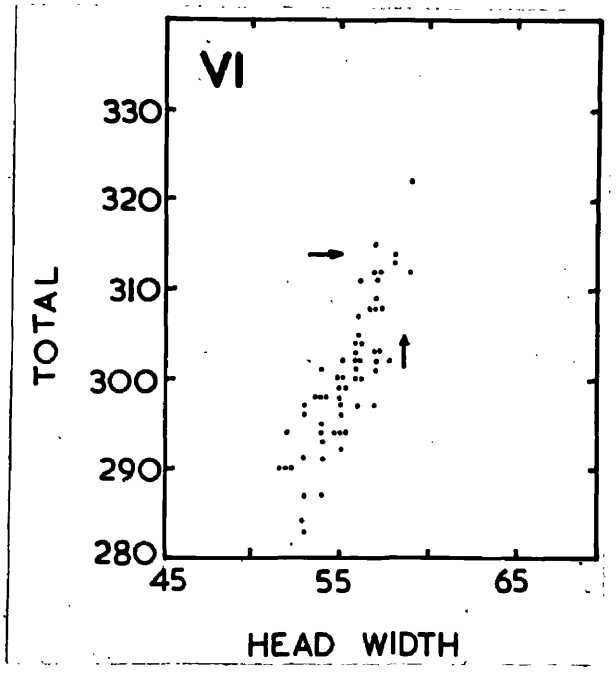

VI
330
320
310
300
290
280
TOTAL
45
55
65
HEAD WIDTH

FIGURE 9

The variation of worker size as measured by the total value of the five measurements made, is shown as the averaged sample value plotted against worker activity, as judged from the fifteen activity samples.

FIGURE 10

A diagrammatic section of the nest type used in these and other experiments.

A - ants

B - black cover

C - cotton wool

D - water dish

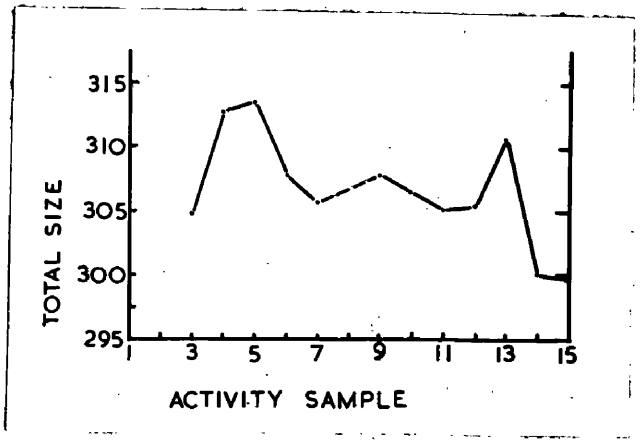
L - light source

P - plaster of paris

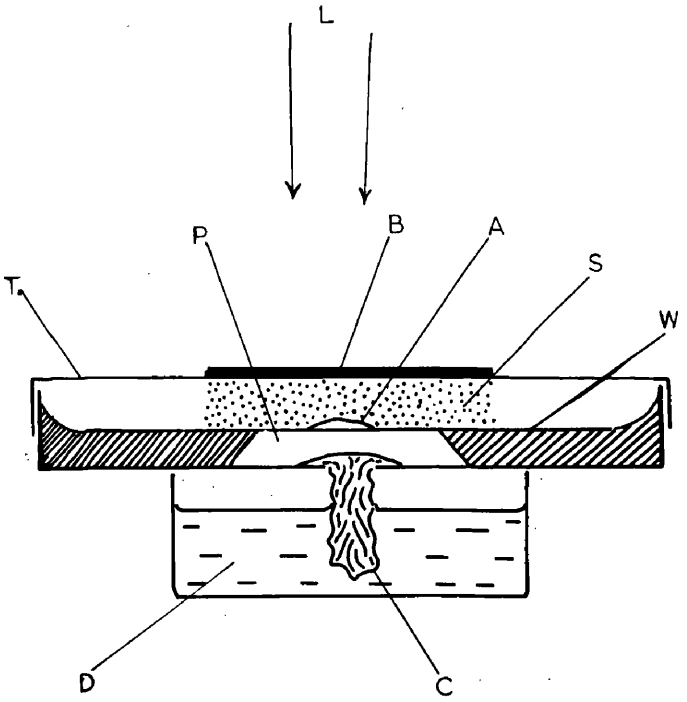
S - shade

T - top of glass container

W - paraffin wax


315
310
305
300
295
TOTAL SIZE
1
3
5
7
9
11
13
15
ACTIVITY SAMPLE

9


L
P
B
A
S
W
T
D
C

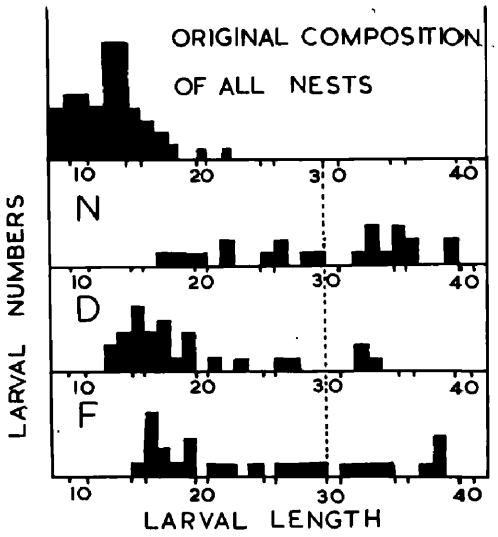
10

FIGURE 11

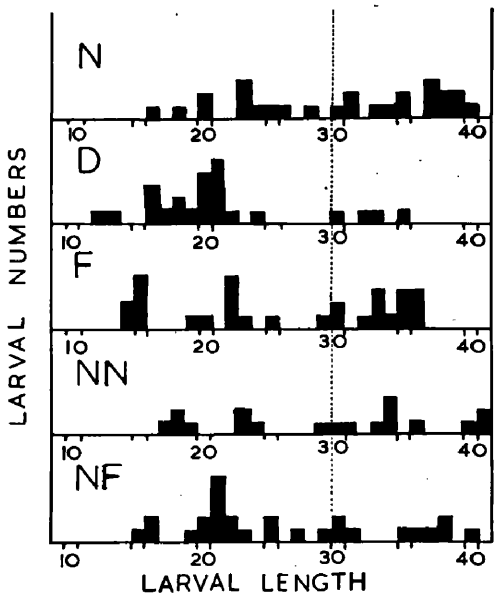
This shows the frequency distribution of larval length in experiment 6, before and after rearing with workers of types F, D and N. The two series of nests used are shown, and included in series B are the results of larval growth in the two nests containing callow workers. (Callow nurses = NN; callow foragers = NF)
1 unit = 0.0909 mm.

FIGURE 12

This shows the frequency distribution of larval length in experiment 7, before and after rearing with workers of types F, D and N. 1 unit = 0.0909 mm.

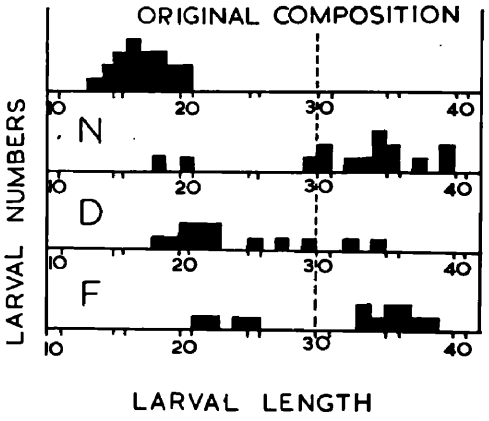


SERIES A



SERIES B

11



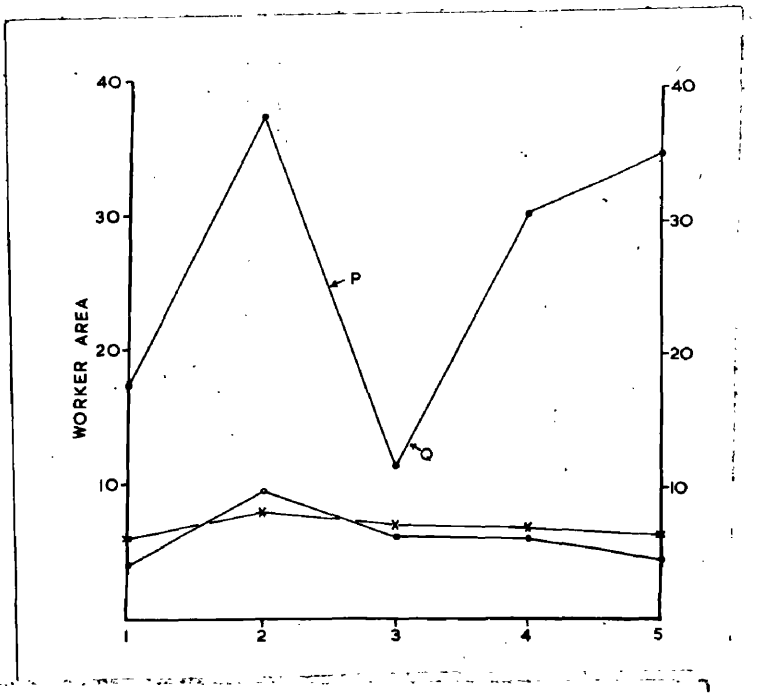
12

FIGURE 13

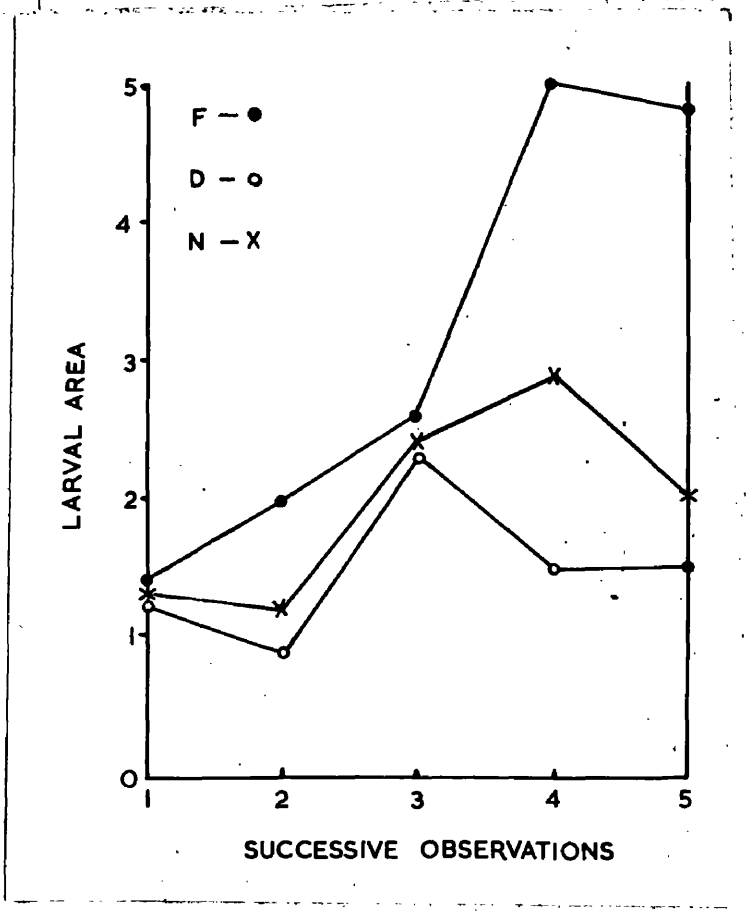
The area occupied by workers of the three types (F, D and N) at five successive observations is shown. The point P indicates the escape of a number of F-type workers from the nest. The full worker complement was restored at point Q. Worker area is measured in arbitrary units of purely relative significance. The key to the worker types is on figure 14.

FIGURE 14

The area occupied by larvae reared by workers of the three types (F, D and N) at five successive observations is shown. Larval area is measured in arbitrary units of purely relative significance.


WORKER AREA
40
30
20
10
P
Q
1
2
3
4
5

13


LARVAL AREA
F — ●
D — o
N — X
0
1
2
3
4
5
SUCCESSIVE OBSERVATIONS

14

FIGURE 15

This shows the relative worker oviposition by the three worker types F, D and N. (E_w = worker oviposition) The calculated total value N+D+F is inserted.

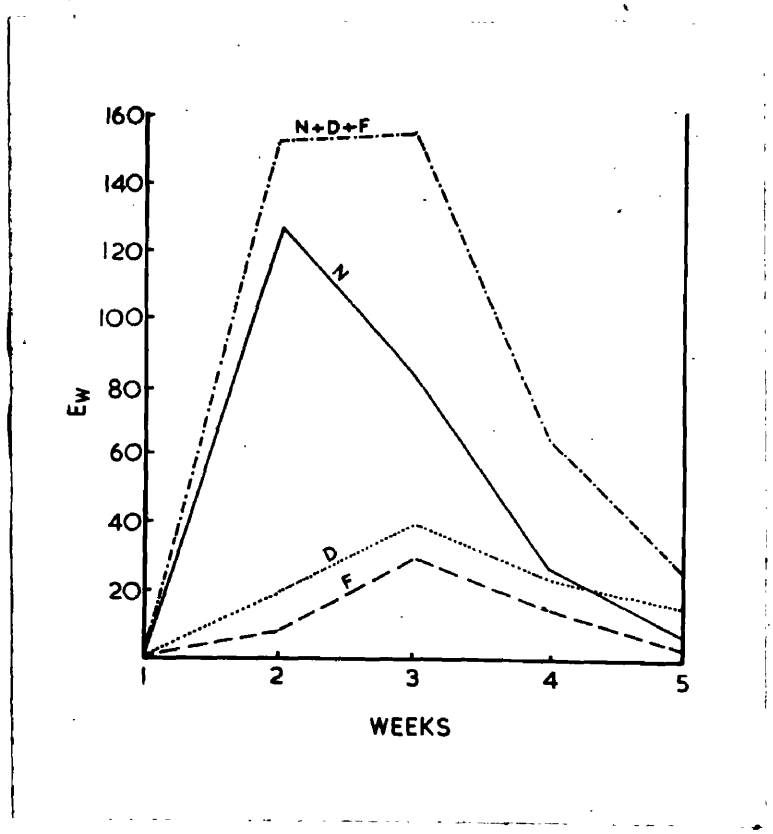
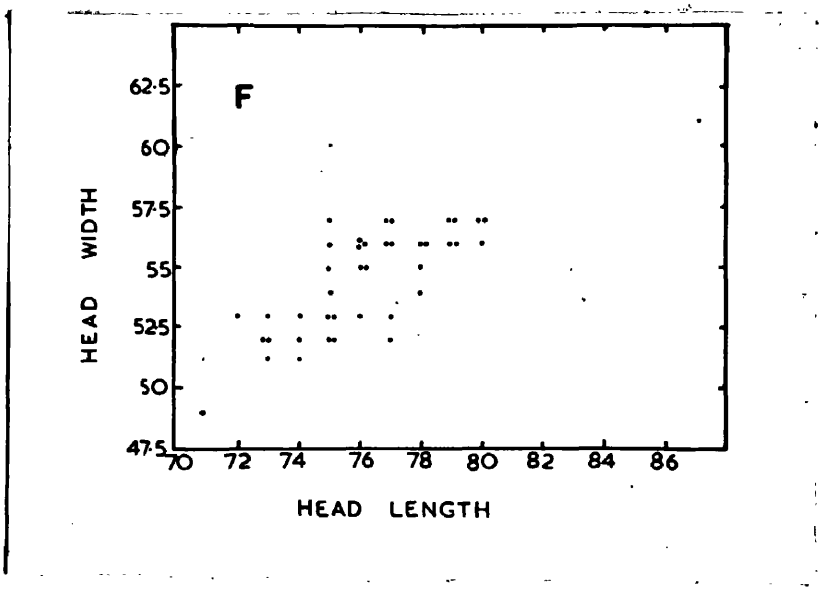

160
140
120
100
80
60
40
20
E_W
N+D+F
N
D
F
1
2
3
4
5
WEEKS

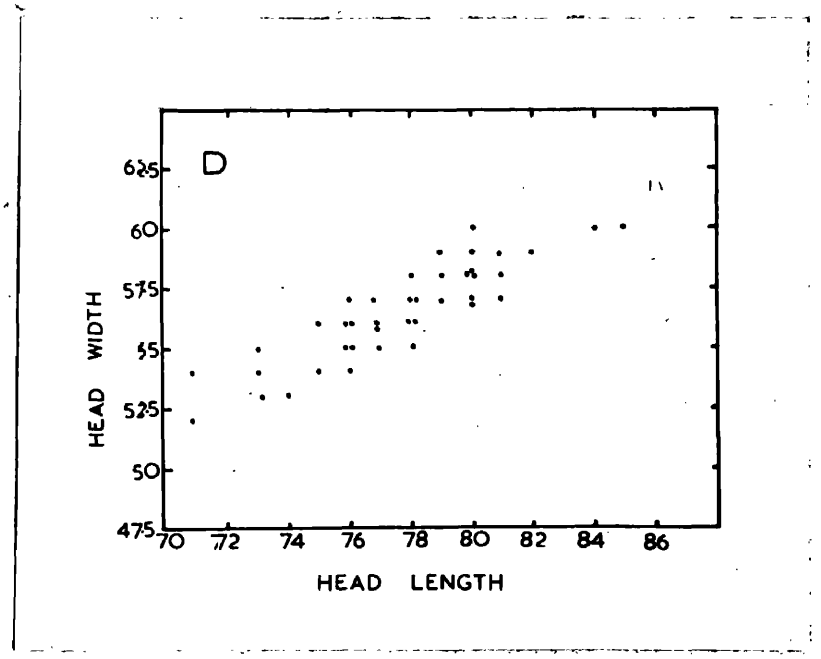
FIGURE 16

This shows the relationship between head width and head length in overwintered workers of the three types F, D and N.

1 unit = 0.0154 mm.

It is apparent that the sample of F-type workers contains few large individuals.


F
HEAD WIDTH
HEAD LENGTH
62·5
60
57·5
55
52·5
50
47·5
70
72
74
76
78
80
82
84
86


D
HEAD WIDTH
HEAD LENGTH
62·5
60
57·5
55
52·5
50
47·5
70
72
74
76
78
80
82
84
86

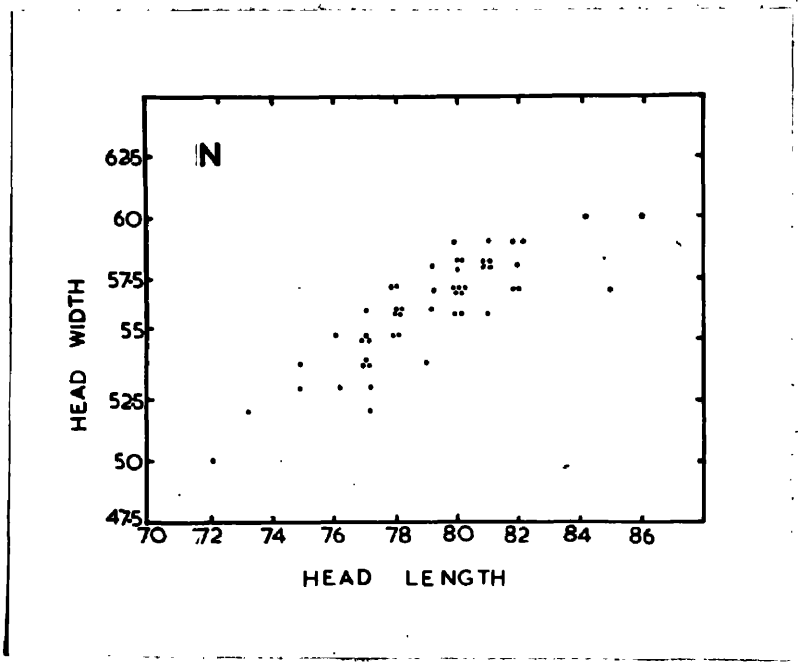
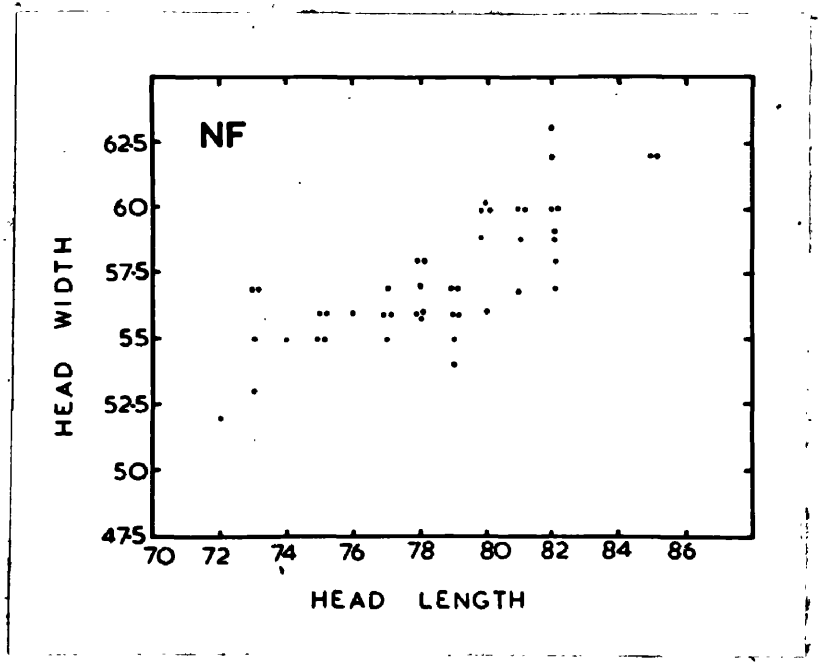

N
HEAD WIDTH
HEAD LENGTH
62·5
60
57·5
55
52·5
50
47·5
70
72
74
76
78
80
82
84
86

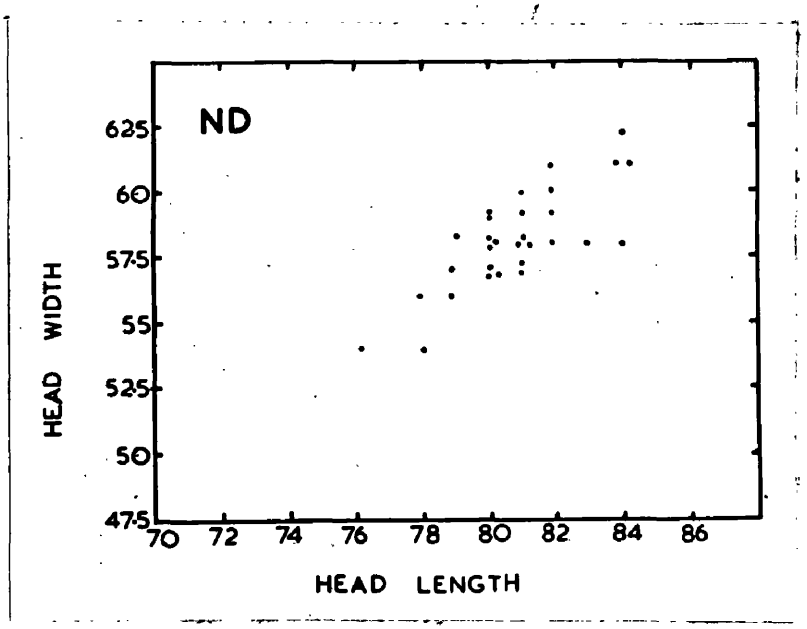
FIGURE 17

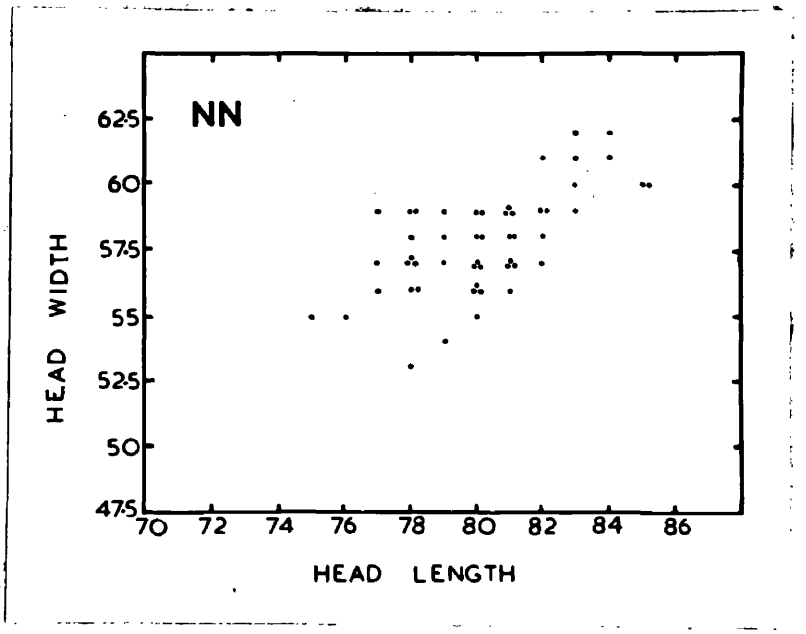
This shows the relationship between head width and head length in callow workers which have been separated into the three types F, D and N.

1 unit = 0.0154 mm.

It is apparent that the sample of F-type workers contains few large individuals.


NF
HEAD WIDTH
625
60
575
55
525
50
475
70 72 74 76 78 80 82 84 86
HEAD LENGTH


ND
HEAD WIDTH
625
60
575
55
525
50
475
70 72 74 76 78 80 82 84 86
HEAD LENGTH


NN
HEAD WIDTH
625
60
575
55
525
50
475
70 72 74 76 78 80 82 84 86
HEAD LENGTH

QUEEN OVIPOSITION IN *MYRMICA*

C O N T E N T S

1. INTRODUCTION

The present study was undertaken in the course of an investigation into the factors controlling dormancy in larvae of Myrmica, carried out in the Zoology Department at Glasgow University during the years 1951-54. Material for this investigation was collected in several localities in South West Scotland.

The species used in the course of this investigation are Myrmica rubra microgyna, Brian and Brian, 1949, Myrmica rubra macrogyna, Brian and Brian, 1949, and Myrmica laevinodis Nyl. There has been considerable taxonomic revision of these species as originally described. Myrmica rubra L. was divided by Nylander (1846) into three forms. Successive reinvestigations of this group as undertaken by Forel (1874), Donisthorpe (1927) and Santschi (1931) are summarised by Brian and Brian (1949), who have shown the necessity for subdivision of the species M. rubra L. The nomenclature and classification of Brian and Brian (1949) has been used throughout this report. There remains considerable dubiety as to the exact taxonomic status of the species which have been used by previous workers in their research. This is unavoidable but does not appear to have caused any confusion with the present work. Within each section of this report, the techniques employed are described and the results are discussed.

The/

The significance of this investigation may be summarised as follows. Brian (1951b) has described the summer population changes of colony components in the ant Myrmica. In all the colonies described, some over a period of years, the distribution of egg abundance, when plotted as a frequency polygon against time, shows a bimodal distribution each year. Bimodal annual egg frequency polygons are also recorded by Lubbock (1892) on Myrmica laevinodis and by Talbot (1945) in Myrmica schenki. As Brian (1951b) points out, this need not imply periodic oviposition rates, as a constant oviposition rate could be masked by larvae eating eggs. In only one case, however, was he able to demonstrate a complete interaction between larval egg eating and egg frequency bimodality, and if the other instances are to be explained it appears that periodic oviposition rates must be assumed.

In considering subsequent experiments it is convenient therefore to distinguish the true rate of queen oviposition from the rate of accumulation of eggs on the egg mass, i.e. the egg-input, as was done by Brian (1953b). The egg-input periodicity is of great significance in the investigation of larval dormancy [the non-dormant larvae are, in nature, derived from eggs accumulated during June while larvae produced from eggs accumulated during August normally become dormant]. The following lines of investigation are therefore apparent.

A./

A. Is there a qualitative difference in the eggs produced at the two maxima, i.e. a blastogenic determination of dormancy?

B. Do the higher midsummer temperatures have an adverse effect on queen oviposition?

C. Is egg input bimodality a manifestation of a physiological cycle or change, inherent in the queen or the workers or both?

D. Finally, could egg input bimodality result from a functional interaction of queen with workers, not being produced by either component in the absence of the other. [The basis for such interaction might be provided by the polyethal worker conditions which have been demonstrated by the present writer in these species (Weir, unpublished)].

Of these four lines of enquiry, A has been investigated by Brian, in experiments, as yet unpublished, the results of which are noted below. The investigation of items B, C and D and the reinvestigation of item A form part of the original work of the present author.*

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* I wish to record at this point my indebtedness to M.V. Brian in allowing me to use and criticise his experimental results in section A of this report, and in indicating to me what he considered were the salient features of those results.

2. EXPERIMENTAL RESULTS

A. Possible Blastogenic Determination of Dormancy.

Brian (personal communication) has shown that eggs from colonies in both vernal and serotinal conditions are developmentally plastic, and can either develop into dormant larvae or produce metamorphosing brood within a few weeks. The ultimate fate of the eggs is not determined by the time at which they are produced. Their fate can be controlled by changes of the socio-ecological environment. These results have been confirmed in numerous experiments by the present writer (Weir, unpublished). If differences exist between queen eggs produced by colonies of varying seasonal ages these are not critical with regard to dormancy, under the experimental conditions here used. There is then no blastogenic determination of dormancy.

B. The Effect of Temperature on Queen Oviposition.

Temperature alone has been chosen for this investigation, out of a number of possible physical variables. It must be realised that in the strictly regulated social environment of the ant nest, microclimatic variation due to humidity and temperature is at a minimum. Nevertheless, it appears likely that if any physical factor does vary significantly within these nests it is likely to be the temperature (Muir, unpublished).

It is convenient to define at this point the terms and symbols which will be used throughout this report with regard to fluctuations in egg production.

ACCUMULATION RATES

The total number of eggs produced by a queen per unit time = T_q

The total number of eggs produced by a group of workers per unit time = T_w

DIMINUTION RATES

The total number of eggs lost by being eaten by a queen per unit time = L_q

The total number of eggs lost by being eaten by a group of workers per unit time = L_w

The/

DIMINUTION RATES (Cont'd)

The total number of eggs lost by being eaten by a group of larvae per unit time $= L_l$

The total number of eggs lost as the result of eclosion per unit time $= L_e$

The effective oviposition rate of a group of queens and/or workers, is then the number of eggs surviving per unit time, i.e. from census to census, which can be defined as the egg input per unit time, or E. The suffix x may be used with E to denote an unknown or variable relationship of E. Specifically definable relationships of E have been denoted by a series of suffixes which can be considered as follows:-

1. In a queen - worker - larva colony from which no periodic egg withdrawals are made, the effective oviposition rate E which may be characterised in this case by the suffixes qwle, is given by:-

$$E_{qwle} = T_q + T_w - L_{qwle}$$

2. Similarly in a queen worker colony from which periodic egg withdrawals are made before the onset of eclosion (three weeks at 25°C), E may be characterised by the suffixes qw:-

$$E_{qw} = T_q + T_w - L_{qw}$$

3./

3. In a worker larva colony, from which weekly egg withdrawals are made, E may be characterised by the suffixes wl:-

$$E_{wl} = T_w - L_{wl}$$

4. In a colony consisting of a single queen, and from which weekly egg withdrawals are made, E may be characterised by the suffix q.

$$E_q = T_q - L_q$$

5. In a worker colony from which weekly egg withdrawals are made E may be characterised by the suffix w.

$$E_w = T_w - L_w$$

In the course of the investigation of temperature effects on queen oviposition, three experiments, designated I, II and III, will be described. In these, queens have been cultured alone or in company with workers. These colony components have been subjected to changes of temperature within their natural range (as known from observations in the West of Scotland during the months from March to September). No larvae have been used in these experiments, and eggs have always been removed prior to eclosion

The results of the individual experiments and the conclusions drawn from them are given after each account. A comparison of I and II is given after II, and a general discussion of the results at the end of the section.

EXPERIMENT I.

Six queens of Myrmica rubra microgyna were collected in early spring (February and March) before egg laying started. The queens were then kept at 22°C for about fifteen days until egg laying began. They were then incubated in six separate tubes in the dark, each supplied with ample sources of sugar and protein, and containing also a damp cotton wool plug. (This is the normal rearing technique used throughout these, and many other experiments, and has been shown to be a very efficient and foolproof method of culturing.) Three cultures were incubated at 20°C and three at 25°C. The groups of three cultures were transposed from one incubation temperature to the other after two weeks. The eggs were removed from the cultures every week, counted, and discarded. Results thus represented the queen input per unit time, E_q. The results of experiment I are shown graphically in fig.1. In view of the close correspondence between the three replicates of each set, the results have been averaged. The time of the temperature change is indicated on the graph by an arrow. This method is used in all subsequent graphs where a temperature transposition occurred.

The noteworthy feature of this experiment was the occurrence of the maximal value during the first week of the experiment and the second week of actual oviposition. Egg laying in all cultures/

cultures ceased within seven weeks and was followed at once by the death of the queen. Solitary queens at 20°C lived longer than those at 25°C and achieved a greater total effective egg production throughout this period. (Fig.1, death follows where the egg input falls to zero).

EXPERIMENT II.

Six queens of M. rubra microgyna , similar in size to those used in experiment I, were placed in tubes as described previously and incubated, three at 25°C and three at 20°C. In this experiment,however,three workers were included with each queen. The colonies were, as before, changed from one temperature to the other after fourteen days. The results are shown graphically as the dotted lines on figures 2 and 3. Each line represents the average of the three replicates, the close agreement between all three justifying this procedure; the arrow indicates the point of the temperature change. No queens died during the eleven weeks of the experiment.

A/

A comparison of the results of experiments I and II shows:-

(a) Increased queen survival in the presence of workers.

The considerable E_{qw} over the eleven weeks of experiment II shows that the solitary queens of experiment I did not die purely as a result of adverse environmental conditions. It appears that they died because the queen by itself is inadequate to deal with an environment, which, were workers present, would represent optimal laboratory conditions for culture.

(b) A significant and large increase in E_x as a result of the change from E_q to E_{qw}, in spite of the generally accepted fact that T_w is small compared to T_q. The increase is effective in two ways:-

1. by increasing the total of eggs produced during eleven weeks,
2. by increasing the length of time during which the input of queen eggs continued.

(c) No polymodality of E_q or E_{qw} is apparent. There is in all cultures a steady decline in egg input throughout the experiments. This decline is effective in two respects:-

1. Solitary queens (E_q) show more rapid decline then queens with workers (E_{qw}).
2. Queens at high temperatures (within each of the above groups) show a higher rate of decline (whether E_q or E_{qw}) than queens at lower temperatures.

(d)/

(d) The effect of temperature changes on E_q or E_{qw} can be considered as follows:-

1. If there is a temperature rise there is an immediate rise in E_{qw}.

2. If there is a fall in temperature there is an immediate fall in E_{qw}.

3. In experiment II there ensues subsequently the stabilisation of E_{qw} to a value comparable to that before the temperature change. Thus, in experiment II compensation for the temperature change is achieved after fourteen days. While relatively complete numerical readjustment of E_{qw} is achieved this does not imply that T_q is the same in both cases. L_w and L_q remain unestimated throughout the experiment, and L_w in particular has been shown to be significantly large under certain conditions (See experiment V below, p.43), while T_w is also unestimated.

4. There is a fall in E_q after both temperature alterations. It is probably more accurate to say that in the case of solitary queens neither of these changes has any immediately detectable effect on E_q. (See section 5 below).

5. In the case of experiment I, the readjustment of the queen physiology to the new temperature is reflected in the temporary increase in E_q in all cultures at 20°C fourteen days after the temperature change. This is directly comparable to the equivalent readjustment noted in section 3 above.

6./

6. While this compensation has a very small differential effect on E_{qw} in experiment II, the partial nature of the compensation in experiment I accounts for the differences in the total egg production during the period of the experiment.

EXPERIMENT III

This experiment was carried out using queens and workers of Myrmica laevinodis. It was desirable that all the queens and workers for this experiment should be as homogeneous as possible in respect of both genetical constitution and previous environmental treatment. It was therefore necessary to use a different species of ant since the numbers of queens which were required for this experiment are only rarely encountered in single colonies of M. rubra microgyna. Thirty queens were used, all coming from one nest which was regionally very isolated. They represent therefore material as genetically uniform as ant material from the field can be. This is particularly important when dealing with species such as M. laevinodis where the pleometrotic habit is combined with the tendency to increase queen numbers by recovery from the region round the nest of fertilised queens of their own and, presumably, other colonies. The queens were separated from the workers in the/

the colony, and divided into ten groups of three. Group selection was completely randomised as the queens were "dealt out" one after the other. The separation was carried out while the queens and workers were narcotised by carbon dioxide in order to avoid the selection of more active queens first and less active ones afterwards. The use of carbon dioxide as a narcotic was subsequently abandoned when experiments showed that deleterious effects were produced by repeatedly exposing workers to the gas. These experiments will be described elsewhere.

Each group of three queens was given twelve workers, similarly randomised, from the original colony of several thousand workers. Five of the groups were then incubated at 25°C and five at 20°C. After one week at the appropriate incubation temperature all five cultures of each series were changed to the alternative temperature. The original colony had been collected in the field in early April when a few eggs were present in the nest. Separation of the queens was not carried out until after a week in order to ensure that none had been seriously injured as a result of collection. The E_{qw} of the first ten days was therefore lost. The variation between the replicates at each weekly egg census will be considered subsequently (Section C, p.23). The results of each set of five replicates have been averaged as before. These results/

results have then been divided by three in order to allow direct comparison with experiment II on the basis of single queens. The results are shown graphically as the continuous lines in figures 2 and 3. The position of the temperature change is again indicated by an arrow on each graph.

There is again no significant (F test : $P > 10\%$) difference between E_{qw} at the two temperatures, and, in this respect, the effects of a change in temperature are the same as were found in experiment II. The different temperatures however do have a measurable effect on the size of the eggs produced. In experiment III the length of all eggs counted was measured and the mean value found for all the replicates at one census. For graphic purposes, the ten replicates have been averaged as two series of five. The results are shown in figure 4. The time of temperature alteration is indicated in each case by an arrow. The eggs produced at 25°C were smaller than those produced at 20°C. Statistically the difference is significant (F test : $P < 1\%$).

There is however a further complication. There is a partial overlap of the size range at any one census, between the summed replicates. Two frequency distributions for a typical census are shown in figure 5. It is apparent that notwithstanding the differences in the means of the distribu-:tions, there is a difference in the type of distribution and the/

the degree of skewness of the distributions. The significance of this difference is obscure. Reverting to figure 4, it is apparent that there is a steady decline in the average size of the eggs produced at 25°C. It should be noted by comparison with figures 2 and 3, that the time of the alteration in average egg size shown by the five replicates which were changed from 20°C to 25°C corresponds to the time of readjust-:ment of the total effective egg production to a value approximating to that before the temperature change. No striking differential viability has been detected between the two size ranges of eggs.

Conclusions Derived from Experiments I, II and III

The following conclusions can therefore be reached with regard to the effects, in these species of Myrmica, of temperature and temperature changes on queen oviposition, in addition to those effects previously enumerated (p.11 above).

a. There is still no evidence to suggest that temperature or temperature variation causes the bimodality of egg frequency polygons which has been reported in any of these species.

b. The effect on E_{qw} of the temperature change is immediate/

immediate, but readjustment occurs in all cases, after fourteen days.

c. The physiological adjustment is incomplete in at least M. laevinodis, where egg size falls as temperature rises.

d. In small M. rubra microgyna queens there is a gradual fall in egg input of the averaged replicates throughout the experiment. Such a fall would correspond to an annual decrease in E_{qw} in this subspecies under the experimental conditions. This effect is not shown by the averaged replicates of the larger M. laevinodis queens.

e. Possibly connected with the preceding item is the higher E_{qw} of the large M. laevinodis queens throughout the experiment (III). This difference is again related to the probable function of the macrogyne and not the microgyne as the colony foundatrix (see below, p.20) and the rapid death of solitary queens of M. rubra microgyna (experiment I).

f. The rapid attainment of maximal E_q, maximal E_{qw}, and maximal average egg size during the first week of all experiments where these were measured, must be considered as significant. The following emerge as possible causes:-

1. The initial maxima are due to intrinsic socio-ecological causes and occur in nature. On this point there is no evidence.

2. The initial maxima are due to the change from conditions in the field to those in the laboratory. If this is the case, the possible causes can be further analysed:-

   A. The laboratory environment may not be conducive to maximal reginal and/or colonial efficiency. It should however be noted that maximal sociological efficiency may not involve maximal egg size or maximal E_{qw}.

   B. The effect of a diurnal temperature rhythm is unknown.

   C. The food supplied in the cultures may be sociologically inadequate or unbalanced. There is strong evidence that ants of these species derive their sugar supplies from myrmecophilous aphids (Muir, unpublished). The effect of seasonal changes in the quality and quantity of honey dew is unknown.

   D. Finally, as a source of sociological variability, the accidental non-random selection of a few workers cannot be overlooked. Polyethal conditions have been demonstrated by the present writer, in/

in these species, and the random fragmentation of such a system may have had very significant sociological consequences.

Comparison of Experiments I, II and III with those Undertaken by Brian (1951b).

Comparison shows that the most notable differences are:-

a. The absence of environmental fluctuation in the present experiments. Brian made his observations on nests which were, as far as possible, under natural conditions.

b. The repeated removal of eggs from these cultures. Brian did not remove eggs from his nests in which, as a result, a large egg mass accumulated.

c. The use of different nest types.

d. The possible occurrence of a social form of functional depression in experiment I. Brian (1953a) has shown that workers in isolation undergo such depression. The degree to which such depression occurs in solitary queens, and, similarly, the degree to which the presence/

presence of juvenile forms such as eggs or larvae would counteract or reverse such depression, is a problem beyond the scope of the present investigation.

e. The use in some cases of a different species of Myrmica. The M. laevinodis queens used in experiment III and by Brian (1951b) may be twice the bulk of the M. rubra microgyna queens used in experiments I and II.

In relation to factor e (above), there is the interesting observation of both Brian (personal communication) and the present writer, who find in nature many more young colonies of M. rubra macrogyna than young M. rubra microgyna colonies. These observations favour the hypothesis that in these species of Myrmica only the true macrogyne is fitted to act as a colony foundatrix. This would account for the rapid death, low E_q, and short period of production of E_q in solitary microgynes. As will be shown subsequently the egg production of these queens must be derived largely from the fat body. The exhaustion of the fat body is followed by the death of the individual queen. The large queen has therefore more chance of survival. Throughout this report further aspects of this problem will be encountered and their significance indicated at the appropriate place.

Discussion of Experiments I, II and III

Field observations indicate that egg frequency bimodality is a feature of all species of Myrmica commonly found in the West of Scotland and used in these experiments. The use of culture tubes has been demonstrated by repeated experiments to provide a close approach to optimal laboratory conditions for formici-culture in this genus. Therefore in any investigation of the possible causes of such bimodality items c and e above can be discarded.

Having considered in some detail the conclusions to be derived from these experiments with regard to temperature effects, it is perhaps suitable to indicate some implications of wider sociological significance, and to relate these to other contemporary research. The significance of the results on queen independence, survival, and productivity have already been dealt with. These must be of significance to the mechanism of colony foundation in these and other genera.

The adjustment of egg size demonstrated in M. laevinodis is a suitable physiological mechanism for a microthermal species which has to overcome low spring and autumn temperatures in order to complete its life cycle within one year. It is of interest to compare this adjustment of egg size with the effects noted by Ledoux (1954) in Oecophylla and Gosswald and Bier (1954) in Formica where differential egg types have far reaching/

reaching effects on the sociology of the colony.

In conclusion it should be pointed out that although these experiments on temperature variation have provided interesting sociological data, they do not directly aid the elucidation of the factors affecting dormancy in myrmecine larvae.

C. Cyclical Activity in Colony Fragments of Myrmica.

The results contained in this section are derived from the continued analysis of experiment III. For a description of this experiment reference should be made to the previous section (B).

The socio-physiological adjustment to temperature, maintaining E_{qw} at a constant level, is so nearly complete in experiment III that a statistical analysis of the results over an eight week period when the temperatures were constant and E_{qw} had been stabilised, shows that greater variability is attributable to the individual colonies and their temporal changes, than is due to experimental temperature differences (Tables 1 and 2). It should however be pointed out that a time-series analysis has not been carried out, so that temporal differences must be considered with some reserve, and no conclusions regarding sequential significance can be drawn. This does not materially affect the subsequent investigation.

TABLE 1 /

TABLE 1

Experiment III

Considering the five components at each temperature as replicates, the following is the analysis of variance of the results.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F	P (PROBABILITY OF OCCURRENCE)
Total	79	26,444			
Temperatures	1	58	58	.21	>10%
Times	7	5,320	760	2.724	< 5% - >1%
Interaction	7	3,170	453	1.62	>5%
Error	64	17,896	279.6		

Temperature then being disregarded, Table 2 gives the results of an analysis of variance of the ten components and eight times of sampling.

TABLE 2/

TABLE 2

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F	P (PROBABILITY OF OCCURRENCE)
Total	79	26,444			
Ten Nests	9	6,084	676.0	2.83	$<1\% - >.1\%$
Eight Times	7	5,320	760.0	3.18	$<1\% - >.1\%$
Error	63	15,040	238.7		

The calculation follows the methods described in Snedecor, (1946), as do other statistical calculations described in this work.

In the investigation of the total egg input of the individual colonies, it is possible to ignore the differing temperatures and consider only the variation between the individual colony fragments and the ways in which they vary throughout the eight week period under consideration. When the censuses from the ten colonies involved are examined individually, it is seen that seven show one or more periods of at least one week's duration during which the egg input drops to a low figure relative to the other weekly recoveries from that colony. For convenience in consideration of the results, these seven have been further subdivided into three groups, depending on the total egg input over the period of eight weeks.

The results are treated as though they represent differen-:ces in the total egg production of the queen as reflected by differences in the egg input of the colony fragments. One should not however ignore the possibility that these changes represent differences, not of productivity, but of egg loss, resulting from egg consumption by either the workers or the queen. The differential consumption would then be assumed to act on a consistent level of egg production and significant differences would pertain to the different colony fragments as before. In general, subsequent experiments show that while differential egg consumption may occur it is unlikely to cause the/

the effects noted in this experiment.

The range in E_{qw} in the four groups may be either wide (R) or narrow (r), and the total egg input during the eight weeks may be low (α), moderate (a) or high (A). The four groups may then be denoted by the letters:-

αR, aR, AR and (ar & Ar)

Two typical graphs of each of these groups are shown in figure 6 [where A = (αR), B = (aR), C = (AR), and D = (ar & Ar)]. The range values are R = sixty eggs and r = twenty to forty eggs. The dotted line on the graphs denotes an E_{qw} of thirty eggs per week. This has been chosen as an arbitrary level below which the total egg input is considered to be in a period of depression. The outstanding differences in graphs A, B and C, can be related to the length of time during which E_{qw} falls below this level. These differences are tabulated in table 3 as if they represented a series of oscillations of varying periodicity. The differences in total egg input can be attributed to the association of long depression periods with low minima and maxima, and short depression periods with high minima and maxima. The four groups (αR), (aR), (AR) and (ar & Ar) present a series passing from a low basal productivity and long period at one extreme, through increasing basal productivity and shortening period, to the other extreme at which the basal productivity/

productivity is again increased and the period is either abolished or is so short that it becomes indetectable.

TABLE 3

GRAPHS	TIME IN WEEKS		UNITS = EGGS	
	WAVE LENGTH	LENGTH OF DEPRESSION PERIOD	MAXIMA AND MINIMA	RANGE
A = (αR)	10 Weeks	5 Weeks	0 - 60	60
B = (aR)	6 Weeks	2 Weeks	10 - 70	60
C = (AR)	6 Weeks	1 Week	20 - 80	60
D = ar & Ar	None Detectable	None	35 - 55 40 - 80	20 40

There are however further implications to be drawn from this experiment which complicate the preceding analysis. These arise from a consideration of the statistical treatment of the results./

results. As has been already indicated these queens represent a relatively uniform mass of similarly treated material, which has been randomly issued to each of ten containers in turn, under the influence of a narcotic (carbon dioxide) so that no activity, behaviour, or other factors can have influenced this random choice. The experimental temperature treatment has been shown to have had no significant effect, yet the probability of occurrence of such a series of eighty results from a uniform population each unit of which is composed of three queens and nine workers, has been shown to be less than 1%. It has, furthermore, been shown that this series of ten colony fragments can be arranged in a series according to the total egg input over the period of the experiment. When this is done certain other variables are also seen to be in array, for instance, the maxima, minima and wave length. The simple explanation of such a set of results would be that there was an unknown variable which influenced all these aspects of colony fragment efficiency. The even distribution over the values found cannot be accounted for unless this variable is a component within each colony fragment.

Considering then the remaining social variables there are three outstanding possibilities:-

1/

1. Variations in queen productivity as a result of heredity, age or previous environment.

2. Variations in worker efficiency.

3. Variability of queen-worker interaction.

These can be further reduced by reconsideration of the experimental set up. If the probability of chance occurrence of such results from these colony fragments each composed of three queens and nine workers is less than 1%, then the selection, by the method described, of queens of comparable type, into each of ten groups, is infinitely more improbable though it cannot be statistically estimated here. It is evident both from the experiments previously described and from the observations of Brian (1951b) that such queen variability is extremely improbable. It can therefore be concluded that the variability between colonies in experiment III is due either to factors (2) or (3) above. It can further be concluded that the effective queen input (E_{qw}) is under the control of one or both of these factors. This is the only explanation of such striking effects as the similarity between both the graphs shown in figure 6.A, and also the similarity between the two graphs shown in figure 6.B (although these latter are completely out of phase with each other).

Discussion of Section C

Conclusions to be derived from this portion of the investigation/

investigation dealing with the possibility of cyclical activity in colony fragments of Myrmica are as follows:-

a. Cyclical activity of some nature has been demonstrated to occur in the laboratory. The characteristics of these cycles have been tabulated.

b. It has been shown that it is extremely improbable that any variation in queen physiology or ethology could account for such cycles.

c. It is considered that the probable causes of such cycles have been reduced to either:-

    1. Worker variation, or

    2. Queen and worker interaction.

    These possibilities are further examined in the next section of this report.

d. There is no evidence that the presence of cycles such as have been demonstrated affects in any way the determination of eggs for dormancy or non-dormancy.

e. There is no evidence of any consistent bimodality in E_{qw} comparable to that noted by Brian (1951), and which would occur within the period of one year.

The wider sociological implications of these findings may however, be worthy of note. It is difficult to assign any definite period in the field for the completion of such cycles as/

as have here been experimentally demonstrated. Nevertheless it is difficult to avoid the conclusion that these are in some measure a reflection of seasonal cycles of productivity.

Turning to the problem of synchronised queen productivity within groups, there appear to be only three possible explanations.

i) The queens are synchronised by some extra-sociological factor (i.e. an allochthonous factor).

ii) The queens are synchronised by some sociological factor outside the queens themselves, such as worker control.

iii) The queens are synchronised by a dominant queen within each group. [Chen (1937) has demonstrated the existence of leaders and followers in workers of the same caste.]

The possible occurrence of this third factor would not detract in any way from the conclusions which have been reached. If through all the individual queens of this subspecies there extends a range of behaviour (Brian & Brian, 1949) such as would imply potential dominance [and perhaps be comparable to the condition described in Polistes (Pardi, 1948) or analogous with the peck order of domestic fowls] then, in every group of three queens, one queen may be dominant, and there is no justification in going beyond the groups of three queens and twelve workers, in/

in this analysis. The differences in E_{qw} between the groups would then show that the dominant queens were sufficiently variable among themselves to justify the conclusions reached above.

D. Worker Polyethism and its Interaction with Queen Oviposition

Worker polyethism has been shown by the present author to exist in colonies of Myrmica scabrinodis and Myrmica rubra. The work of Ehrhardt (1931) has shown the presence of this socio-physiological mechanism in colonies of Myrmica laevinodis. This polyethal condition provides a basis for any investigation concerning worker variability in these species. It is not implied that these polyethal factors are the only possible source of worker variation which may affect queen productivity, but they are the only source of consistent worker variation so far demonstrated.

It is necessary at this point to consider briefly some of the conclusions reached with regard to worker polyethism. The present writer's conception of this subject is that there are numerous grounds for the separation of workers into ethological types designated "foragers" (F), "domestics" (D), and "nurses" (N). These have been discussed elsewhere. Relevant at this point are the following items:-

1. It has been shown that workers of type F have a much higher protein foraging potential than those of either D or N. D have higher foraging potentials than N, which have very little.

2. The brood rearing efficiency of these types is very/

very different. Type D is notably unsuccessful in brood rearing.

3. A gradient of brood "attractiveness" can be shown to exist among these types. N. being most "attracted" by the brood mass.

4. E_w differences are noted subsequently in experiments IV and VI (p.35 and p.43 below).

Three experiments (IV, V, VI) are described in this section. These deal with the effects of numerical variation in colony fragments comprising workers of varying ethological types (as defined previously) and queens or queen eggs. The factual data obtained from each experiment are given after the description of the experiment. Conclusions derived from these three experiments are given at the end of this section.

EXPERIMENT IV

Seventeen queens of Myrmica rubra microgyna were used, along with a number of workers from the same nest. There is a size range between queens of different colonies of this subspecies (Brian & Brian, 1949). These queens belong to the larger size range, were all of apparently uniform size and all still healthy after fourteen days at 20°C. The nest was collected/

collected in early spring and, owing to a period of unusually fine weather early in the year, the queens were laying eggs before the experiment began. It is not possible to say how much of the E_{qw} was lost in this fashion. For the experiment the queens were randomised as before, this time without recourse to narcosis, and incubated in tubes at 25°C. Different numbers of workers were placed in these tubes and censuses taken after seven day intervals. Five queens were incubated alone. Three queens were incubated each queen with two workers; three queens each with five workers; three queens each with ten workers and, finally, three queens each with twenty workers. The queen replicates in each series were unavoidably confounded with worker variability. Three worker series were then obtained, each with four components comprising two, five, ten, and twenty workers. In order to estimate E_w over the same period, as compared with E_{qw}, three corresponding series of tubes were incubated containing only the appropriate numbers of workers to act as controls.

Results derived from experiment IV are described in two parts:-

a. Variations of E_{qw} and E_w

b. Variations of egg size.

IV.a Variations of E_{qw} and E_w/

IV.a Variations of E_{qw} and E_w

The results of the egg censuses during a two week period are shown in figure 7 [where W_1 = first week, W_2 = second week, W.N. = worker number. The terms E_x^N, E_x^D and E_x^F are used subsequently to indicate egg input in cultures containing queens and workers, or workers alone, derived from the N, D, and F series respectively. These conventions are used where necessary in the text and throughout the following series of figures]. The E_{qw} of the worker series each week have been averaged to facilitate comparison between the weekly censuses. Values of Eqw from all three worker series are averaged for the first week, but only those for groups D and F during the second week. The reasons for this are dealt with below.

There is an optimal value of E_{qw} in the first week at a worker/queen ratio of five. [Disregarding the initial value where no workers are present, there are significant (F test : $P < 5\% > 1\%$) differences between E_{qw} results for differing worker/queen ratios] There is no statistically detectable optimum during the second week, but E_{qw} reaches a maximum at a worker/queen ratio of between five and ten.

Figure 8 shows the individual values of E_{qw} during the first week for each of the three worker series. During this first week E_w of the controls was nil in every case. As is obvious from figure 8, there is a high degree of correlation between/

between the E_{qw} resulting from all three worker types.

Figure 9 shows, inter alia, the individual results of the second week E_{qw}. In this case the worker series N has achieved a much higher value with its queens than have the other groups. The control censuses of E_w for the second week are graphed in figure 10, from which it can be seen that while individual cultures of types F and D workers contain small numbers of eggs, series N shows a highly significant regression of E_w on W.N.* This value of E_w^N could be considered to be incorporated with the corresponding value of E_{qw}^N with certain theoretical observations based on the observations of Bier (1954), the subtraction of E_w^N from E_{qw}^N should then give a value for a theoretical $[E_{qw} - E_w]^N$. This calculated value $[E_{qw} - E_w]^N$ has been inserted in figure 11, and also in figure 9, where it is seen to be comparable, if not identical with values of E_{qw}^F and E_{qw}^D. The completely erroneous nature of this interesting comparison will be considered subsequently.

With regard to the values of E_{qw}^F and E_{qw}^D during the second week, it is considered probable that these series will contain a few potential egg-laying workers as noted in the relevant E_w series (figure 10). The sporadic occurrence of egg laying in the F and D series at this time could be attributed/

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* $e = 3.515w - 1.014$ where, as in Brian (1953b), e = eggs and w = worker number. Standard error of the regression coefficient = .014 eggs per worker.

attributed to incomplete separation of the worker types concerned.

With regard to queen oviposition during these two weeks in cultures not containing any workers, it is noteworthy that not all the eggs laid were assembled as an egg mass. The ability of a queen to form such a mass is a variable, the controlling factors being at present unknown. This aspect of brood rearing will be discussed more fully elsewhere.

IV.b Results derived from egg size measurements

The examination of eggs from experiment IV showed that size differences could be detected not, as might be expected, between queen colonies with varying numbers of workers, but between colonies which included queens and those which did not. The frequency distribution of the sizes of a random sample of eggs from queen and queenless colonies of the worker series N, are shown as histograms in figure 12. From this it can be seen that the eggs of a queen worker colony have a much lower average egg size than those of a worker colony. The statistical analysis of egg sizes from this experiment over the ensuing weeks show that a significant (F test : $P < 1\% > .1\%$ difference exists between the average size of the eggs produced in queen and queenless colony fragments. Further investigation shows that there are three characteristic shapes associated with eggs from/

from worker colonies and queen-worker colonies (figure 13). Table 4 shows the dimensions of these eggs and their frequency of occurrence in nests.

TABLE 4

Frequency of Occurrence	EGG LENGTHS (1 Unit = .0232 mm)		
	LARGE 31 - 34 Units	MEDIUM 26 - 28 Units	SMALL 21 - 24 Units
In Worker Colonies	Frequent	Frequent	Frequent
In Queen-Worker Colonies	Very Infrequent	Very Frequent	Frequent

The long (31 - 34 units) eggs normally found only in worker colonies are bilaterally symmetrical and one surface is hollowed. This shape is seen also in medium sized (26 - 28 units) eggs recovered from both worker and queen-worker colonies/

colonies, though these eggs are less curved. Finally, small (21 - 24 units) eggs recovered from both worker and queen-worker colonies are more or less globular, and there is no flattened surface. Comparison of the histograms in figure 12 shows that there is an overlap of the frequency distributions so preventing separation of these egg-size groups on the grounds of differing intra colonial origin. Such a situation could be explained by:-

a. the suppression of worker oviposition in the presence of the queen.

b. Selective use of worker eggs as food by the queen or the workers.

c. Alteration in size of worker eggs in the presence of the queen.

These qualitative differences in the eggs show that E_{qw} in these cultures is not simply the effective egg input of the queen (as estimated from other contemporary cultures where worker oviposition is at a minimum), plus the effective egg input of the relevant number of workers, but reflects some interaction of the queen on the workers or the worker eggs.

EXPERIMENT V /

EXPERIMENT V

Two hundred and eighty workers of Myrmica rubra microgyna were separated randomly into groups of twenty. These were then incubated with different numbers of eggs from a queen-worker colony at 25°C. The workers were removed from 10°C one week prior to the experiment and kept at 25°C for that time. Throughout the experiment, which lasted five days, it is unlikely that any workers oviposited, since no eggs were laid by the workers during a control period of five days following the end of the actual experiment. The workers could then be described as being in a pre-vernal condition* during which oviposition did not occur. The worker groups were arranged as two series of seven, each pair being given respectively, two, five, ten, fifteen, twentyfive, forty and eighty eggs per group.

The percentage loss of eggs in each culture is shown by the continuous line in figure 14 as the average of the two values for each level of egg abundance. The dotted line in this figure represents the results of a corresponding set of seven/

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* Brian (1954) has used the terms vernal, aestival and serotinal, to describe changes in the "condition" of workers throughout a natural season of 26 weeks, as found in the West of Scotland. The present author has used the term pre-vernal to describe workers in the condition immediately prior to vernal (as defined by Brian) and equivalent to March or early April in nature.

seven cultures at 20°C. The percentage loss at 20°C is comparable to that at 25°C. Worker variability probably accounts for the irregular nature of the graph. The actual losses are given in table 5. It is then apparent that while workers must, under certain conditions, treat queen eggs as potential juvenile forms, non-ovipositional prevernal workers treat queen eggs as utilisable food material.

TABLE 5

	INDIVIDUAL CULTURES						
Initial egg abundance	2	5	10	15	25	40	80
Actual Losses at 25°C (Averaged)	2	4	10	11	15	30	35
Actual Losses at 20°C.	2	5	10	14	14	40	34

EXPERIMENT VI /

EXPERIMENT VI

Three groups of five M. rubra microgyna queens of the upper size range (Brian & Brian, 1949) were used. Each group of five queens was cultured for five weeks with thirty-five workers. The three worker types used are again characterised by the letters F, D and N. A control experiment was carried out, whereby three groups of thirty five workers of types F, D and N respectively were cultured for five weeks without queens.

The results of experiment VI are shown in figures 15, 16 and 17. From these it is apparent that the cultures of worker type N have a much higher E_{qw} than those of types D or F. The striking resemblance between $E_{qw}{}^{F}$ and $E_{qw}{}^{D}$ and the calculated value $[E_{qw} - E_{w}]^{N}$ during the second week of experiment IV has here been utilised and in figure 17 the calculated values of

$$\frac{E_{qw} - E_{w}}{\text{Queen Number } (QN)}$$

for all three worker types are shown. (Division by the queen number is necessary because of the death of two queens in the second last week of the experiment).

It is obvious from figure 17 that there is a close similarity between the $\left[\frac{E_{qw} - E_{w}}{QN}\right]$ graphs of all the worker types over a period of five weeks. This is similar to the observations on experiment IV where the $[E_{qw} - E_{w}]^{N}$, $E_{qw}{}^{D}$, and $E_{qw}{}^{F}$ graphs all showed a striking correspondence for differing worker/queen ratios/

ratios throughout the two week period under review. This correspondence, although previously shown to be erroneous, must therefore be taken as significant and indicative of some relationship in view of the repeated occurrence of such results.

Discussion of Experiments IV, V and VI

It is possible using the results derived from experiments V and VI to offer some explanation of the results obtained in experiment IV. These will be considered in two sections as follows:-

Section A.

Considering series F and D only in experiment IV, it is now possible to account for the relatively low optimal value of the worker/queen ratio in respect of E_{qw} during the first week and the subsequent increase in this value and in E_{qw} during the second week. (Figures 7, 8, 9; F + D only).

During these two periods the workers of the F and D series are in a pre-vernal condition comparable to that of the workers used in experiment V. Although not ovipositing they are however actively foraging or otherwise supplying the queen with food. The addition of two workers causes a large increase in E_{qw} on both occasions and the addition of five workers causes a further,/

further, though smaller, increase. The non-linearity of this increase might be attributed to the increased loss of queen laid eggs due to greater worker egg eating when five workers are present. During the first week the presence of ten and twenty workers per queen causes an actual decrease in E_{qw} due, presumably, to the loss of eggs by worker egg eating being greater than the increase in queen input attributable to the presence of the additional workers. The stabilisation of the graph between the values of ten and twenty workers per queen might be attributed to other factors, such as the accessibility of the egg mass and the queen to the workers. Observation suggests that under these conditions the maximum number of workers which were within antennal touching distance of the egg mass at any one time was eight. A state of equilibrium may then be reached depending on how frequently the workers round the egg mass change places with those outside antennal touching distance of the egg mass, the queen remaining either immediately beside the egg mass or acting as an apparent centre of a subsidiary nucleus of workers. It has not however been possible to devise a technique to measure the dynamic effects on egg production of variations in proximity to the egg mass of the queen in the presence of varying numbers of workers.

With the aid of wire mesh or bolting silk, attempts were made to separate queens and varying numbers of workers from workers with egg masses in adjacent tubes. These attempts were unsuccessful/

unsuccessful in the respect that if the mesh was sufficiently wide to allow the passage of food it was also sufficiently wide to allow the transference of eggs. Where the mesh was fine enough to prevent this transference, the adjacent colonies showed no great interest in each other. This line of investigation has not been pursued.

The state of equilibrium reached in the F and D cultures during the second week is on a higher level than that during the first week. This fact and also the change of the optimum worker/queen ratio could be accounted for by the increase in worker physiological efficiency during the period of the experiment. The workers are now on the verge of oviposition and it must be presumed that at this point they undergo a physio-ethological change with regard to queen-laid eggs. Experiments have shown that vernal workers when ovipositing have a relatively high degree of brood rearing efficiency (50% egg survival) given queen laid eggs (Weir, unpublished). Consumption by workers of queen laid eggs must therefore fall to a low level at this time, since much of the 50% egg loss must be attributed to larval egg eating. This leads to a relatively greater E_{qw} in the F and D cultures compared with the first week. Ten workers can now contribute substantially to the food requirements of the queen without their consumption of queen laid eggs detracting from the total egg input. Twenty workers per queen, however, cause a reduction in the size of the egg mass.

Section B.

The E_{qw} achieved by the cultures of N type workers in experiment IV presents a different problem. The situation during the first week is exactly comparable to that shown by the F and D types of workers. During the second week, however, the consistent high rate of worker oviposition is associated with a corresponding increase in E_{qw} from the queen-worker series. It has been shown previously that the hypothesis that the increased E_{qw} simply represents the E_{qw} of the equivalent F and D series plus the E_w of the N series is untenable on the grounds of egg size. Nevertheless the calculated value $[\frac{E_{qw} - E_w}{QN}]^x$ of experiment VI appears to have a real significance.

Turning now to consideration of the results of experiments IV, V and VI as a whole, there are three possible explanations.

1. The worker laid eggs which may be produced in the queen-worker colonies are being converted by the queen into queen laid eggs. Such a conversion of worker eggs by queen ingestion has been demonstrated with the aid of vital dyes (see experiment VII below). Criticism of this result could be based on the fact of excessive numbers of worker eggs being introduced periodically into the nest.

2./

2. Worker oviposition being wholly or partially inhibited by the queen (Bier, 1954), queen oviposition is then augmented as a result of some worker effect (in proportion as the workers would lay eggs in the absence of the queen).

3. Worker oviposition is not inhibited by the queen presence but the average size of the eggs laid is affected. The large eggs normally laid by workers in the absence of the queen would be replaced by smaller eggs when the queen was present.

E. Observations on Individual Queens Following Dye Labelling.

These observations are based on vitally stained food supplied to the colony. They are described in the form of two experiments (VII and VIII) and the results derived from these experiments are then discussed.

EXPERIMENT VII

This experiment using the dye nile blue sulphate was carried out to clarify the fate of worker eggs in a queen-worker colony. Two queens and four workers were cultured for three weeks in a tube supplied as usual with drosophila-medium and sugar. In addition a number of vitally stained blue eggs from worker colonies were added every three days. After three weeks the colony components were dissected. Both queens were found to have the mid gut stained a pale blue colour. No workers showed any trace of the development of this colour in the mid gut. It was concluded, on admittedly scanty evidence, that the queen ingests these worker eggs either directly or via the workers. While this experiment throws some light on the fate of worker eggs introduced into a colony fragment it does not necessarily reflect the conditions obtaining when worker eggs are produced continually by the workers in a colony.

EXPERIMENT VIII/

EXPERIMENT VIII

This experiment, which is largely composed of observations utilising vital dyes, does not consist of one coherent set of treatments. It represents instead numerous observations on individual queens, spread over a period of several years. It is however convenient to treat these observations together and regard the whole as an experiment.

The wide range in behaviour of queens towards eggs which have just been laid is noteworthy. In general, queens which lay only a few eggs when in solitary confinement do not assemble these eggs into an egg mass (e.g. queens which have been cultured for some time in isolation). Those solitary queens which have a high egg input do form an egg mass (e.g. some queens when first cultured in isolation). The queen is quite capable of eating her own eggs, and newly laid eggs appear to be eaten quite readily. Where eggs are scattered through the nest, however, and allowed to remain scattered for a period of hours, the queen does not feed on the isolated eggs and does not appear, in fact, to be aware of their presence. If these eggs are, however, gathered up and placed in an egg lump which the queen encounters, she may either tend the egg mass or gradually consume it. The isolated queen therefore shows two possible reactions towards her own eggs:-

A./

A. to regard these as potential larvae, i.e. to care for them, or

B. to regard them as realisable food, i.e. to eat them.

Such reactions would be influenced by the available food resources. These different reactions are similar to those shown by workers of varying seasonal conditions, e.g. prevernal as opposed to vernal.

In all cases the isolated queens have been supplied with ample quantities of micro-crystalline or semi-liquid sugar and quantities of Drosophila larvae (live). While workers appear to survive for indefinite periods on this food, the solitary queens are unable to do so. The relevance of this question to colony foundation in the micro-thermal species of Myrmica has been considered previously, and will be discussed subsequently.

The actual physio-ethological mechanism underlying this inability is of interest. Some solitary queens of Myrmica laevinodis, Myrmica rubra macrogyna and Myrmica rubra microgyna have been observed in isolated culture to attack and kill adult Drosophila. In one case a queen was observed to kill five adult Drosophila within one hour. Some of these have been observed being malaxated and the mangled remains of others have been detected in culture tubes. Vital dyes such as nile blue sulphate/

sulphate, neutral red, trypan blue, etc., have also been used in these investigations. The incorporation of solutions of nile blue sulphate in the wet plug of cotton wool which fills the bottom of the culture tube and which provides a drinking supply, results in the production of blue eggs by the queen. Since the colour is incorporated with the yolky material of the egg, this shows that the queen is actively drinking from the water supply. Incorporation of the dye in the sugar and protein supply produced no result.

It can therefore be concluded that the solitary queen will not ingest as food sufficient quantities of sugar or protein to augment her egg production. The presence of one or two workers will however cause the blue colour to appear in the queen eggs when the dye is incorporated only in the sugar and protein. In the presence of workers the queen can then utilise the protein and sugar resources of the culture which were previously - at least in the case of the protein and probably also in the case of sucrose - physiologically (but not apparently ethologically) inaccessible.

Prolonged culture of queens in isolation resulted in the death of all queens concerned, eggs being removed periodically (e.g. experiment I). Post-mortem and premortem examination of queens in isolation shows exhaustion of the fat body, compared with queens from queen-worker cultures.

3, CONCLUSIONS

The results obtained within each of the five preceding sections have been discussed, and their significance stated, relative to the particular problem under examination. These results will now be reconsidered as a whole, under the following headings:-

i. Oviposition and colony establishment by solitary queens.

ii. Segregation of egg production by queens.

iii. Cyclical activity in colony fragments.

iv. Ovipositional control in Myrmica (general hypotheses in relation to the original problems, and the sociology of Myrmica as a whole).

i. Oviposition and colony establishment by solitary queens.

Brian (1951b) has shown that solitary queens of M. laevinodis collected in the field can establish colonies under laboratory conditions which resemble those in nature. Eggs laid by the solitary queen are aggregated into an egg mass and cultured by the queen alone until eclosion. Some of the/

the larvae may undergo metamorphosis and produce workers within twelve weeks, other larvae being overwintered before metamorphosis. The workers assist the queen in producing and rearing the next generation of the brood.

Experiments by the present author (I, IV and VIII above) under strictly regulated laboratory conditions show that the queen may not always aggregate her eggs and form an egg mass, nor, after aggregation, are eggs always tended as potential brood material (p.51 above). Individual eggs are only recognised when newly laid, i.e. when held in the mandibles [the newly extruded egg is removed from the abdomen by the mandibles] or when recently deposited from the mandibles. When E_q is low, egg masses are rarely formed and single eggs are left scattered in the culture tube. If these eggs are gathered into an egg mass by some other agency, the queen may then tend the eggs or it may systematically destroy the egg mass by eating the eggs (p.51 above). Egg masses are usually formed only during the first few weeks of culture of solitary queens, i.e. when E_q is high (experiment I). Formation of an egg mass of four or five eggs is usually necessary before the eggs are tended, and it appears from observation that this egg mass is initiated by the production, in quick succession, of the requisite number of eggs during this period of high E_q. An egg mass of four or five eggs in the culture tube can be

"recognised"/

"recognised" by the queen, although the same number of eggs scattered individually throughout the culture tube may not be recognised(p.51).

The establishment of an egg mass which will be tended by a solitary queen then depends on a high E_q, a low L_q, and a lack of environmental disturbance. Environmental food is not necessary nor even realisable (experiment VIII, p.52), since the exhaustion of the fat body of isolated queens (p.53) shows that their egg production is derived largely from stored reserves in the fat body or redundant internal organs. The high E_q, low L_q, and redundant internal structures [e.g. the thoracic musculature (Janet, 1907)] are all characters of queens one year old. The solitary queen has then to:-

[1] Lay eggs, some of which eclose to larvae

[2] Survive without access to allochthonous food until the eggs eclose.

[3] Feed the larvae either on allochthonous food procured by the expenditure of her own energy reserves, or on autochthonous food (eggs or glandular secretions) by the utilisation of her stored food material.

[4] Survive long enough to allow pupation and worker emergence.

In the case of Brian's queens these four conditions were successfully fulfilled. When the workers render allochthonous food/

food accessible to the queen (p.53), the colony may be considered to be successfully established.

From Brian's experiments (1951b) on M. laevinodis it appears that the minimal essential survival time of the solitary queen is in the order of ten weeks in the field*. The corresponding time in the laboratory at 25°C may be in the order of six weeks (Brian,1954). The failure of any isolated queens to reach this minimal survival period in culture at 25°C (experiment I, p.10) can be attributed to critical differences between these experiments and those of Brian (1951b). The three relevant factors in the present series and not in Brian's experiments, may be summarised as follows (p.19):-

[a] The periodic removal of eggs,

[b] The possible incidence of functional depression,

[c] The absence of environmental fluctuation.

Of these three, [a], the periodic removal of eggs from the cultures might alone produce the effects noted.

Both microgynes and macrogynes have failed (experiment VIII) to survive for the requisite six week period at 25°C, but differences in the time and conditions of collection preclude/

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* Solitary queens overwinter before egg laying commences (Brian, 1951b). The ten week period referred to above is the time from the onset of oviposition to the establishment of the colony (i.e. the period of high energy expenditure).

preclude the possibility of individual comparisons. In these experiments the energy normally expended in the several processes involved in colony establishment is being utilised for the repeated production of an egg mass. If the eggs were left in the culture tubes they might be regarded as potential juvenile material and accordingly cultured, if the queen was sustained by internal utilisation of the fat body and other reserves. Alternately, only some of the eggs might be so cultured, but continuous oviposition and reconsumption of eggs would provide the queen with a continuous supply of food. The periodic removal of eggs from the nest would inevitably eliminate whichever of these systems was in operation, causing the rapid exhaustion of food reserves in the queen, followed by the death of the queen. Effects such as the greatly increased death rate, the non-achievement of maximal egg input, the short duration of oviposition, and the rapid decline in E_q (p.11), as compared with the corresponding data for similar queens cultured with workers (E_{qw}) (p.11), show the inability of the solitary queen to remain in equilibrium with the laboratory environment. The addition of only two or three workers (experiment II, p.10; experiment IV, p.37) which will supply food to the queen and so increase enormously the survival capacity of the colony, (in this case its ability to persist unaltered, for an indefinite period under the prevailing conditions), permits the/

the maintenance of equilibrium with this environment.

The larger egg input, the usual absence of any apparent seasonal decline in egg input (p.17), and the larger egg size, together with the much greater bulk of the fat body of the macrogyne - here observed in M. laevinodis, though at least the two latter facts are true of M. rubra macrogyna - show that the macrogyne may be more fitted to act as a colony foundatrix than the microgyne. The much higher frequency of occurrence in the West of Scotland of young colonies of M. rubra macrogyna compared with M. rubra microgyna (p.20) appears to confirm this observation.

Similarly the numerically greater egg input of solitary queens at 20°C (p.12), and the production of larger eggs at 20°C than at 25°C by queens with workers (p.16 above), shows that sociological efficiency as measured by the maximal bulk of eggs produced is greatest at 20°C. Culture at 20°C then presents a closer approach to optimal laboratory conditions than does culture at 25°C. It is possible that solitary queens at 25°C are unable to establish colonies as a result of this consistently high temperature.

To recapitulate then, the mechanism of colony establish-:ment by solitary queens of the ant Myrmica has been partially investigated. The first eggs are derived from the internal resources of the queen, which itself survives on these reserves/

reserves until the first workers are produced. It seems that the macrogyne may be more fitted to act as a colony foundatrix than the microgyne, and that 20°C is preferable to 25°C for laboratory culture of queens.

ii. The segregation of egg production by queens.

The object of this section of the discussion is to show that the food which is utilised for queen egg production has a multiple origin. These different sources of food for queens are associated with different levels of queen egg production.

It has been shown in section (i) of this discussion (above) that the eggs laid by solitary queens are derived almost exclusively from the internal organs of the queen. This source of queen egg production is characterised on the average by a low egg input. This low egg input (E_q) probably reflects a correspondingly low value of egg production (T_q). This low value of egg production derived from the internal resources of the queen may be designated $T_q(1)$.

The further discussion of this section of the results is based on the three possible explanations of the results of experiments IV, V and VI enumerated on p.48. These are, briefly/

briefly:-

(1) The alimentary conversion by the queen of worker eggs into queen eggs, on an egg for egg basis.

(2) The inhibition of worker oviposition in the presence of the queen, and the augmentation by increased worker feeding, of queen input, on an egg for egg basis.

(3) A change in the size of eggs produced by workers in the presence of the queen.

There is no evidence to favour the possibility of an alteration in worker egg size in the presence of the queen (hypothesis (3) above). All the available evidence supports either (1) or (2) or both, and therefore (3) has been disregarded.

In addition to these three hypotheses which deal with discrepancies among groups including ovipositional workers, it should be remembered that E_{qw} is also augmented to some extent by pre-ovipositional workers (experiment IV, p.45). These observations are now collated with those of experiments VII and VIII and with contemporary researches of other workers.

Bier (1954) on Leptothorax, has shown that in the presence of the queen there is an inhibition of worker oviposition/

oviposition. This effect cannot produce any possible confusion during the first week of experiment IV since none of the workers in the control experiments (i.e. in the absence of the queen) were laying eggs. The only possible influence of the queen on worker oviposition during this period is the stimulation of worker oviposition by the presence of the queen. No such effect has ever been demonstrated in ants or indeed in any social Hymenoptera. In fact, in several widely separated Hymenoptera, e.g. Leptothorax (Bier, 1954), Apis mellifera (Hess, 1942; Millen, 1942), Polistes (Deleurance, 1950a), the converse has been demonstrated. It can therefore be concluded that during this first week of experiment IV, E_{qw} is derived completely from T_q and varies only with worker number. During the second week of experiment IV, differential worker oviposition occurred. The corresponding differences in E_{qw} cannot be accounted for simply by the suppression of worker oviposition in queen-worker colonies, unless other social effects occur. The three possible explanations have already been enumerated and experiment VII shows evidence favouring the hypothesis that the queen converts worker eggs to queen eggs. There is NO evidence favouring the suppression of worker egg laying in queen-worker colonies. On the contrary, there is evidence to show that at least some worker eggs are laid in queen-worker colonies. This may be tabulated as follows:/

follows:-

[1] Large eggs (length 30 - 33) have been found in queen-worker colonies (p.40). Eggs of this size are characteristically produced in worker colonies. The lack of development in these eggs when recovered from queen-worker colonies suggests that only those which have beenlaid a short time previously are recovered. Presumably other eggs of this size are destroyed after a short time in queen-worker colonies*.

[2] Workers stained with vital dyes have been cultured along with normal unstained queens and vice versa. The production of vitally stained worker eggs has been observed in these nests for periods of up to seven days after the admission of the workers. The absence of eggs after this time probably indicates the exhaustion of the vital dye, not the suppression of worker oviposition.

[3] Brian (1953b) observed "corpora lutea" in workers from colonies containing an actively ovipositing queen.

[4] Brian (1953b) observed workers ovipositing in a queen-worker colony.

In/

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* The egg size differences described in these nests of Myrmica rubra microgyna may not always be detectable in nests from other localities. It is probable, however, that the same mechanisms of ovipositional regulation exist in colonies which do not show these size differences.

In view of these facts it is probable that at least some worker oviposition does occur in queen-worker colonies.

With the queen/worker ratios used in the experiments described above, the value of T_w is probably small compared with T_q, except in cultures of N type workers at certain periods. This worker oviposition is not imcompatible with the observations of Bier (1954) when it is recollected that socially, Myrmica may well be a more primitive genus than Leptothorax on which Bier worked. The efficiency of worker ovipositional suppression may not have been achieved in Myrmica where the mechanism of alimentary conversion of worker eggs, if present, is probably more wasteful. It appears as a result, that hypothesis 1 (p.48, alimentary conversion of worker eggs) must be accepted, though worker oviposition may also be partly suppressed (see p.49 above, hypothesis 2).

Further, considering the known physiological and ethological differences between the three worker types (p.34), it has been shown that the theoretical values of $[E_{qw} - E_w]^x$ in experiments IV and VI are significantly alike (p.48). But it will also be remembered that,on the basis of egg size alone, the E_{qw} of N type workers cannot represent the E_{qw} of the F or D type workers plus the E_w of the N series, in spite of the numerical similarity of the calculated values (p.48).

A co-ordinated explanation utilising all the previous results/

results can only be made as follows. The like values of $[E_{qw} - E_w]^x$ throughout experiments IV and VI, suggest that T_q, in a series of cultures over a period of time, is divisible into three components. These may be designated $T_q(1)$, $T_q(2)$ and $T_q(3)$. The occurrence of these three components is associated with three levels of egg production as measured by the egg input. The components are:-

$T_q(1)$ = The very low egg production attributable to the isolated queen and derived from her fat body (p.56).

$T_q(2)$ = The queen egg production resulting from the presence of preovipositional (prevernal) workers. This varies with worker number but reaches an optimal value in the region of a worker/queen ratio of ten (pp. 37, 45 et seq.).

$T_q(3)$ = The queen egg production derived from the conversion of worker laid eggs. This also varies with the worker number but does not reach an optimal value below a worker/queen ratio of 20. It may then show an asymptotic approach to an optimum at a worker/queen ratio near infinity, dependent on queen physiology (pp.38,48).

It is difficult to partition the egg input during a period when all three components may be contributing to T_q. The three levels of T_q associated with the collective incidence of these components/

components can then be designated:-

$T_q^{(1)}$ = the very low E_q of the isolated queen.

$T_q^{(1+2)}$ = the moderate E_{qw} of a queen with prevernal workers (non-ovipositional).

$T_q^{(1+2+3)}$ = the high E_{qw} of a queen with vernal (ovipositional) workers.

$T_q^{(1)}$ Varies only with the age of the queen.

$T_q^{(1+2)}$ Varies with the number of workers and the seasonal "condition" of the workers. It is independent of worker type.

$T_q^{(1+2+3)}$ Varies with the number of workers and the seasonal "condition" of the workers. It is dependent on worker type. It is apparently proportional on an egg for egg basis with T_w (assuming L_w is constant).

The value of L_w is small in conditions where $T_q^{(1+2+3)}$ occurs, in view of the physio-ethological change in worker behaviour noted previously (p.47). The value of L_w, however, is significantly larger in conditions where $T_q^{(1+2)}$ occurs.

It has been shown that $T_q^{(1+2)}$ is independent of worker ethological type. Yet the foraging potential of these three worker types is known to be different (p.34). Type F has a higher/

higher foraging potential for crude environmental protein (allochthonous protein) than has D or N. This fact must be related to the uniform values of $\frac{[E_{qw} - E_w]^x}{QN}$ in experiments IV and VI where $T_q^{(1+2)}$ occurred. It is difficult to avoid the conclusion that the queen cannot utilise any of the additional allochthonous food collected by F type workers. The allochthonous food is either utilisable only in small quantities, or it is not realisable by the queen at all, and must be trans-:formed in some way by the workers. $T_q^{(1+2)}$ is then dependent solely on worker number (over a very short range) and seasonal condition.

The significance of worker condition in relation to brood rearing has been considered by Brian (1954) who has described three seasonal conditions. The use of the term prevernal to describe a fourth condition has been introduced by the present writer. The actual physiological factors which must control the differences between these conditions remain undetermined. The possibility of food transformation and/or the seasonal production of a specialised salivary secretion by the workers, is supported by much of the experimental evidence presented here. While no glandular cycles have so far been demonstrated in worker ants, other work of the present author supports the hypothesis that such cycles must exist. If $T_q^{(2)}$ can be attributed to salivary secretions of the workers, then $T_q^{(3)}$ must/

must be derived from a different source. Once more, it is difficult to avoid the conclusion that $T_q^{(3)}$ is derived very largely from alimentary conversion of worker eggs.

iii. Cyclical activity in colony fragments.

Experimental evidence has shown that cyclical behaviour of various kinds may occur in colony fragments under laboratory conditions. The variable properties of the cycles described in Section 2.C above suggest that they represent the interaction of at least two separate factors. It may be supposed that either individual worker efficiency or variable queen-worker interaction, or both, are operative. Variation in individual worker efficiency might be operative in several ways, e.g. in feeding the queen or in the actual consumption of queen eggs. There is evidence (Paper I of this thesis) to show that individual workers produce widely different quantities of worker laid eggs. The random fragmentation of a colony containing workers with a range of egg laying capacity might well produce the observed variation of maxima and minima noted in table 3. Such variation can therefore be discounted in the case of graphs A, B and C (p.28 above).

The probability of the synchronisation of queen egg input has been noted previously (p.32 above). The possible incidence of/

of queen non-synchronisation in certain colonies would account for the uniformity of egg input noted in graph D (table 3), if cyclical activity is assumed to be shown by the remaining graphs.

There remains the problem of the significance of this cyclical behaviour under natural conditions. No such behaviour has been noted in the field by other observers. The nature of the experiment however is such that no accurate comparison with natural conditions can be made. Nevertheless, one possible explanation of the observed cyclical activity is here suggested; an explanation based on results and observations of other laboratory experiments. Worker polyethism has been demonstrated in this species by the present author (Paper I of this thesis). Superimposed on these polyethal worker differences are differen-:ces of seasonal condition (e.g. workers in vernal, aestival and serotinal condition). Worker physiology (e.g. worker oviposition) may vary both with polyethal worker type (forager or nurse) and also with seasonal condition (vernal and serotinal). The significance of polyethal worker conditions in cyclical colonial activity has already been dealt with. It is tempting to suggest that worker seasonal change (i.e. the change with time from pre-vernal to vernal to aestival to serotinal conditions) is responsible for the observed cyclical activity of queen egg production. The restriction of potential worker energy output to queen feeding presupposes that workers are always ready and able/

able to feed the queen. Such may not be the case. Experimental evidence has shown that workers may produce a special queen food over a restricted period of time. The physiology or ethology of the workers then alters and this supply of food is restricted. However, the natural sequence of events following the feeding of the queen (i.e. the production of larvae) is here thwarted by the removal of eggs. The normal change in worker physiology may however follow automatically, with a resultant fall in queen egg input. The queen egg input is then restored to its original level only after worker physiology has been readjusted to the absence of larvae. Such a sequence of events would explain to some extent the cyclical behaviour observed in these colony fragments.

iv. Ovipositional control in Myrmica (General hypotheses in relation to the original problems, and the sociology of Myrmica as a whole).

In the three previous sections of conclusions the ultimate significance to queen oviposition of the worker ants has been shown. The control of queen oviposition can only be understood in relation to the social control of colony growth and develop-:ment as a whole.

The segregation of queen egg production in respect of the three possible food sources demonstrated, has shown that, once a colony/

colony containing workers has been established, the queen is dependent on these workers for the food necessary to enable her to produce eggs. Her egg production varies with polyethal worker type and with worker seasonal condition.

It should however be remembered that the effects of worker polyethal types have only been investigated in the laboratory. In nature these worker types appear to be mixed throughout the colony and the mobility of the queen may enable it to keep in contact with all worker types. This problem has not been investigated.

The demonstration that the queen may receive a special worker produced food, or worker treated food, cannot be considered separately from other work of the present author on larval feeding habits (Paper III of this thesis). In this work on the incidence of larval dormancy, it has been shown that non-dormant larvae receive a special worker produced or worker treated food which may be of glandular, or ingluvial origin. The time of production of this larval food and the time of production of the special queen food are identical. It may then be suggested that this is, in fact, the same or a closely related food. The wider sociological implications of such an observation are discussed subsequently (Paper III of this thesis) Nevertheless, it is apparent that should these limited foods be identical, bimodal frequency polygons of egg abundance may be explained by the preferential allocation of this food, first to the/

the queen, then to certain larvae, and finally to the queen again. The seasonal behaviour of the colony may then be tabulated as follows:-

Prevernal colonies

Worker foraging commences. Queen egg production (TQ^2) then starts. Eggs at this time are few in number, and do not accumulate since they are eaten by workers as food. Eggs therefore represent a means of food storage and circulation at this time. Overwintered larvae start to grow (and develop?).

Vernal colonies.

Intense worker foraging. Queen egg production reaches a maximum ($TQ^2 + TQ^3$). Eggs accumulate. Eggs are not regarded by workers as food (Paper I of this thesis) but as potential juvenile material. Overwintered larvae come to pupation. The possibility exists that certain overwintered larvae may receive special worker produced food during this period, since it first becomes available at this time. There is high nurse oviposition at this time, accompanied by decreasing worker fat body.

Aestival colonies.

In early aestival conditions a foraging peak may be expected in nature, as a dynamic response to the recruitment of a new batch of callow nurses (Paper I of this thesis). Worker oviposition/

oviposition declines. The non-dormant brood approach pupation, being fed on a special worker food, and queen oviposition is low. In late aestival conditions queen oviposition is restored at the same time as the non-dormant brood pupate.

Serotinal colonies.

In early serotinal colonies, it may again be supposed that there is increased foraging as a dynamic response to the second batch of callow nurses. There is an increase in the nitrogenous composition of the larval food and a change in the quality of this food. There is no queen or worker oviposition at this time. Dormant larvae are produced, and the worker fat body accumulates.

These aspects of myrmicine sociology are dealt with individually in papers I and III of this thesis

SUMMARY

The factors affecting queen oviposition in Myrmica rubra microgyna have been investigated experimentally.

The effects of high and low temperature, of temperature change, and of worker presence are described. It has been shown that both worker ethal type and worker seasonal condition affect queen egg production.

The sources from which the queen ant derives the food material for oviposition have been segregated. Three possible sources are described. The influence of natural variation in these sources has been considered.

A general theory of queen ovipositional control by the social mechanism of the colony is postulated, and related to other work by the present author.

FIGURE 1 RESULTS OF EXPERIMENT I

The total egg input of two groups, each of three small solitary queens of Myrmica rubra microgyna, is shown as the average of the three replicates comprising each treatment. All queens died within a week of the cessation of oviposition, and none survived for eleven weeks. The time of temperature change is shown by the arrow.

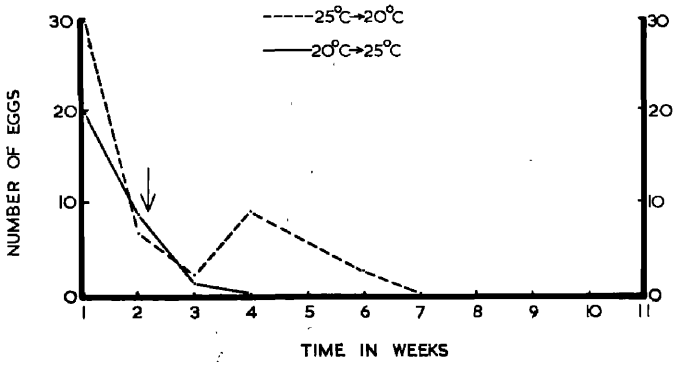
FIGURES 2 & 3

The results of experiments II and III are combined in these two figures, which show the egg input of groups of workers with a queen taken from the averaged value of three replicates of each temperature treatment in experiment II, and five replicates of each temperature treatment in experiment III.

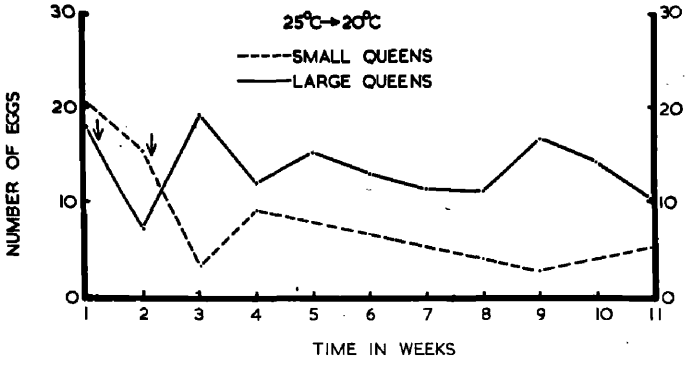
Small queens - Myrmica rubra microgyna (Exp.II)

Large queens - Myrmica laevinodis (Exp.III)

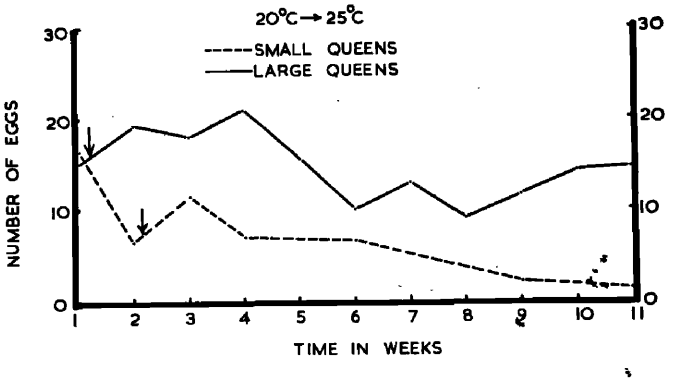
The time of temperature change is shown by an arrow on each graph.


-----25°C→20°C
——20°C→25°C
NUMBER OF EGGS
TIME IN WEEKS

1.


25°C→20°C
-----SMALL QUEENS
——LARGE QUEENS
NUMBER OF EGGS
TIME IN WEEKS

2.


20°C→25°C
-----SMALL QUEENS
——LARGE QUEENS
NUMBER OF EGGS
TIME IN WEEKS

3.

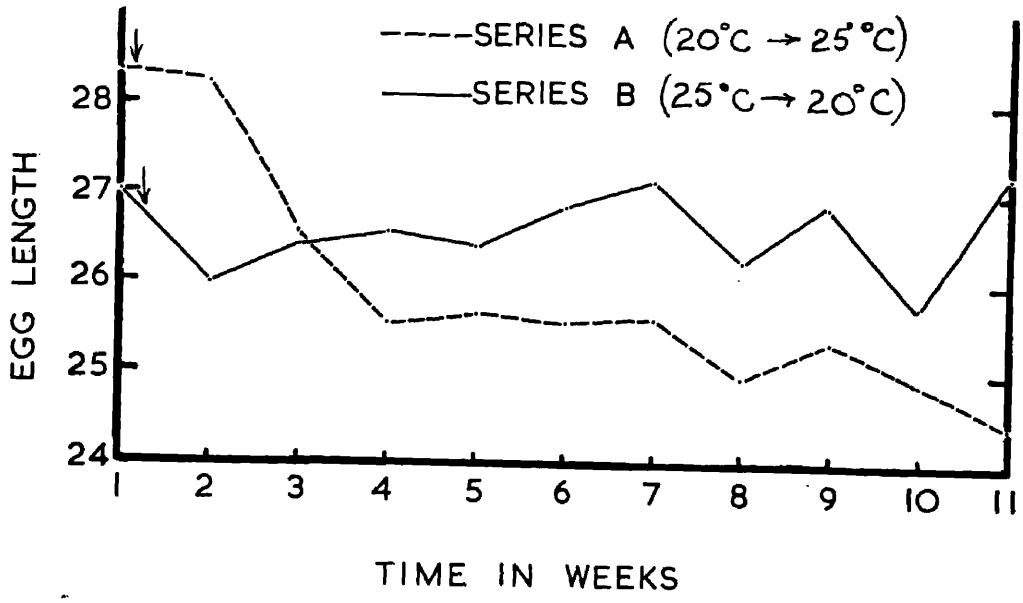
FIGURE 4

These graphs show, as the average of the five replicates of each temperature treatment, the variation in average egg size in experiment III. The time of the temperature change is indicated by the arrow.

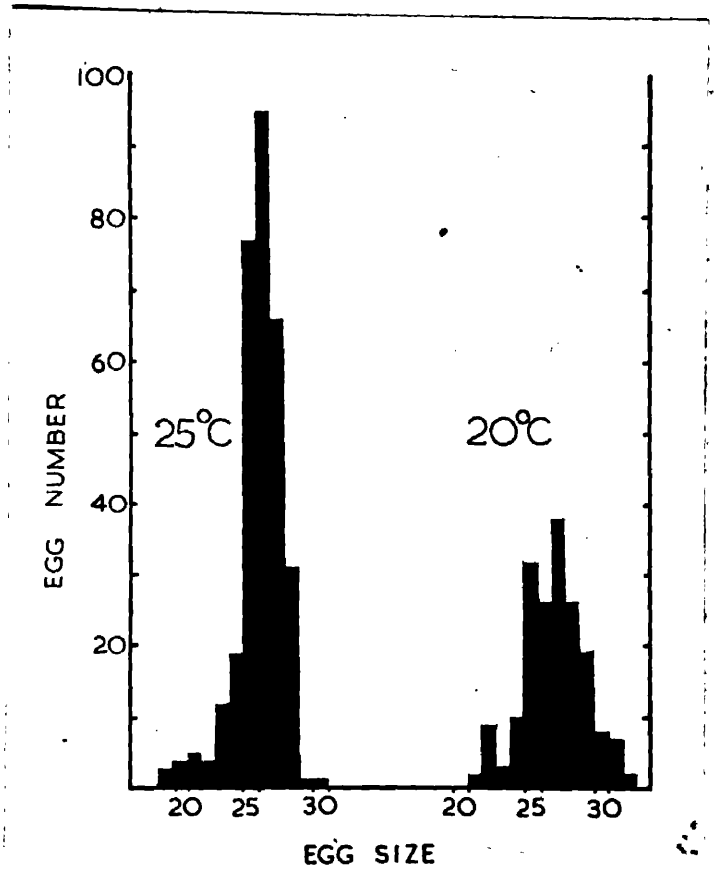
FIGURE 5

These histograms show the differences in experiment III between the frequency distribution of egg sizes at two temperatures. In addition to the lower mean value at 25°C, the degree of skewness of the distributions is different, that at 20°C being a normal distribution and that at 25°C resembling a Poisson distribution.

[1 unit = .0232 mm.]


----SERIES A (20°C → 25°C)
——SERIES B (25°C → 20°C)
EGG LENGTH
28
27
26
25
24
1
2
3
4
5
6
7
8
9
10
11
TIME IN WEEKS

4.


100
80
60
40
20
EGG NUMBER
25°C
20°C
20
25
30
20
25
30
EGG SIZE

5

FIGURE 6

This shows the variation in egg input of some of the individual cultures of experiment III during a period of eight weeks. They have been divided into four main groups according to the total egg input during this eight week period:-

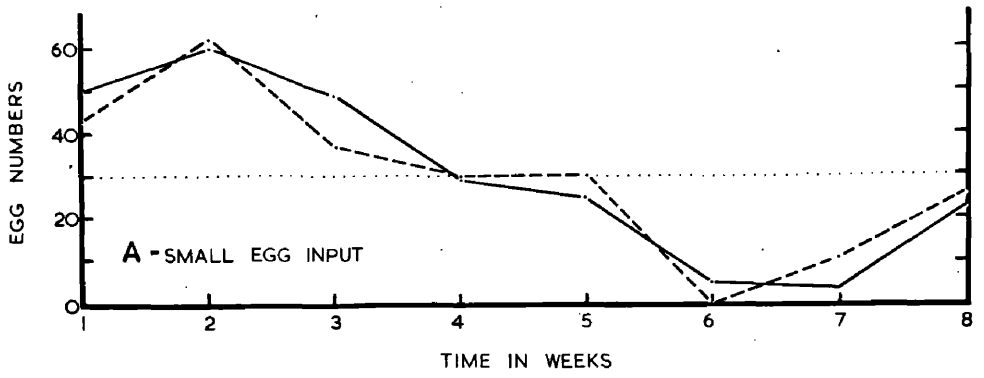
A - Small egg input

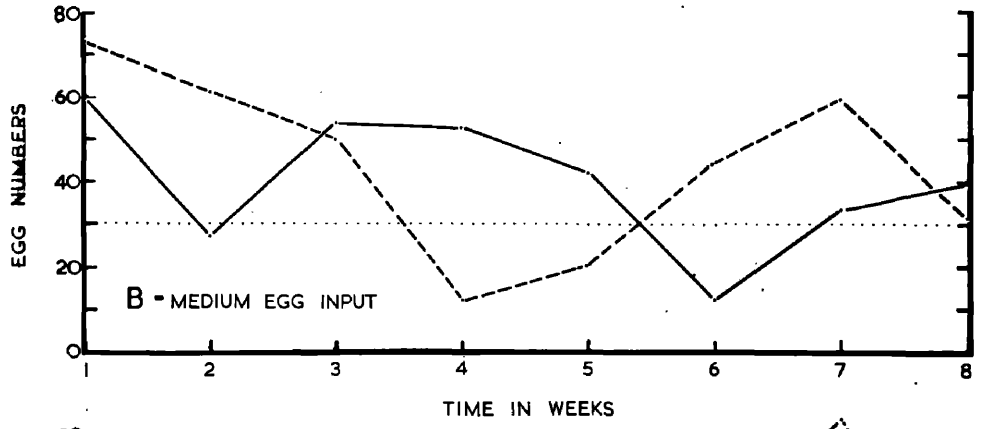
B - Medium egg input

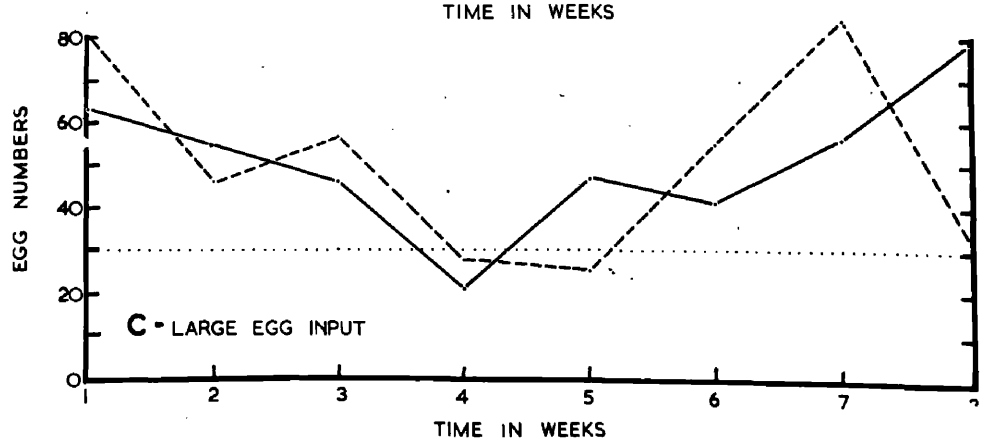
C - Large egg input

D - Large and uniform egg input

The dotted line at 30 eggs per week indicates that this is the arbitrary level below which the E_{qw} of the cultures is considered to be in a state of depression.


EGG NUMBERS
60
40
20
0
A - SMALL EGG INPUT
1
2
3
4
5
6
7
8
TIME IN WEEKS


EGG NUMBERS
80
60
40
20
0
B - MEDIUM EGG INPUT
1
2
3
4
5
6
7
8
TIME IN WEEKS


EGG NUMBERS
80
60
40
20
0
C - LARGE EGG INPUT
1
2
3
4
5
6
7
TIME IN WEEKS

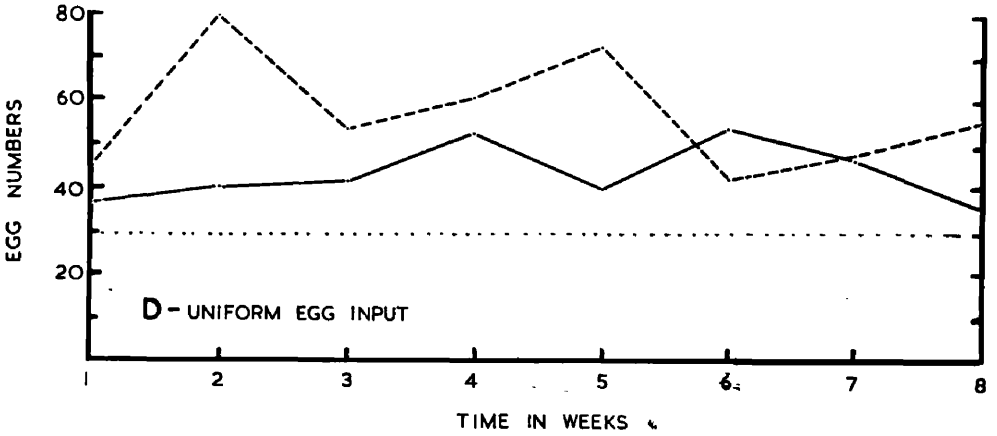

EGG NUMBERS
80
60
40
20
D - UNIFORM EGG INPUT
1
2
3
4
5
6
7
8
TIME IN WEEKS

FIGURE 7

These graphs show the averaged egg input of the three series of replicates (the three worker series F, D and N) in experiment IV.

W_1 = first week

W_2 = second week

W.N. = worker numbers

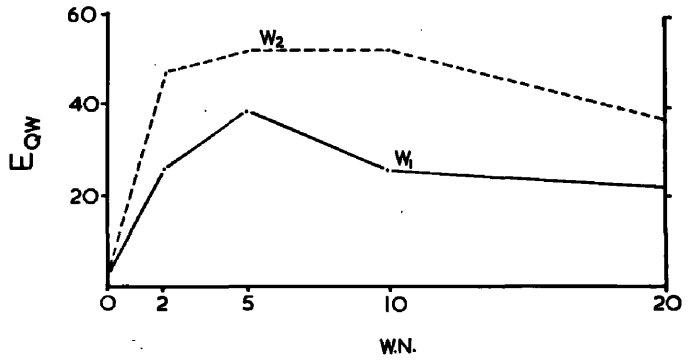
The value for the second week does not include worker series N.

FIGURE 8

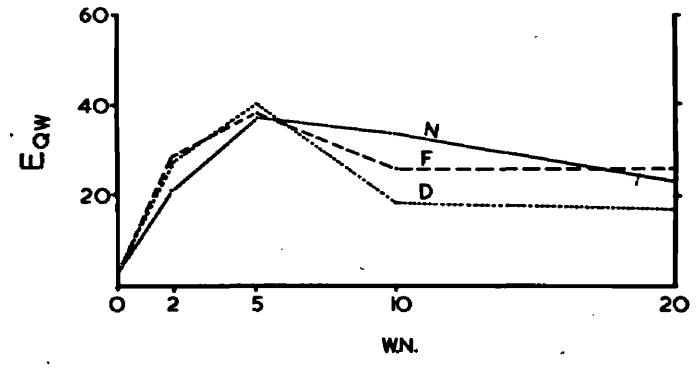
This shows the egg input (E_{qw}) of the cultures of different worker types during the first week of experiment IV. N = Nurses: D = Domestics: F = Foragers: W.N. = Worker number.

FIGURE 9

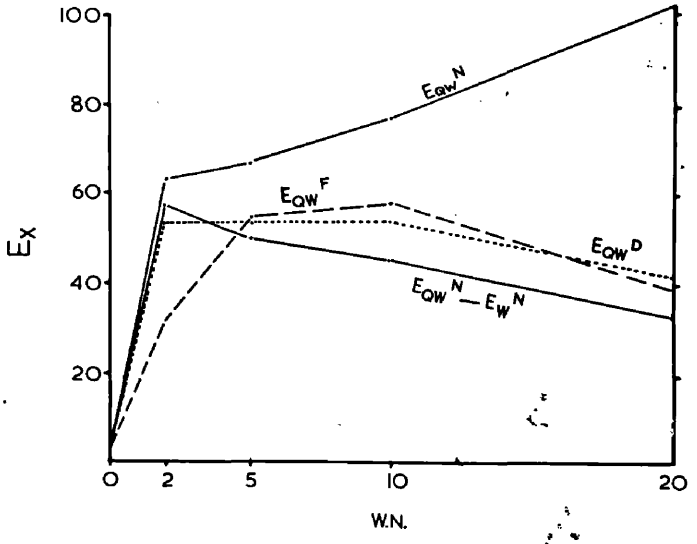
This shows the egg input (E_{qw}) of the cultures of individual worker types during the second week of experiment IV, (N = Nurses: D = Domestics: F = Foragers: W.N. = Worker number), and also a calculated value $[E_{qw} - E_w]^N$ (see figure 11 below).


60
40
20
E_{QW}
W_2
W_1
0
2
5
10
20
W.N.

7


60
40
20
E_{QW}
N
F
D
0
2
5
10
20
W.N.

8


100
80
60
40
20
E_X
$E_{QW}{}^N$
$E_{QW}{}^F$
$E_{QW}{}^D$
$E_{QW}{}^N - E_W{}^N$
0
2
5
10
20
W.N.

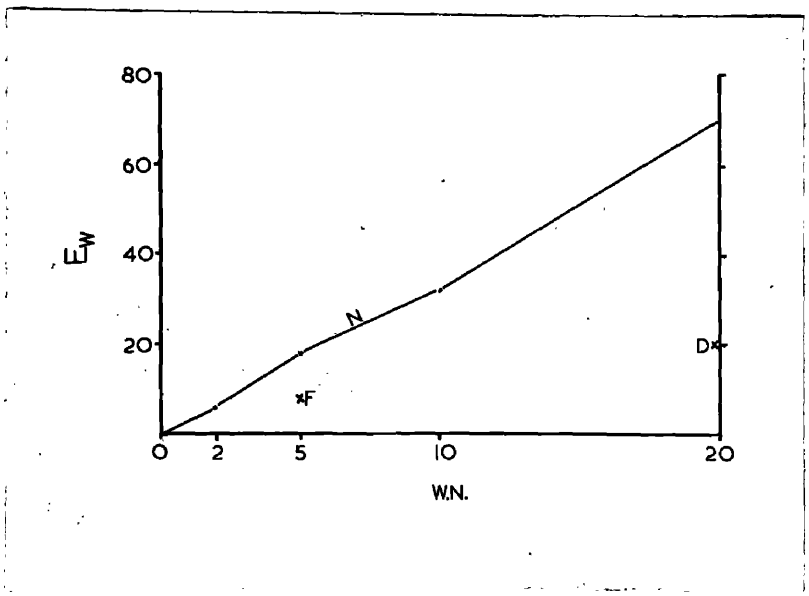
9

FIGURE 10

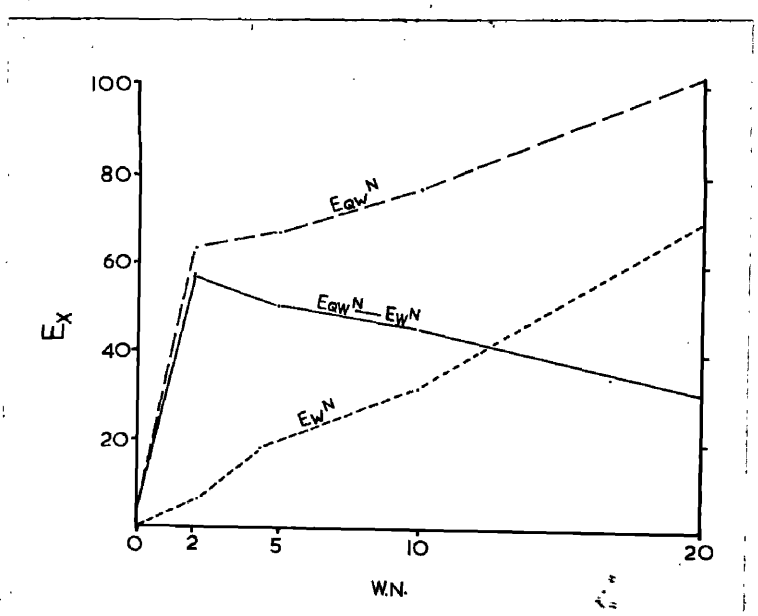
This shows the egg input (E_w) of the worker cultures during the second week of experiment IV. Sporadic egg occurrence in one culture only is indicated by the cross, with the worker type shown beside it.

FIGURE 11

This shows the derivation of the calculated value $[E_{qw} - E_w]^N$ in experiment IV (see figure 9 above) for $E_{qw}{}^N$ and $E_w{}^N$ during the second week of the experiment.


80
60
40
20
E_W
N
D
*F
0
2
5
10
20
W.N.

10


100
80
60
40
20
E_X
$E_{qw}{}^N$
$E_{qw}{}^N - E_W{}^N$
$E_W{}^N$
0
2
5
10
20
W.N.

11

FIGURE 12

These histograms show difference in egg sizes between eggs derived from queen-worker cultures (E_{qw}), and those from purely worker cultures (E_w). The egg lengths of 25 units and 30 units are alone indicated as these show the critical differences which are found.

[one unit = 0.0232 mm.]

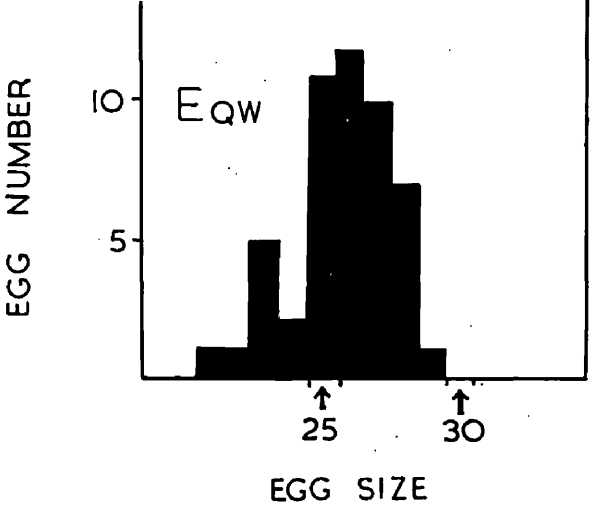
FIGURE 13

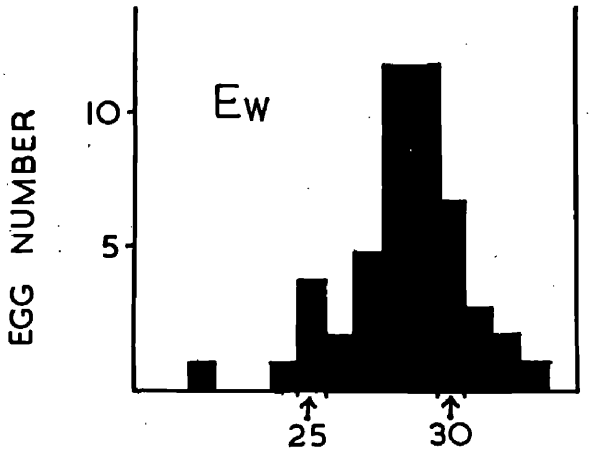
This shows the variation in shape throughout the range in egg size encountered in these cultures. The large eggs (length 30-31 units) which are characteristic only of worker colonies are shown, along with small (length 22-23 units) and medium (length 25-26 units) sized eggs characteristic of both queen-worker and worker colonies.

[one unit = 0.0232 mm.]

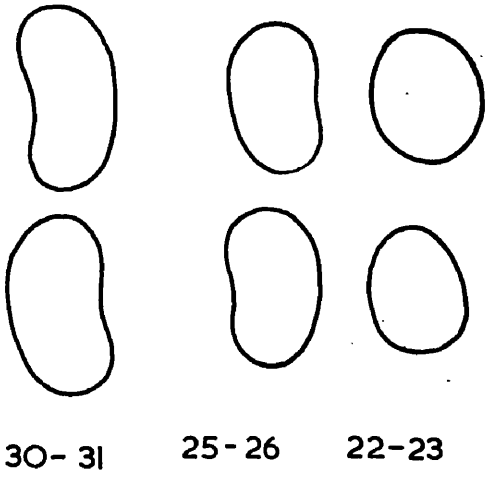
FIGURE 14

This shows the percentage loss in eggs attributable to worker egg eating in experiment V. The continuous line shows the average value of the two replicates at 25°C. The dotted line shows the corresponding value at 20°C.

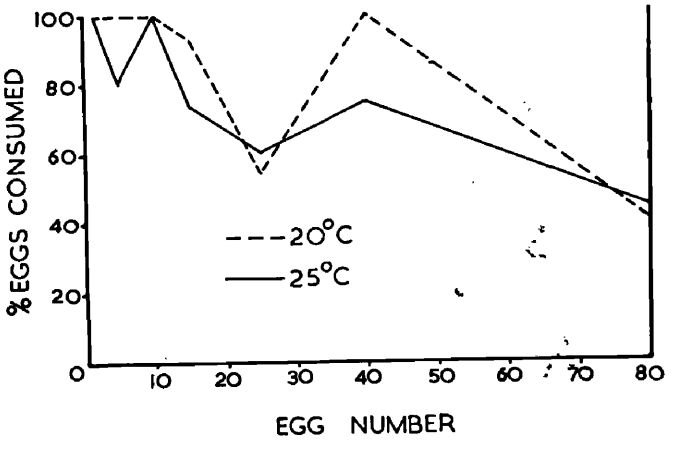

EGG NUMBER
10
5
EQW
25
30
EGG SIZE


EGG NUMBER
10
5
EW
25
30
EGG SIZE

12


30-31
25-26
22-23

13


%EGGS CONSUMED
100
80
60
40
20
0
10
20
30
40
50
60
70
80
---20°C
——25°C
EGG NUMBER

14

FIGURE 15

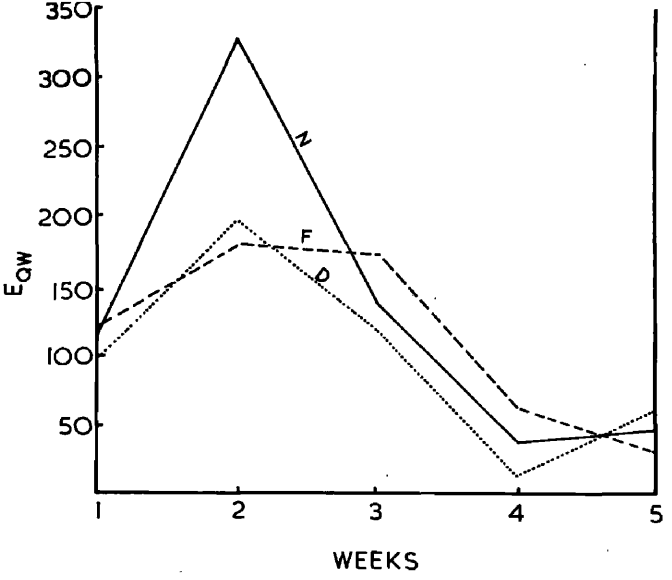
The egg input, during a period of five weeks, of the three groups of queens and workers used in experiment VI is shown graphically

FIGURE 16

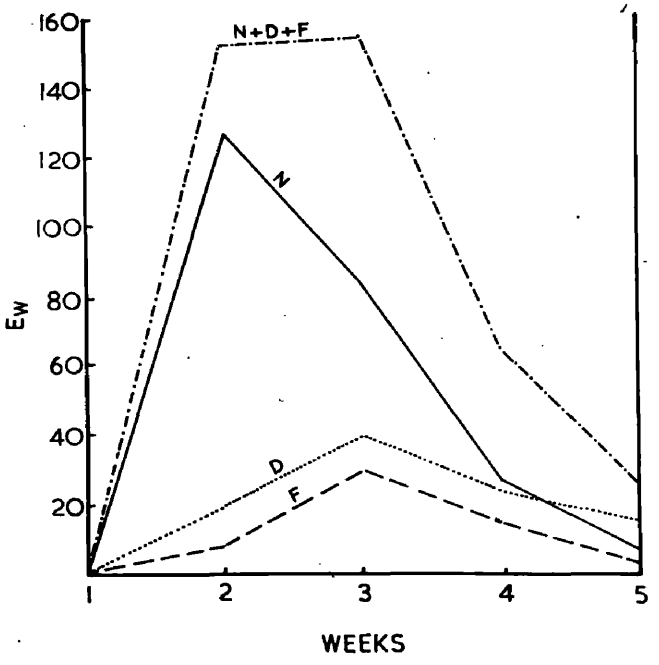
The egg input of the three groups of workers used in experiment VI is shown graphically. N = Nurses: D = Domestics: F = Foragers. The value (N+D+F), calculated by summation, has also been inserted.

FIGURE 17

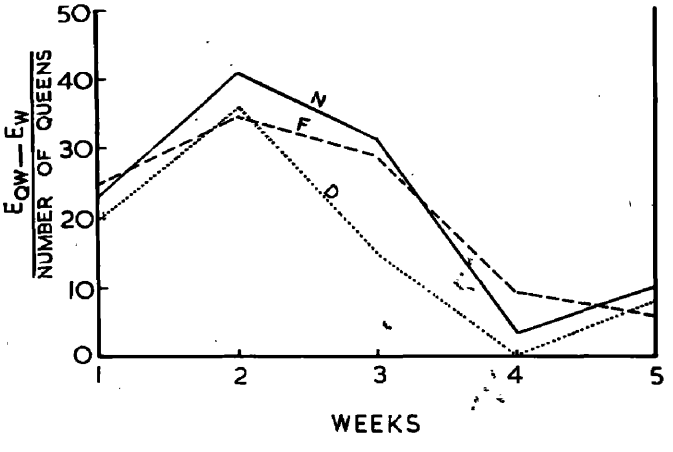
The calculated egg input given by the value $\frac{[E_{qw} - E_w]^X}{QN}$ is shown for each of the three worker series (N = Nurses: D = Domestics: F = Foragers) during the five weeks of experiment VI.


350
300
250
200
150
100
50
E_{QW}
N
F
D
1
2
3
4
5
WEEKS

15


160
140
120
100
80
60
40
20
E_W
N+D+F
N
D
F
1
2
3
4
5
WEEKS

16


50
40
30
20
10
0
$\frac{E_{QW}-E_W}{\text{NUMBER OF QUEENS}}$
N
F
D
1
2
3
4
5
WEEKS

17

CONTENTS

1. INTRODUCTION

The experimental results described in this paper form part of an investigation into the socio-physiological factors controlling dormancy in female larvae of the ant Myrmica, which was carried out in the Zoology Department at Glasgow University during the years 1951-1954. Results are reported on the growth of female larvae in groups and as individuals and the sociological significance of these and preceding observations by the present author is described.

All ants used were collected in the West of Scotland and belonged to the species Myrmica rubra, as subdivided by Brian and Brian (1949) into M. rubra macrogyna and M. rubra microgyna.

Brian (1951b, 1951c, 1954) has described the possible variations in the path of female larval development. Briefly Brian has shown that female larvae may undergo metamorphosis in the summer of the year of eclosion and become workers (larval path-development via non-dormancy) or may be overwintered and undergo metamorphosis in the year following eclosion (larval path-development via dormancy). These latter larvae may metamorphose to either workers or queens, caste determination being trophogenic and plastic in post-dormant larvae (Brian, 1952,1954).

The structure and function of the retrocerebral endocrine system of the female larva has been investigated by the present author/

author (Weir, unpublished; appendix III of this thesis) as has also that of the alimentary canal (Weir, unpublished; Appendix IV). The results of these two investigations are discussed with reference to the experiments described below. The relevance of the investigation by the present author of worker polyethism (Paper I of this thesis) and of queen oviposition (Paper II of this thesis) is indicated in the text.

The present paper attempts to relate information derived from the above sources with other original work on larval growth by the present author, and proposes a mechanism of socio-physio-:logical control of dormancy in Myrmica.

2. EXPERIMENTAL RESULTS

A. The egg mass and larval growth inside it.

The socio-physiological conditions controlling the formation and establishment of the egg mass have already been described (Paper II of this thesis).

Observations show that the egg mass must be continuously maintained by the workers during the period prior to egg eclosion. Neglect of the eggs by the workers results in the loss of the hydrophobic reaction of the egg cuticle in nest conditions. The individual eggs lose coherency and the egg mass/

mass gradually "collapses". Eggs in this condition are licked by workers before replacement in an egg mass and thereafter show a hydrophobic reaction. It therefore appears that the workers normally apply some glandular secretion to the surface of the eggs. In large egg masses, composed of three or four hundred eggs, workers may leave "gaps" which allow antennal access to most regions of the egg mass.

Brian (1951b,1953b) and Weir (Paper II of this thesis) have considered the problem of worker egg eating. Worker oviposition has also been examined by Brian (1953b) and by Weir (Paper I of this thesis). There remains little doubt that worker oviposition is of fundamental importance in the establishment of the egg mass, in its continued existence, and in early larval growth.

Experiments using vitally stained workers (water soluble nile blue sulphate was used) have shown that worker eggs, in undisturbed colonies, may be placed round the outside of a "core" of earlier queen laid eggs. (Queen oviposition may begin seven to ten days before worker oviposition.) Alternatively, worker eggs may accumulate in separate regions of the egg mass. In the latter case it appears that workers may be able to differentiate queen laid eggs from worker laid eggs.

Four colonies containing egg masses with central "cores" of queen laid eggs surrounded by an uneven layer of mixed queen and worker eggs were observed, and the method of worker disruption of the egg mass was noted. In two cases, disruption of the egg mass/

mass occurred immediately after observation was started and was probably due to excessive and abnormal light stimulation. Workers seized portions of the egg mass, tore them from the main bulk, and carried them off. When these fragments were later reconstituted, there did not appear to be any systematic structure in the reconstituted mass. In the remaining two cases however, workers only disrupted the egg mass after larval eclos-:ion. Larval flexing movements, while they may have caused some disturbance, were not directly responsible for the break up, which was carried out by the workers. Larvae were removed, laid on their backs, and some residual eggs placed on or near them. The fate of other residual eggs from the egg mass is unknown.

Two egg masses were examined immediately the first larval eclosion was observed. The eggs could be divided into two categories:

A. Those which showed recognisable signs of development,

B. Those which did not.

These results are shown as follows:-

Qualitative Composition of the Egg Mass.

	Non-developing (B)	Developing (A)	Totals
Egg Mass I	125	130	255
Egg Mass II	52	72	124

When/

When eclosion begins in an egg mass from a colony of normal queen/worker ratio (i.e. one comparable to a small natural nest), there are approximately equal numbers of developing and undeveloped eggs in the mass.

B. Condition of early larval growth and survival.

Measurement of the head capsule of larvae removed from egg masses showed that three instars were recognisable.

First instar larvae are recognised by the possession of, at most, two hairs. (These are always on the head). The points of development of other hairs are sometimes visible as a cuticular thickening on the thoracic and abdominal segments, but these are not elongated to form hairs. Second instar larvae show between six and thirty four cephalic, thoracic and abdominal hairs. Third instar larvae possess a very large number of hairs (over two hundred in some cases) with considerable individual variation in their length and number on different larvae.

First instar larvae may be divided into two categories designated IA and IB, characterised as follows:-

IA: Length 10-11 units (1 unit = 0.0625 mm.)
No air in tracheae. Head disproportionately large compared with the body. Recognisable external differentiation and tapering in dorsal view of abdominal segments.

IB/

IB: Length 12-22 units (1 unit = 0.0625 mm.)
Air in tracheae and tracheoles. Body proportions closer to those of older larvae. Abdomen without recognisable complete external segmentation, and untapered.

Both these conditions are recognised in first instar larvae within the egg mass. IA larvae are newly-eclosed larvae and experimental observation shows that the consumption by the newly eclosed larva of one egg, can convert the the IA larva to a IB larva. Larvae in IB condition appear capable of consuming an indefinite number of eggs. Larvae in IA condition appear to function largely as automatic feeding machines. Stimulation of these larvae results in mandibular opening and closing, flexing movement of the body, and the initiation of pharyngeal sucking. This results in the consumption of adjacent eggs in the egg mass since the mandibles of a first instar larva are in fact sclerotised (contrast: Brian 1951b) and are capable of piercing other eggs at any stage of development, although some may be eaten more easily than others.

Some of the experimental difficulties associated with the early instars are demonstrated as follows.

Experiment 1

This shows the necessity of eggs for the survival of first instar larvae of type IA (newly-eclosed). Sixty-four of these first instar larvae were cultured in groups of four, each group with/

with ten vernal* Myrmica rubra microgyna workers, for eight days, at 25°C. To eight of the cultures ten worker laid eggs were added, eight cultures received no eggs. The experiment was undertaken in four blocks; the larval survival and egg recovery after eight days is shown in Table I, as the total recovery from each block.

TABLE I

A. Larval survival in experiment 1 after eight days.

	With ten worker eggs per culture.	Without eggs.
Blocks 1 + 2	9/16	1/16
Blocks 3 + 4	7/16	1/16

B. Egg recovery in experiment 1 after eight days.

	With ten worker eggs per culture.	Without eggs.
Blocks 1 + 2	30/40	-
Blocks 3 + 4	18/40	1

The necessity of eggs for significant larval survival may be/

* The terms vernal, prevernal, aestival, serotinal, as used in the present report are defined in Paper I of this thesis.

be explained by:-

1. The use of eggs as the sole larval food during the early part of this instar.

2. The necessity for a significant bulk of brood before worker recognition is possible. Worker neglect under the conditions of experiment here used causes the rapid death of first instar larvae.

3. The inability of workers to distinguish first instar larvae from eggs. As a result first instar larvae of type IA receive no worker supplied food and are dependent on eggs for food, although if given worker food they might survive.

Experiment 2.

This shows the greater mortality under adverse conditions of first instar larvae compared with third instar larvae. Twelve cultures each comprising ten serotinal workers, and thirty larvae, were reared, six at 25°C and six at 20°C. Larvae in half the cultures were of type IB and were cultured with thirty queen eggs, while larvae in other cultures were small serotinal third instar larvae. Larval survival after fourteen days is shown in Table II. Larval mortality with these worker/larva (W/L) ratios and serotinal workers is heavy.

TABLE II/

TABLE II

Larval survival after fourteen days in experiment 2.

		3rd Instar Larvae			1st Instar Larvae		
		Culture			Culture		
		A	B	C	A	B	C
Temperature	25°C	9	15	11	0	4	1
	20°C	16	17	14	12	8	8

Extreme larval mortality occurs among first instar larvae at 25°C, reared by serotinal workers. Third instar larvae however show moderate survival capacity (40%) under these conditions. More than twenty experiments have been performed along these lines and led to the general conclusions enumerated below.

These are as follows:-

1) It is probable that in undisturbed nests in nature there is some systematic organisation of the egg mass.

2) Larvae are not directly responsible for the disruption of the egg mass though they may influence the workers which do this.

3) There are three larval instars.

4) Eggs are necessary for the survival of first instar larvae.

5)/

5) The necessity for eggs may operate either by their necessity for food or by their necessity to form a recognisable brood mass which will not be ignored by the workers.

6) Even under conditions of egg abundance, the action of adverse environmental and sociological conditions shows that first instar larvae are less resistant than third instar larvae.

The inherent sensitivity and high mortality of first instar larvae (experiments 1 and 2 above) led to attempts to devise better cultural techniques for rearing these larvae (experiment 3 below). High larval mortality is not confined to Myrmica, e.g. Peacock et al. (1954) working on Monomorium consider that only 25% of young larvae become adults.

Experiment 3.

Eighteen cultures of M. rubra microgyna were used each comprising twenty first instar (IB) larvae and twenty aestival workers all cultured at 25°C. The cultures contained variable numbers of:-

A. Small, overwintered, third instar larvae, of the same nest.

B. Worker pupae from the dormant brood of the same nest.

C. Worker prepupae from the dormant brood of the same nest.

There were three control cultures which contained only the

first/

first instar larvae. The survival of first instar larvae in all cultures after fourteen days is shown in table III.

TABLE III

Larval survival in experiment 3 after fourteen days.

		OTHER FORMS OF BROOD PRESENT IN CULTURES		
		3rd instar larvae overwintered	Prepupae from overwintered larvae	Pupae from overwintered larvae
Number of other forms present	2	-	5	-
	5	1	3	1
	10	1	4	3
	25	2	5	5

Larval survival in the three control cultures was as follows:- 0, 0, 1.

The results of experiment 3 are inconclusive. They show that, under laboratory conditions, there is a significant difference in larval survival between cultures containing prepupae and those containing third instar larvae. It is tempting to suggest that in colonies in nature, the incidence of prepupation is associated with a change in worker occupation from the feeding of third instar larvae to the care of young larvae. In several cases young larvae have been observed on the/

the ventral thoracic surface of prepupae. [The placing of eggs on the ventral thoracic surface of the larva is a characteristic form of worker behaviour associated with the feeding of larvae. Third instar larvae at some stages have a ventral thoracic depression into which eggs may "fit" so securely that they have to be "prised" loose with a needle. This forms an interesting comparison with the praesaepium of certain sub-tropical formicine larvae.] The accumulation of more eggs and young larvae on a prepupa may result in the formation of a brood mass. This may be compared with the association of eggs and pupal cells in Bombus (Brian & Brian, 1948). The significance of this effect in nature is unknown.

Experiment 4.

Young larvae in nature may be, at various times, subjected to the influence of other brood forms, e.g. prepupae, pupae and third instar larvae due to their contemporaneous presence in the nest (experiment 3 above). Also, at times, callow (recently emerged) workers are associated with the brood mass, along with aestival overwintered workers. An experiment (4) was designed to show whether callows produced greater survival of first instar larvae than did aestival overwintered workers under the same conditions. The experiment comprised eight cultures (each con-:taining twenty M. rubra microgyna workers, and thirty second instar larvae) which were reared for fourteen days, four at 20°C and four at 25°C. The larval survival during this experiment is shown in table IV, and the average length of larvae in table V.

TABLE IV

Larval survival after fourteen days in experiment 4

	25°C		20°C	
	Trial A	Trial B	Trial A	Trial B
Aestival Overwintered Workers	18/30	14/30	25/30	29/30
Callows (three weeks old)	18/30	12/30	7/30	16/30

TABLE V

Average size of larvae after fourteen days in experiment 4

	25°C		20°C	
	Trial A	Trial B	Trial A	Trial B
Aestival Overwintered Workers	34.2	31.2	24.4	23.7
Callows (three weeks old)	32.6	34.0	25.1	22.1

(1 unit = 0.0625 mm.)

Results show that at 25°C the survival capacity of the larvae with both worker types is very similar. At 20°C however, the survival capacity of the larvae with overwintered aestival workers is much higher than with callow recently emerged workers. The results of the larval growth increases show, as might be expected, that larvae with both types of worker grow faster at 25°C. There is no evidence in experiment 4 to show that callow workers exert any critical influence on larval survival at this time, except possibly under adverse environmental conditions of low temperatures. (Such permanently low temperatures are unlike-:ly over long periods in mid-summer in the West of Scotland.)

Experiment 5.

Brian's observations (1951b) show that certain larvae produced from the spring egg peak become dormant, along with all larvae from the summer oviposition peak. The differentiation of larval path development in groups of varying sizes has been repeatedly investigated. Experiment 5 describes such larval path divergence in one group of 20 first instar larvae, 20 vernal workers, and 4 prepupae. This is compared with larval path development in 10 groups each composed of 2 first instar larvae, 3 workers, and 2 prepupae, all groups being cultured at 25°C. The unequal worker/larva ratio is unavoidable if the effects of worker depression noted by Brian (1953a) are to be avoided. The results of this experiment are shown in figures 1 and/

and 2. In figure 1, the differential increase in size shown by certain larvae between the 10th and 14th days, indicates that these larvae are potentially non-dormant and will metamor-:phose during the current season. Three larvae did in fact metamorphose. Within the same period of time in which the non--dormant larvae of the large group metamorphose, it is apparent from figure 2 that no larvae in the small colony fragments do become non-dormant. Also, the length of the larvae within each small group remains very similar throughout the experiment, but there is wide variation in the average larval size between all the small groups at any one census. The overall size variation shown by larvae within these small groups produces a larval size range comparable to that shown by the large culture, if the rapid brood are discounted. It is difficult to assign these differences in dormant larval growth to any one cause, but it is apparent that either inherently "good and bad" worker groups or inherently "good and bad" larval groups must exist. The probable existence of "good and bad" worker groups has been demonstrated elsewhere (Paper I of this thesis, sections 3 & 4).

It may be indicated at this point that while over two hundred larvae have been individually examined and over two hundred reared individually or in groups of two larvae, no non-dormant larvae have ever been reared from the first instar either in isolation or in groups of two larvae. While certain isolated larvae have metamorphosed during the same year as egg eclosion,/

eclosion, these have all shown critical differences compared with the non-dormant larvae produced in large groups as described above (figure 1) or have been isolated in the third instar. These differences are described subsequently. The worker/larva ratios used for these experiments have never greatly exceeded those ratios known to prevail in nature (Brian, 1953a).

Conclusions from experiment 5 and from similar observations during the laboratory production of large numbers of non-dormant larvae show that under these laboratory conditions:-

1. Non-dormant larvae occur normally in larval groups.
2. The range of weights of dormant larvae at dormancy differ and the differences can be attributed to:-
   a) Quantitative effects such as bias in larval groups (sections 3 & 4 of Paper I of this thesis);
   b) Qualitative effects such as differences in worker or larval efficiency (sections 3 & 4 of Paper I of this thesis).

C. The growth of larvae in groups.

The mechanism controlling the production of non-dormant larvae in larval groups was further investigated in experiment 6.

Experiment 6.

It comprised fifteen cultures in three series of five cultures containing different numbers of aestival workers and larvae./

larvae. The five cultures in each series contained 5, 10, 15, 35 and 75 first and second instar larvae, along with 5, 10, 15, 35 and 75 eggs respectively. Cultures in each of the three series contained 10, 25 and 50 workers respectively. The experiment was carried out at 25°C. The individual cultures were in glass basins with smaller basins containing sugar and Drosophila larvae. There was an ample supply of food from these sources throughout the experiment. In certain cultures, the larvae were heaped by the workers on the floor of the basin.

The results of this experiment must be considered in relation to those of Brian (1953a). Whereas Brian investigated changes in the brood rearing efficiency of workers by variation of the worker/larva ratio while the size of the brood mass was initially constant, in this experiment (6) both the worker/larva ratio (the W/L ratio) and the size of the brood mass are varied.

The results of experiment 6 are considered, first in respect of the total brood survival as a percentage of the original quantity of brood (Table VI). The percentage survival decreases with increasing size of the brood mass and increases with increasing worker number, so confirming in part the observations of Brian (1953a) for cases where there is adjustment of the size of the brood mass to worker number.

TABLE VI/

TABLE VI

Percentage Survival of larvae from initial brood mass in experiment 6, after thirty days.

		WORKER NUMBER		
		10	25	50
Larval Number	5	100%	100%	80%
	10	60%	80%	100%
	15	40%	100%	100%
	35	22.8%	31.4%	42.8%
	75	22.6%	25.3%	30.6%

These results show no differential variation with the W/L ratio between the three brood group sizes in respect of the percentage survival, after thirty days, of the initial brood mass.

The rate of production of pupae and prepupae shows considerable variation. Table VII shows the numbers of pupae and prepupae present after fourteen days. This can also be expressed as a percantage of the total number of pupae and prepupae produced in thirty days (Table VIII).

TABLE VII/

TABLE VII

Pupae and prepupae produced after fourteen days in experiment 6

Initial Larval Number	WORKER NUMBER		
	10	25	50
5	1	2	-
10	1	4	3
15	2	4	6
35	1	5	5
75	3	5	6

TABLE VIII

Number of pupae and prepupae produced during fourteen days as a percentage of the total number produced during thirty days.

Larval Number	WORKER NUMBER		
	10	25	50
5	25%	66%	0%
10	50%	80%	37%
15	40%	40%	85%
35	25%	71%	71%
75	27%	45%	50%

Certain distributional anomalies in table VIII appear to be caused by variation in the sizes of pupae and prepupae produced, e.g. the low values in those cultures containing 50 workers and 5 or 10 larvae, similarly with the culture of 25 workers and 15 larvae. These data are unfortunately incomplete and cannot be tabulated. It is apparent however that the initial size of the larval brood mass may be affecting the rate of production of pupae and prepupae. The causes of such an effect are obscure.

Examination of the total number of non-dormant brood produced during 30 days shows that, if expressed as a percentage of the brood numbers at 14 days, those having been adjusted by the workers, they decrease both with worker number and with brood-mass size (Table IX below). Similar figures relative to the initial size of the brood mass show even greater decreases with the above factors.

From these results it appears that the adjustment of larval numbers which had taken place after 14 days was insufficient to ensure that 100% of the then available larvae became non-dormant and metamorphosed. Both increased numbers of available larvae and the W/L ratio affected the result.

Worker brood rearing success in this experiment (6) in respect of dormant larvae is shown in figure 3. In this figure, the average size of larvae remaining after 30 days is graphed against the W/L ratio. While the complete data show a consider-

:able larval size range, the average size of these residual larvae/

TABLE IX

Column A: The larval numbers in the brood mass after being subjected to worker adjustment for 14 days.

Column B: The total number of non-dormant brood produced during the 30 days of the experiment as a percentage of A.

		10 Workers		25 Workers		50 Workers	
		A	B	A	B	A	B
Larval numbers	5	5	100%	6	100%	5	80%
	10	7	71%	8	100%	14	79%
	15	6	100%	18	72%	18	50%
	35	8	87%	15	53%	18	61%
	75	19	73%	26	61%	34	47%

larvae varies with the absolute size of the brood mass as well as with the W/L ratio.

In conclusion, with regard to experiment 6, it may be said that aestival worker brood rearing success varies (in respect of non-dormant brood and dormant brood both of which were produced) both with the worker/larva ratio and the absolute size of the brood mass. With increasing size of the brood mass in experiment 6, worker brood rearing was less efficient in respect of larval survival, and the percentage of non-dormant brood produced. Further, with increased numbers at the same W/L ratio, worker brood rearing was less efficient measured by the average size of the residual larvae. Such results might be attributable to shortage of environmental food, yet in all cultures, sugar and Drosophila larvae were present. If poor larval brood rearing was caused by food shortage, this shortage was due to worker negligence and not to the experimental design. The only apparent advantages in brood rearing efficiency from increased worker numbers were the production of larger prepupae, and an increased rate of production of prepupae and pupae. These factors have not been effectively measured in experiment 6.

Two possible explanations of the results of experiment 6 are given here. These are not mutually exclusive.

A./

A. Worker negligence causing a shortage of environmental (allochthonous*) food in circulation and resultant larval starvation. Such negligence might increase with worker number for the same W/L ratio, and might depend on such factors as the accessibility of the workers to the larvae.

B. Brood piling by workers accompanied by lack of larval circulation by the workers. The larger the brood mass, the smaller the chance of any one larva being fed, but those larvae which as a result of stoichastic factors were on the surface, would receive the benefits of any worker food available.

In both these cases the opposed effects of 1) increased rate of production of non-dormant larvae with increasing worker number, and 2) decreased residual larval size with increased worker number are difficult to explain except in terms of autochthonous food produced by aestival workers in limited quantities.

A special food may be produced by workers in amounts proportional to the number of workers present. This may be allocated preferentially to a restricted number of larvae in any group. As a result, the percentage of non-dormant brood produced from these preferentially fed larvae may be dependent on/

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* Larval food in Myrmica may be considered to have a dual origin:

A. Autochthonous or sociological food, e.g. eggs or worker glandular secretions.

B. Allochthonous or environmental insect food, e.g. insect protein, or sugars.

on the degree of worker accessibility to them, i.e. dependent on the number of larvae on the surface of the brood mass. Therefore, for any particular worker/larva ratio, increased worker and larval numbers would result in increased numbers (or increased rate of production) of non-dormant brood. This increase may not be proportional to worker number, but to the interaction of the worker number and the surface area of the brood mass. The average size of residual dormant larvae would also be likely to vary inversely in such circumstances, since large brood masses, with less accessible larvae in the centre (i.e. unfed larvae) also result from group size variation at the same worker/larva ratio.

It should be noted that seasonal differences in the degree and effects of brood piling may occur. Aestival or serotinal workers produced from pupae during the previous year become domestics (Paper I of this thesis). Domestics have been shown to have strong brood piling tendencies, while they do not circulate the brood. Therefore the incidence of such effects as noted in aestival condition may be different with vernal workers which are, in nature, nurses. However, the natural incidence, seasonal or otherwise, of brood-piling and its effects is not yet known.

D. The measurement of larval growth and development./

D. The measurement of larval growth and development.

In studies on the growth of individual larvae during their period of determination for dormancy or non-dormancy, various measurements have been made on these larvae. These measurements include:-

i. The total area

This was done by projection of the lateral view from a slide of standard depth, the larva being held down under water by a coverslip. For small larvae this gives a rapid and accurate measurement of bulk, and is also useful in the detection and measurement of quantitative differences in the volumes or condition of various internal organs (e.g. the bladder).

ii. "Girth", i.e. Weight/length or area/length

Girth is used as a measurement in preference to length or weight alone for the following reasons. Experiments show that an early third instar larva first elongates and then expands laterally in the course of growth and development. Measurement of length alone fails to reveal this lateral expansion, and measurement of weight alone is unreliable since worker neglect prior to lateral expansion may result in evaporation and/or in the accumulation of large quantities of urine, both of which cause variation in weight alone but not in girth. Also accurate measurement of weight in these small larvae is difficult (a first instar larva may weight 0.05 mgm., and an early third instar larva 0.3 mgm.).

iii. The area of the residual food in the gut.

This has been achieved by projection as in i above. The area measured is the area enclosed by the secondary peritrophic membrane (Weir, unpublished, Appendix IV).

iv. The length of the malpighian tubules occupied by white crystalline material*.

This is also achieved by projection as in i above. The length of the individual tubules occupied is measured and summed for each individual larva. In Myrmica larvae, both the length of tubule filled with these white crystals and the width of the tubule filled are variable. This is not the case in certain other insects, e.g. Rhodnius (Wigglesworth, 1953).

v. The apportionment of the larval brain between the head capsule and the prothorax.

Microscopic examination of the larva in side view under water on a slide of standard depth shows that the posi-:tion of the brain may vary with the state of develop-:ment. This condition has also been described (Brian, 1952,1954) in dormant and post-dormant larvae. Measurements are made of the percentage of the brain which lies behind the posterior border of the head capsule. There is no evidence to show what regulates this movement. It seems probable that it is caused by at least two factors. These are:

- the degree of turgor of the body during develop-:ment. The present author (Weir, unpublished, Appendix II) has shown, as has Brian (1954) that the degree of movement of the brain may be affected (before a critical stage** is reached) by the degree of hydration of the larva.

- the change in shape of the brain, in an immediately post-critical condition, as a result of differential growth in certain regions of the brain. (Weir, unpublished, Appendix III of this thesis).

The/

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* No chemical tests have been undertaken on this material. It is presumed to be crystalline uric acid.

** This critical stage is described in Appendix II of this thesis.

The effect of such factors as synchronous growth in closely apposed imaginal rudiments is unknown but is probably secondary.

vi. The measurement of imaginal buds as developmental markers.

Imaginal buds (e.g. those of the wings and legs) have been utilised by Brian (1952,1954) to describe changes in growth and development in post-dormant larvae. In the course of the experiments described below and others undertaken by the present author, it became apparent that measurement of larval development by these buds was inadequate in larvae prior to dormancy in view of their small size. Measurement of the antennal bud shows signi-:ficant growth prior to the critical stage and it has been used here as a developmental marker. The early growth of this bud in larvae of Myrmica agrees with the observations of Tiegs (1922) on Nasonia.

The growth and development of the antennal bud has not been followed to completion, since the present investigation is concerned with only pre-dormant larvae. It is hoped to describe elsewhere certain unknown structural peculiarities of this and other imaginal buds.

E. The significance of worker seasonal condition.*

Experiment 7.

This was undertaken to investigate differences in the brood/

* Some preliminary experiments on this point had been performed by Mr. M.V. Brian who kindly supplied the unpublished results of them. More detailed results obtained by the present author and presented here have made it unnecessary to quote Brian's figures.

brood rearing potentiality of workers of three seasonal conditions. Six cultures were used, each containing 25 larvae, 25 worker-laid eggs, and 25 workers. Workers were of three kinds - vernal overwintered workers, serotinal overwintered workers and aestival callow workers (produced three weeks previously from overwintered larvae). Three of the larval groups were of first instar larvae, three of early third instar larvae. The results of this experiment after seven days at 25°C are shown in figure 4. The three larval developmental paths attributable to the three worker types are shown. The paths of first instar and early third instar larvae correspond in all three cases. For clarity only one average developmental path is shown, and the length of path indicated represents the maximal distance that any larva has proceeded along this path.

The individual results of this experiment show that larvae have made widely different progress along different develop-:mental paths. Serotinal workers show least success, callow workers produce an intermediate group, and vernal workers produce the best growth and development. Differential larval growth and development has occurred in all cultures, due presumably to biased or preferential feeding, and certain larvae in all cultures show little progress. The significance of this experiment however lies in the differing path develop-:ment produced in those larvae which show the greatest growth and development in each culture.

Experiments/

Experiments and observations referred to have shown that a critical larval developmental stage can be related accurately to the length of the antennal bud. Within a period of seven days from the beginning of this experiment (7) certain third instar larvae had passed this critical developmental stage. This implies that these larvae have been determined for non-dormancy. The maximal distance that a group of larvae can travel along a developmental path may alone differ, since the time taken for workers to influence all the larvae in the group may be such that the "condition" of the workers themselves undergoes change. It is concluded from this experiment that different larval developmental paths are associated with workers of different seasonal "conditions".

Experiment 8.

This experiment was designed to elucidate certain aspects of experiments 6 and 7. In both of these the effect of differential larval growth, or perhaps even preferential worker treatment of larvae in groups, was obscuring the significance of worker condition in larval path development.

Experiment 8 consisted of ten cultures each containing three early third instar larvae, and ten workers. Workers were from two sources, eithal all vernal or all serotinal. No workers derived from brood of the current season were included. The average larval path development in the two series of cultures/

cultures of experiment 8 is shown in figure 5, after 14 days at 25°C. In this experiment the small larval groups largely over-:came the problem of biased feeding and nearly all larvae were obviously influenced by worker treatment. No larvae cultured by serotinal workers reached the critical larval developmental stage. All except one of the larvae cultured by vernal workers reached and passed this critical stage.

It was concluded from this experiment that all larvae in the early third instar may enter upon either of the possible developmental paths. Selection of certain larvae in a group for any particular developmental path is then probably stoichastic, and not controlled by critical larval differences (i.e. path determination prior to experimentation) in the early third instar, although larval differences (e.g. of size) may bias the probability of entrance upon or selection for any particular developmental path, by induction of bias in their treatment by the workers.

Experiment 9.

This was designed to investigate the mechanism of seasonal worker control of larval path development.

A colony containing 20 workers and one queen of M. rubra microgyna was removed from the field in early aestival condition and cultured at 25°C. Callow workers produced from overwintered larvae/

larvae were removed from the colony as were any residual pupae and prepupae derived from the overwintered brood. Measurements were made at intervals of eight days on all third instar larvae produced in this colony. All third instar larvae which had reached or passed the critical developmental stage at each census were removed and discarded after measurement. The results of this experiment are shown in figures 6 and in table X.

TABLE X

The initial condition of larvae during experiment compared with larvae removed during the three successive censuses

		MEASUREMENTS UNDERTAKEN				
		% Brain outside head capsule	Length of antennal bud	Area of wing bud	Area of limb bud	Volume of malpighian tubule occupied by uric acid crystals
Initial values	Max.	0.5	35+5	5	9	20
	Av.	0.3	32+4	4	8	10
	Min.	0.1	25+3	3	4	4
Census 1	Max.	1.0	150	15	4S	22
	Av.	1.0	145	10	3-4S	7
	Min.	1.0	120	6	3S	2
Census 2	Max.	0.4	35+5	6	9	27
	Av.	0.3	30+3	3	6	20
	Min.	0.2	23+2	2	3	6
Census 3	Max.	0.4	30+4	4	6	34
	Av.	0.2	25+5	3	4	26
	Min.	0.0	20+2	2	2	11

For ease of assimilation the tabulated figures of wing bud area, limb bud area, and uric acid volume have been derived by calculation from the original data and no absolute values can be assigned to the units. At the first census, the onset of limb bud segmentation has prevented accurate measurement of the considerable areal increase and the numbers of segments (S) are indicated instead.

In measurement of the antennal bud length 1 unit = 0.00294.

In successive groups of larvae reaching the critical stage, there was a steady increase in the area of residual food in the gut proportional to the total area of the larva, associated with a change in colour of the residual material from pale yellow to dark brown. The amount of uric acid produced by the larva in-:creased as did the bulk of the fat body, and decreases in the rate of development as measured by the antennal bud length, the brain movement, wing buds and limbs were noted.

It is concluded that, during the aestival worker condition, which marks an intermediate stage between vernal workers producing non-dormant larvae and serotinal workers producing dormant larvae, there is a change in worker physiology or ethology causing a change in the quality and perhaps quantity of food fed to larvae. This change in feeding may control larval path development and can occur without the influence of callow workers.

The changes here observed account to some extent for the difficulty in interpretation of measurements of the length, weight and girth of the larva. Increases in larval girth and weight may be attributed either to:-

1) The increasing bulk of the imaginal rudiments in non-dormant larvae.

2) The increasing bulk of the residual food in the gut, of the urine, and of the fat body in dormant larvae.

F. The importance of nitrogenous food material and the time of induction of dormancy.

Experiment 10.

The cause of these changes has been further investigated in experiment 10. In this experiment three groups of ten aestival workers were pre-treated for 14 days prior to the experiment as follows. One group was fed on protein alone (Drosophila larvae), one group on sucrose, and one group on both. Each of these three groups then reared ten early instar larvae on the same diet that it had previously received for 14 days. Differences in the larval gut and urine after this period were noted as follows:-

Protein only:
- voluminous dark brown gut;
- urine voluminous, cloudy, containing numerous white uric acid crystals;
- malpighian tubules white opaque and full of uric acid crystals.

Sugar only:
- moderate sized gut, pale brown in colour;
- small amount of urine, no uric acid crystals in urine;
- malpighian tubules with very small numbers of uric acid crystals.

Sugar & Protein:
- moderate sized but, pale brown in colour;
- voluminous urine, a few crystals of uric acid;
- malpighian tubules containing some uric acid crystals.

Thus appearances resembling those observed in experiment 9 can be produced by alteration of the carbohydrate/nitrogen ratio of the food. Sugar, however, does not, in this case, produce the pale yellow colour observed in the gut of larvae reared by vernal workers.

Experiment 11.

Analyses of total nitrogen content by the micro-kjeldahl method of Ma and Zuazaga (1942) have been made on larvae at certain developmental stages and after culture by certain seasonal worker types. The series of analyses described below were made on larvae from one colony of M. rubra microgyna throughout one season. The colony was collected in vernal condition and analyses were made of the overwintered brood. Subsequently, analyses were made of non-dormant larvae, but in figure 7 it is convenient to show these analyses as a continuous seasonal series (i.e. in the reverse order).

From figure 7 it is apparent that, while larval wet weight and dry weight show linear arithmetic increases throughout the season, the total nitrogen content shows logarithmic increases with both these values. The superposition of the values for critical stage larvae and for prepupae of the same ratio of dry weight/wet weight may or may not be fortuitous. It is nevertheless interesting and might suggest that the post-critical intake of nitrogenous material was related in some way to the pre-critical intake.

For instance, it is difficult to avoid the conclusion that the rate of incorporation of nitrogenous material in post-critical larvae shows logarithmic increases in the series non-dormant workers ⟶ dormant workers ⟶ queens, if the time elapsing between the critical stage and prepupation is constant.

If/

If this time increases, then it follows that the nitrogen incor-:poration in the prepupa is a factor of the time elapsing between the critical stage and prepupation. In both cases the situation is confused by the ejection at prepupation of a meconium which may be largely formed in the critical stage larva, and which varies considerably in size, and presumably in nitrogen content.

These estimations of total nitrogen must include a high proportion of intrinsic nitrogen in the cuticle and internal organs. A suggested value (probably an underestimate) has been inserted in figure 7, and this shows that the percentage change in non-intrinsic nitrogen content between these larval groups may well be significantly different. The amount of nitrogenous material ingested prior to the critical stage may control larval path development for dormancy or non-dormancy, but the amount of nitrogenous material ingested between the critical stage and prepupation also appears to be significantly different. It seems, since group E in figure 7, as analysed in the prepupal condition, appeared to represent brood undergoing queen path development, that the larval ingestion of nitrogenous material may well represent the physiological trophogenic mechanism controlling caste determination. This queen path determination as opposed to worker path determination in post-dormant larvae has been investigated by other means by Brian (1952,1954). Observation of the size range and developmental conditions of larvae and prepupae has allowed the several paths of larval development to be related to larval nitrogenous composition (Figure 7).

Line/

Line A → B indicates the change from the critical stage to prepupation in non-dormant larvae, line C → D the equivalent change in medium sized dormant larvae producing workers, and line D → E, the equivalent change in large dormant larvae producing queens.

The form of the curve of total nitrogen content (a logarithmic increase) as opposed to the linear regression of wet weight on dry weight, suggests that another component of the dry weight shows a reciprocal logarithmic increase relative to wet and dry weights. This might be caused by carbohydrates. The relationships of these quantities suggest that this unknown component of the dry weight may occur maximally in non-dormant larvae and prepupae.

Artificial diets have been used in experiments to determine whether simple changes in the carbohydrate/nitrogen (C/N) ratio is used to control larval path development from the first instar. None of these experiments have been successful with first instar larvae. The results of two experiments using second instar larvae are shown in table XI. Each culture in each experiment contained four second instar larvae and ten pre-treated workers. Workers had been reared on a diet of an appropriate C/N ratio for ten days prior to the experiment.

TABLE XI/

TABLE XI

The average length of larvae [where 1 unit = 0.0625 mm.] surviving after fourteen days in two experiments using a series of fixed C/N ratios. [The numbers of larvae surviving in each culture is shown in brackets below the average larval length.

	CULTURES							
	1	2	3	4	5	6	7	8
Casein in gms. (Nitrogenous food)	-	.1	.5	2	2	2	2	2
Sugar in gms. (Carbohydrate)	2	2	2	2	.5	.1	.01	-
Experiment I	42 (3)	40 (4)	27 (3)	30 (1)	32 (2)	- (0)	- (0)	- (0)
Experiment II	39 (4)	39 (3)	37 (1)	30 (2)	- (0)	32 (1)	21 (2)	- (0)

The significance of these results is obscured by the differential larval mortality which occurred throughout. Comparable experiments using casein, sugar and oleic acid in various proportions showed that none of these were as successful for larval brood rearing as Drosophila larvae and sugar. It appears that a "live" stimulus may be necessary before workers will consume or perhaps recognise proteinaceous material as food.

Vital dyes as markers for larval food have been extensively used in this investigation. While results derived solely from these observations using vital dyes must be regarded with caution, they appear to confirm that carbohydrates (sucrose) are preferentially fed to non-dormant larvae. The first incidence of worker feeding was detected in late first instar larvae. These showed traces of dyes associated with sucrose. It is unlikely judging by intensity of colour and gut volume, that this diet consisted solely of sugar. Such trophic relationships of workers and larvae have been extensively considered by Le Masne (1953). It is possible from the above observations that non-dormant larvae are fed on a sugar-rich diet which may be mixed with the salivary secretions of the larvae themselves, of certain of the workers, or with eggs. The diet of dormant larvae as investigated by this means was more uncertain. Quantities of protein marked material are however included in the diet.

In/

In the course of numerous experiments on larval growth which will not be described in detail but are similar to those described above, the measurement and observation of the growth of 180 individual larvae has produced the following conclusions.

1) Larvae enter dormancy in a condition where the brain may lie between 0.0 out of the head and 0.6 out of the head.

2) Measurement of the length of the antennal bud provides a more accurate measurement of the degree of pre-dormant development. Larvae may enter dormancy when the length of the antennal bud is between 22+3 units and 35+5 units (1 unit = 0.00294 mm.), where 22 or 35 refers to the length of the bud, and 3 and 5 refer to the thickness of the basal portion of the sheath as seen in optical section (Appendix V)

3) Larvae in which the brain is more than 0.6 out of the head, or in which the antennal bud is longer than 35+5 units, can if given suitable cultural conditions (optimal laboratory conditions), undergo metamorphosis within a short period of time (c.f. experiment 5).

4) A correlation exists between larval size (total bulk) and the size of the antennal bud. The position of the brain shows greater variation relative to larval size.

5) The relationship of larval bulk to the growth of the antennal bud and brain position is different in pre-dormant as compared with post-dormant larvae.

6)/

6) In pre-dormant and dormant larvae there is an arithmetic relationship of larval bulk and linear measurements of the antennal bud. In post-dormant larvae the relationship of linear measurements of the antennal bud to the measurement of larval bulk becomes logarithmic, the antennal buds showing a disproportion-:ate amount of growth associated with the differentia-:tion of the imaginal bud into the adult structure.

7) The point of transference from one form of growth to the other can be accurately defined in terms of the imaginal rudiments. This transference occurs when the brain is between 0.4 and 0.6 out of the head, and when the antennal bud and sheath are 35+5 units in length. Examination of the retrocerebral endocrine system shows that at this point neurocolloid is detectable for the first time in the third instar in the corpus paracardiacum (Appendices II and III).

8) This critical developmental stage as defined above with reference to the growth of imaginal rudiments is therefore critical also with regard to the time of release of the growth and differentiation hormones (Appendix II).

9) Experiments and observations on larval growth in several series of larvae of varying developmental conditions show that once a larva has passed this critical developmental stage, metamorphosis may follow rapidly, in optimal laboratory conditions, and also in many cases, in sub-optimal laboratory conditions.

10)/

10. It is difficult to avoid the conclusion that this critical developmental stage represents the earliest possible time of liberation in the third (the final) instar larva of the "growth and differentiation" hormone.

11) The duration of the period of liberation of neuro-:colloid may be relatively short and is probably complete by the time the brain leaves the head.

12) Critical stage pre-dormant larvae and critical stage dormant larvae do not have neurocolloid in the corpus paracardiacum. This appears only in critical stage non-dormant larvae or critical stage post-dormant larvae (Appendices I, II and III).

13) There is a range in size (and therefore development) of dormant larvae removed from the field. The causes of such a size range have been investigated both by the present author in experiments described below, and by Brian (1955). In both cases the effects of such a larval size (and development) range on the incidence of dormancy and the possible incidence of endocrinal diapause (Appendix I of this thesis) have been investigated.

G. Larval size and development at dormancy in relation to the possible incidence of endocrinal diapause.

A range in larval size and development at dormancy is found in late serotinal colonies in the field. Considered in relation to laboratory experiments there are apparently two possible explanations of such larval series.

A./

A. Biased feeding of larval groups. Experiments previously described have shown that a size range of larvae is inevitably produced after the culture of an initially uniform group of larvae by a group of workers. The causes of such differential growth in laboratory nests may not be attributed to purely laboratory artifacts such as brood piling (Paper I of this thesis). There is some doubt as to whether brood piling occurs in nature where differential growth of a uniform larval group may be due to other causes.

B. Differences in the time of production of larvae in the field. It is known (Brian, 1951b) that some dormant larvae may be produced from the first (vernal) egg peak. All larvae produced during the second (aestival) egg peak become dormant. Such a range in the time of production of larvae may well provide a basis of larval differential growth and development in nature prior to dormancy.

Experiments with serotinal (dormant) larvae have been undertaken with the following aims. To determine:-

1. The ability of sub-critical pre-dormant and dormant larvae to grow and develop towards the critical stage.

2. The ability of critical pre-dormant and dormant larvae to undergo post-critical growth and development.

The growth of 35 small and medium sized M. rubra microgyna larvae each cultured in isolation with four workers of varying seasonal conditions, showed that all small larvae and certain medium sized larvae could increase in girth, without becoming post-critical./

post-critical. Certain small larvae could also become post-critical and undergo metamorphosis, if reared with vernal workers.

The growth of small and medium sized larvae in groups of three also produced the same results. Further clarification of this problem is derived from an experiment carried out by Brian (1955). A short account of this experiment is given here:-

Twelve cultures each containing 20 larvæ and 40 workers of M. rubra macrogyna were cultured, six at 20°C and six at 25°C, larval size and worker condition also being varied throughout. The variation in the proportion of larvae of each size group undergoing metamorphosis varies both with the condition of the workers and with temperature. Considering, for example, only those cultures reared by vernal workers at 20°C:-

Large larvae:	4 metamorphosed,
	16 remained dormant at the same size as at the start of the experiment.
Medium sized larvae:	7 metamorphosed,
	14 remained dormant with slight bulk increase.
Small larvae:	11 metamorphosed,
	6 remained dormant

Brian's results show that numbers of larvae metamorphosing in each size group also vary with worker quality (vernal as opposed to aestival, vernal workers causing a higher percentage meta-:morphosis) and temperature (25°C as opposed to 20°C, 25°C causing an increased percentage metamorphosis).

Therefore it appears that if a condition of physiological diapause (endocrinal diapause: Appendix I of this thesis) exists/

exists in certain of these female larvae, then:-

1) Workers can break this diapause,

2) The rigidity of this diapause is not comparable to that seen in insects such as Cecropia (Williams, 1952) since it can be broken to varying extents by workers of different seasonal conditions working under differing environmental conditions.

If however the work of Brian (1955) is accepted as proof of the incidence of endocrinal diapause, (even under optimal laboratory conditions [25°C, vernal workers] 100% metamorphosis was not achieved in all larvae of all size groups; specifically, 66% large larvae, 50% medium sized larvae and 100% small larvae metamorphosed under these optimal conditions) then it is apparent that diapause will be restricted to large and medium sized larvae and, even then only to certain of these larvae. The incidence of diapause may then be considered to be a factor of the bulk of the gut or of the fat body or both.

In an experiment (12) by the present author the growth and development of 30 individual larvae was measured. Each larva was cultured with four vernal workers on a diet of protein (Drosophila larvae) for a period of 65 days. All workers had been pre-treated for 14 days by feeding on protein alone. 10 larvae were large, 10 medium sized and 10 small. The results of this experiment are shown in figure 8 as the average value of the four resulting developmental paths, in a plot of the growth of the antennal bud against time.

In/

In experiment 12, metamorphosis was confined to large larvae (4/10 metamorphosing rapidly). No other larval size groups showed prepupation even after 60 days. There was however considerable increase in bulk in all groups. This result is irreconcilable with the incidence of diapause solely in relation to the bulk of the fat body. It is of interest to note that all larvae showed development past the critical stage defined above (and in Appendix II) and ceased to develop at a subsequent stage, characterised as follows.

The brain at this stage has largely left the head and lies between 0.7 and 0.9 in the prothorax. The antennal bud length varies between 52 (bud) + 5 (sheath) and 65 (bud) + 20 (sheath) where 1 unit = 0.00295 mm. The limb buds have either two segments or are at an incipient three segmented stage. It was difficult to measure accurately the area of the wing buds in view of the large bulk of fat body. This stage appears to be similar to that described by Brian (personal communication) in Myrmica rubra macrogyna where the limb bud is also two segmented. The relationship of Brian's observations on individual ontogeny, to the above observation that larvae can remain indefinitely at this developmental stage (if supplied with a proteinaceous diet under these environmental conditions), is unknown, but the extreme importance of qualitative trophogenic factors in individual ontogeny is apparent.

Nine/

Nine of the larvae from experiment 12 were sectioned and showed that, although neurocolloid had been produced (i.e. the growth and differentiation hormone had been liberated), metamorphosis could not be completed in certain larvae under the adverse conditions of low temperature (20°C) and only proteinaceous food (or lack of carbohydrate). The high worker/larva ratio (4) and the vernal nature of the workers had however enabled the critical stage to be passed. Therefore while vernal workers can achieve the liberation of the growth and development hormone, carbohydrate is also necessary either to workers or to larvae for successful metamorphosis.

A decrease in gut area following the starvation of all cultures for one week (after 40 days), shows that although there is throughout a constant ratio of total area to gut area in all larvae, the larvae are being fed continuously but are unable to show increases in growth or development. Following this period of starvation all larvae were hand-fed with sugar solution. No increased growth or development resulted within one week. The following week sugar was added to all cultures, and all larvae became prepupae within seven days.

Such observations are not obviously reconcilable with those of Brian (1955) and it is apparent that further experimentation is necessary before a clear understanding of these results is forthcoming. It may be considered probable that the large fat body in these larvae contained sufficient stored/

stored carbohydrate to enable metamorphosis to take place. In the case of certain large larvae metamorphosis was achieved without any carbohydrate in the diet. It is concluded, from this experiment that sugar caused a change in worker behaviour or physiology allowing the completion of larval metamorphosis.

3. DISCUSSION

The experimental observations described in this paper concern the factors affecting growth and development in female larvae of Myrmica, and tend to show that these observations can only be interpreted in terms of:

1) a special food produced or prepared by the workers,

2) Variations in the nitrogenous composition of larval food.

The present discussion is concerned with the role of these two trophic factors in inducing non-dormancy or dormancy in female larvae of Myrmica and also with their occurrence, distribution and socio-physiological regulation.

These factors are considered under the following headings:-

I/

I. The structure and differentiation of the egg mass;

II. The food of young larvae;

III. The importance of larval grouping, and the incidence of biased feeding;

IV. The importance of worker condition, of nitrogenous composition of larval food, and the significance of un-:natural laboratory conditions;

V. The possible incidence of larval diapause;

VI. The significance in Myrmica of the factors reported to cause diapause in other insects;

VII. General conclusions on the trophic control of female development, and its significance in other aspects of ant sociology.

Finally the possible socio-ecological significance of the occurrence of temporal plasticity in individual larval ontogeny is briefly discussed.

I. The structure and differentiation of the egg mass.

In Paper II of this thesis three possible sources of queen food have been demonstrated. These all cause egg production and may be classified as:-

1. Food derived from the queen fat body;
2. Food derived from autochthonous worker material (not eggs);
3. Food derived from worker-laid eggs.

In/

In undisturbed vernal colonies the successive temporal incidence of these three sources of queen egg production appears to cause the structural differentiation of the egg mass into two main regions, namely an inner core of queen eggs and an outer region of a mixture of queen and worker eggs. This is caused by the onset of worker oviposition after queen oviposition has been in progress for some time (Paper II of this thesis). An egg mass is not a permanent feature in the colony, since all egg masses are eventually disrupted, and it should be emphasised that this layered condition has only been observed in certain vernal colonies, never in aestival colonies. In aestival colonies a few worker eggs have been observed scattered throughout masses of queen eggs. It may well be, that in nature the high egg production of vernal nurses during this time results in the survival of male larvae from worker eggs; these might then become dormant along with female larvae derived from aestival egg-masses. The general absence of male larvae from many experimental cultures of young vernal larvae may well be explicable in terms of differential mortality in these young larvae under laboratory conditions.

Observations show that differences in the structure of the egg mass do not exercise a critical qualitative control over the determination of larvae for dormancy or non-dormancy. Nor is such determination controlled by the growth of larvae in the egg mass prior to its disruption, since experiment shows that

larvae/

larvae in the early third instar are still plastic in this respect. Under experimental conditions however, workers appear able to culture and feed late first instar larvae. Nevertheless there is little doubt that in nature the egg mass is disrupted by the workers on the detection of second instar larvae and that this provides the first opportunity for the direct control of individual larval development by the workers.

II. The food of young larvae.

Experiments using vital dyes as food markers, coupled with controlled media experiments, show that a sugar-rich diet is best for workers rearing small groups of young larvae.

Larvae in egg masses probably feed solely on eggs, of all sources and ages, though larval cannibalism may occur. Eggs therefore represent almost the entire natural diet of certain of the first instar larvae (since the egg mass is not broken up till the detection by workers of second instar larvae). But isolated late first instar larvae may be fed by workers on a sugar-rich diet. The composition of this diet is discussed in section IV below. The disruption of the egg mass soon after the occurrence in it of a few second instar larvae produces larvae ranging in size from small first instar individuals to moderate-sized second instar individuals. At the time of the first larval eclosion there are approximately equal numbers of undeve-

:loped/

undeveloped and developing eggs. Consequently, at the time the egg mass is disrupted there may be but few eggs left.

III. The importance of larval grouping and the incidence of biased feeding.

The effect on subsequent growth of a range in the initial size of larvae in groups has been considered elsewhere (Paper I, of this thesis). It has been shown that significant differences in growth (and development) are attributable to these initial differences. More significant perhaps, in natural conditions, is the demonstration of the delay in the incidence of meta-:morphosis in certain larvae caused by neglect resulting from the biased feeding of other larvae. If there is (as has been shown here [pp.35-36]) a critical qualitative temporal change in larval food, the result of temporal delay in larval development following biased feeding may expose retarded larvae to different trophic conditions compared with those larvae which metamorphosed initially.

A range in larval size is characteristic of egg masses at the time of disruption. Experiments here described show that the resulting differential feeding may become preferential and result in hyperbolical development. The measurement of this change is difficult since qualitative differences in food obscure weight relationships, as does also the differential growth/

growth of the imaginal rudiments after the critical developmental stage. It is not possible or justifiable to relate changes in growth to initial weight relationships since the weight increases shown by the larvae are largely due either to fat body and gut or to imaginal buds, but not to both in the same proportion. It is difficult to avoid the conclusion that this preferential effect, observable in aestival colony fragments, is a social device enabling maximal utilisation of a limited autochthonous food, the properties of which would be lost if spread throughout the entire group. It is then apparent that if special autochthonous food is produced in limited quantity by workers, the effect of worker number (irrespective of worker/larva ratio) may be critical, as may also larval group size (accompanied by brood piling). These two may interact throughout such an experiment as 6 described above, and show the difficulty of accepting the results of laboratory experiments as being comparable to field conditions.

Brian (1953a and personal communication) has shown that for vernal workers, variation of the worker/larva ratio does not affect the numbers of non-dormant larvae produced. In certain respects, however, Brian's experiments may not have investigated the conditions prevailing in nature during the production of non-dormant brood. For example:-

1. The size of the group may be critical at any particular worker/larva ratio.

2./

2. Vernal workers, as used by Brian, are not in nature contemporaneous with non-dormant larvae. Non-dormant larvae occur first with aestival workers. The possible possession of special sociological qualities by vernal workers has been shown by Weir (Paper II of this thesis) in respect of queen oviposition, and by Brian (personal communication) in respect of post-dormant larval growth.

The factors controlling the accessibility of larvae to workers appear largely structural and dependent on nest construction. While the incidence of such structural effects as brood piling in nature is doubtful, there can be little doubt that differential growth and preferential feeding of larvae in groups are of the utmost importance in the determination in nature of larval path-development.

IV. The importance of worker condition, of nitrogenous composition of larval food, and the significance of unnatural laboratory conditions.

Temporal changes observed in larvae during aestival condition leave little doubt that there is a qualitative as well as a quantitative change in the food fed to larvae at this time.

Evidence for the production by vernal workers of a special autochthonous food rich in carbohydrate or some non-nitrogenous substance, possibly mixed with worker or larval glandular secretions, may be listed as follows:-

1)/

1) Nitrogen analyses of larvae and prepupae show that an increasing proportion of the dry weight is composed of nitrogenous material, and the logarithmic nature of this increase may reflect a reciprocal decrease in non-nitrogenous material (e.g. to maximal extent in vernal, non-dormant larvae).

2) Increased uric acid excretion in serotinal larvae shows increased nitrogenous food at this time.

3) Increased darkening of the gut contents in serotinal larvae shows nitrogenous increases also.

4) Similarly, sections of the larval gut show different food material in serotinal as compared with vernal workers.

5) Dye marking of larval food shows that a higher proportion of sugar is fed to vernal larvae.

6) Dye marking also shows that a higher percentage of proteinaceous material is fed to serotinal larvae.

7) Dye marking shows that sugar fed to vernal larvae is diluted with a colourless substance in the larval gut. This is unlikely to be water alone as Brian and Brian (1951) suggest but may be a worker glandular secretion.

8) Many of these effects described above can be controlled experimentally by variation of the carbon/nitrogen ratio of worker food.

9) These effects are contemporaneous with the reduction of nurse fat body.

10) These effects are also contemporaneous with certain effects on queen oviposition noted elsewhere (Paper II of this thesis, see TQ^2).

11)/

11) These effects are also contemporaneous with the decline in worker oviposition (Paper I of this thesis).

12) Finally, areal measurement of the larval gut shows that in reaching similar developmental conditions vernal and aestival larvae have ingested different quantities of food. It appears that there must therefore be qualitative differences in this food.

The simultaneous incidence of certain effects, namely queen oviposition (TQ^2, Paper II of this thesis), worker oviposition (Paper I of this thesis), the production of non-dormant brood, and the presence of a worker fat body, suggests that all three effects (worker egg input, queen egg input, and special auto-:chthonous larval food) are all derived from or at least closely connected with worker fat body, and its seasonal changes. Similarly, the effect of such special food (produced by vernal workers) on certain overwintered queen potential larvae may be critical and affect the caste potentiality of the larvae. This has not so far been investigated. [Brian and Brian (1951) have investigated certain natural effects (e.g. insolation) controlling sugar supply, and detailed the possible consequences of such food shortages. It may be pointed out that, if as seems probable (Paper I of this thesis) foragers are characteristical-:ly smaller than the average worker size, then, on the basis of Brian's observations, food shortage may cause increased foraging by means of worker preference mechanisms.] Brian (personal communication) suggests that certain queen potential larvae/

larvae may be fed excessively by vernal workers. A dual mecha-:nism of special autochthonous food preferentially distributed to certain larvae by workers (producing non-dormant larvae) could be of advantage sociologically. This device would utilise in the most profitable fashion a temporary surplus of a caste-determining worker produced food, the utilisation being achieved by preferential feeding as may be the case in queen potential larvae.

The change in larval food in aestival condition reflects the critical nature of worker condition, which varies with time. The seasonal worker condition changes can be independent of worker polyethism, though the converse is not the case. No such major differences as variations in worker locomotor activity, behaviour, size, melanisation, or ovipositional potential are ever limiting factors with regard to the induction of dormancy or non-dormancy. There remain therefore, purely seasonal effects due to changes of worker physiology. This variation in worker physiology may at times be dependent on the presence in the colony of a system of worker polyethism, e.g. under natural conditions the greatly increased worker utilisation of crude protein in serotinal colonies is almost certainly connected with changes in the foraging potential of these colonies. Such changes are explicable (e.g. increased foraging as a dynamic reaction to periodic nurse recruitment, nurses being produced from both the worker pupae of the overwintered and non-dormant broods)./

broods). The elucidation of the trophic function of this system of worker polyethism has been obscured in the laboratory by such factors as the provision of ready-made nests and the presence of excessive amounts of all environmental food, both of which result in enforced brood rearing. Such enforced brood rearing is also inefficient since it causes nurse dilution with possibly resulting inefficience of brood rearing.

V. The possible incidence of larval diapause.

Brian (1955) has shown that in Myrmica rubra macrogyna there is an increasing incidence of apparent diapause* with increasing larval size at dormancy. There is also variation with worker condition and temperature. The work of the present author shows that if the larval diet is artificially restricted to protein, larval ability to complete development may vary directly with larval bulk. The following may be noted:-

1) These experiments are subject to the criticisms noted in IV above, namely the supply of excessive food and nurse dilution. Optimal conditions for metamorphosis may not therefore have been supplied.

2) In Brian's experiment the use of a larval group may have permitted the incidence (in a sub-optimal worker group) of preferential allocation of limited worker food.

3)/

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* For definition of this term as used here see Appendix I.

3) All larvae developed to a post critical stage in the experiment by the present author, but only large larvae were able to complete metamorphosis. It may be suggested that the composition (or derivation) of the larval fat body was affected either directly, or via the workers, by continued protein feeding with the result that only large larvae (which had previously elaborated their fat body on a balanced diet) retained enough carbohydrate to complete metamorphosis.

4) Finally, it may be indicated that the partial compression of the larval salivary reservoirs (Weir, unpublished, Appendix IV of this thesis) in large larvae by the increasing bulk of residual material in the gut may cause such effects as autocatalytic indigestion, or lack of attractiveness to workers (on a trophallactic basis).

Consideration of these four items shows that there is insufficient evidence to support the existence of an endocrinal diapause in these larvae. Examination of the retrocerebral endocrine system (Weir, unpublished, Appendix III of this thesis) shows no detectable differences between large, medium and small larvae. The developmental plasticity observed in Brian's experiment and confirmed in part by other experiments by the present author shows that a mechanism of sociological diapause* is much more probable.

This latter mechanism could be related to the following factors:-

1)/

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* See Appendix I for definition of this term.

1) Larval developmental plasticity with trophogenic control of the time of metamorphosis.

2) Social control of the allocation of limited trophic material among the larvae in a group.

3) Seasonal variation in trophic materials as a result of worker physiological differences.

Further, it appears that while non-dormancy is a corollary of a carbohydrate rich diet including a special glandular food, dormancy may reflect:-

a) Larval starvation and subsequent dormancy following cold restriction.

b) Excessive proteinaceous food resulting in a form of larval diapause (Appendix I of this thesis).

VI. The significance in Myrmica of the factors reported to cause diapause in other insects.

Contemporary literature reporting cases of restricted growth and development in insects (diapause or dormancy, etc., see Appendix I of this thesis) is extensive, and has recently been reviewed by Andrewartha (1952). The factors reported to cause insect diapause may be considered briefly in nine categories as follows. It should however be realised that the precise status of the term diapause as used by other authors in observations summarised below is, in some cases, unknown.

1) The "diapause factor"./

1) The "diapause factor".

Such a factor (X), influenced by temperature, was postula-:ted by Bodine (1932) working on the eggs of Melanoplus, while Salt (1947) working on Cephus postulated two factors (X and Y). However, in relation to Bodine's work it may be remembered that Andrewartha (1943) has shown more recently the significance of yolk condition coupled to temperature variation in controlling diapause in the eggs of Austroicetes.

2) Genetic control.

Obligatory diapause may occur in many species, e.g. Austroicetes cruciata (Andrewartha, 1943). The incidence of diapause in multivoltine and univoltine strains of the same species may be under genetic control (Arbuthnot, 1944, working on Pyrausta nubilalis).

3) Maternal effects.

The incidence of diapause in eggs may be controlled by maternal factors (suboesophageal ganglion) although the imago laying the eggs is itself apparently unaffected by the possible presence of a diapause factor in the blood, (Fukuda, 1953; and Hasegawa, 1952, on Bombyx mori). This maternal effect is confused with voltinism in Bombyx mori (item 2 above).

4) The gradient-factor theory./

4) The gradient-factor theory.

Proposed by Novak (1951, 1954) this postulates an intra-:cellular growth factor present in certain larval cells. Novak has also discussed the possible physiological similarities of this factor to the allatal hormone. His theory has been criticised by Hinton (1953).

5) Photoperiodic variation.

The significance of this effect has been little investi-:gated. It is known to be effective in aphids (Marcovitch, 1924, and Wadley, 1931) although in these insects the effect of alternation of generations is confusing. Pbotoperiodic control of diapause is also reported in certain mosquito larvae (Anopheles, Orthopodomyia) by Baker (1935).

The relevance of the five effects described above to con-:ditions in Myrmica is uncertain. There is no evidence to favour the existence of an X or Y factor (1 above). If a genetic influence occurs, this has been masked by differential larval mortality, since larvae in the first two instars appear developmentally plastic. Maternal factors, if they occur, are not critical and can be disregarded, again on the grounds of egg developmental plasticity unless, as before, differential larval mortality is critical. There is no evidence to favour the gradient-factor theory. The effect of photoperiodic variation has not been investigated and the relevance of such an effect to natural conditions in Myrmica would be obscure in view of the normally subterranean or, at least, unlighted nature of the normal and nest. Nevertheless, it should be remembered that while/

while these five factors may not influence larvae directly, some may be operative on other components of the nest (e.g. photo-:periodicity on worker activity) and by this means affect larval growth. The relevance to conditions in Myrmica larvae of certain other effects previously reported in other insects is known and these are enumerated below.

6) Temperature restriction.

The normal incidence in temperate climates of low winter temperatures causes, in many species of insects, restriction of growth and development. Where this can be resumed immediately on raising the temperature to a suitable level, this be desig-:nated cold restriction and does not constitute diapause (Appendix I of this thesis). Diapause may however be induced by low temperatures, e.g. in Heliothis armigera (Ditman, Weiland and Guill, 1940). Similarly, Prebble (1941) showed that, under certain conditions, low temperatures favoured diapause in multivoltine strains of Diprion hercyniae. Some effects of low temperature on Myrmica have been described above, and it appears that workers at low temperatures are less able to initiate larval metamorphosis or, under certain conditions, to enable the completion of metamorphosis, compared with workers at high temperatures. This effect may be purely a worker effect independent of larval physiology. Also it appears that the condition observed in small larvae in winter is entirely one of cold restriction. The provision of workers and food, even in sub-optimal conditions often causes growth anddevelopment.

Small/

Small larvae can always benefit from the provision of optimal conditions for growth, so no condition of diapause can be said to exist in these small larvae.

7) Environmental variation in food and water.

The widespread occurrence of these effects in nature is often apparently associated with the incidence of diapause. In Loxostege stictalis diapause is sometimes induced after unfavour-:able food although high temperature can avert diapause (Stein-:berg and Kamensky, 1936). In hymenopterous parasites (Cryptus, Spalangia) larval diapause is associated with the rate of oviposition and may result from partial reabsorption of the yolk (Flanders, 1953). Trophic effects are also reported in Platyedra (Squire, 1940) and Euproctis (Grison, 1947) among others. It does not appear that egg size is critical in Myrmica unless this effect is masked by differential early larval mortality.

In Myrmica, trophic effects have been conclusively demonstrated, but the causes of such effects in nature are obscure. Similar trophic variation in social insects is reported from Polistes (Deleurance, 1950b), where temperature and possibly worker age is effective in controlling the food fed to larvae by workers. At the end of the season this food is unsatisfactory and the larvae fail to grow, but instead of producing diapausing larvae as in Myrmica, the workers kill off the remaining larvae. In Myrmica, however, increased

proteinaceous/

proteinaceous food in serotinal nests results, in certain larvae, in the elaboration of the fat body and the accumulation of much residual food material in the gut. It is perhaps more accurate to say that non-dormancy is induced by a glandular, carbohydrate-rich diet, since the normal life history must be considered as that leading to the reproductive individual (i.e. via dormancy). The trophic mechanisms controlling both the distribution of glandular food produced by workers, and the mechanism of protein foraging by workers has been investigated by the present author (Paper I of this thesis). The incidence of glandular carbo-:hydrate-rich worker food is dependent on worker seasonal con-:dition and, in nature, on the other social demands for such food. (It may also be fed to overwintered queen potential larvae, and to queens.) The protein supply in the colony in nature may be dependent on a system of worker polyethism or division of labour with worker age, as in Apis, and is probably affected by such factors as periodic worker recruitment to a dynamic system. This mechanism of worker polyethism may not be operative under laboratory conditions, where seasonal changes of worker physiology are sufficient by themselves to cause trophic changes. Neither worker activity nor behaviour are critical in this respect under laboratory conditions.

8) Asthenobiotic autointoxication.

This has been postulated for Lucilia sericata by Roubaud (1922), but has been partially discounted after the work of Cousin/

Cousin (1932) who showed the significance of environmental conditions in controlling the incidence of diapause in that species. Nevertheless, such autointoxication resulting from metabolic waste may have real significance in larvae of Myrmica where the gut is closed and accumulation of residual waste material within the secondary peritrophic membrane has been observed. The rate of such accumulation must overtake the rate of excretion of such material (if any is excreted at all) via the malpighian tubules. As a result, the area of the gut (in lateral view) may reach a maximal value equivalent to 1/5th of the total area of the larva as viewed from the side under these conditions. Thus, in addition to a bulk of indigestible material in the gut, there is also a shortage of space in the gut for digestible material, and it is possible to envisage a form of diapause resulting from the inability of the larva to ingest any more food. Such a condition might produce the results noted in the experiments here described.

9) Variation in size of fat body.

More recently Andrewartha (1952) has postulated a condition where the fat body of the larva is developmentally intractable, and causes larval diapause. Such an effect cannot, in Myrmica, be dissociated from the preceding observations on the gut, since both are cumulative and simultaneous. The same comments are applicable to this problem as are applicable to the accumulation of/

of residual waste in the gut. Such increasing bulk of intractable fat body may also cause the growth anomalies noted in the experiments described above.

The confusion arising from inaccurate use of the terms diapause, dormancy and quiescence has been indicated elsewhere (Appendix I). Similar confusion exists with regard to the mechanisms controlling these various forms of developmental restriction. Hinton (1953) has dismissed as a possible cause of larval diapause the accumulation of developmentally intractable fat body in the larva (Andrewartha, 1952), which he considers is normally utilised in hibernation. Similarly Hinton discards the autointoxication theory of Roubaud (1922).

The larval fat body in Myrmica cannot be regarded solely as a food reserve enabling survival during hibernation, since there is a wide range in size of larvae prior to hibernation, and larvae of all sizes can survive till the spring. Larval size depends both on the accumulation of residual wastes in the gut, and on the elaboration of a large fat body.

It is impossible, in the present investigation, to disregard the significance to larval growth and development of the trophic changes observed. Nitrogenous residues are accumulated in the gut and a large fat body is elaborated at certain times and under certain conditions in certain larvae (e.g. medium sized/

sized larvae fed on a protein diet at low temperatures by vernal workers) which subsequently show inability to undergo metamor-:phosis. It is then not possible to disregard as the possible environmental cause of the sociological diapause here observed either:

a) the autointoxication theory elaborated by Roubaud (1922), or

b) the theory of developmental intractability of the fat body.

This does not in any way detract from Hinton's observation that in insects showing endocrinal diapause there may be two hormonal mechanisms allowing the breakage of diapause, since the mechanism of diapause breakage in Myrmica is unknown. Neverthe-:less, it is important to realise that in any one insect there may be several levels at which diapause may be induced, and several causally related mechanisms operating in succession to achieve this effect. For instance, an environmental control of diapause such as that demonstrated in Lucilia sericata by Cousin (1932) may well be operative in Myrmica larvae and resultant trophic differences may lead to a condition of larval auto-:intoxication from residual proteinaceous waste (such as pro-:posed by Roubaud (1922)) accompanied by the accumulation of a large fat body (possibly developmentally intractable (Andrewartha, 1952)). Such fat-body accumulation or any one of the previous effects may control or influence the hormonal mechanisms/

mechanisms of the larva (Appendix III of this thesis). It is wrong to attempt to restrict causality to any one of these factors, all of which can be demonstrated, with varying degrees of certainty, to be present in Myrmica larvae. The fundamental origin of these differences lies in environmental variation (in this case sociological) and may well do so in many other insects. This sociological diapause may not be strictly comparable to that demonstrated in insects undergoing hormonal (endocrinal) diapause, but shows the possibility of fallaciously attributing to these cases of diapause a solely endocrinal cause, without reference to the ecology of the insect.

It may be suggested that in these larvae the retrocerebral complex may not be the limiting factor in growth and development prior to and during hibernation, i.e. there may not be a rigid and inflexible physiological diapause (endocrinal diapause) as described in such genera as Cecropia (Williams, 1952). Instead there may be a flexible sociological diapause. Growth and development in predormant larvae is dependent on and varies with such socio-physiological factors as worker seasonal condition. In addition, individual pre-dormant larval ontogeny is influenced by effects of larval physiology (e.g. size) and their interaction with worker condition. Thus, while potential post-dormant larval ontogeny may be affected by vernalisation (Brian, 1954), the number of larvae undergoing such vernalisation is under social control. The resultant queen potentiality represents/

represents the diversion of potential colonial strength from the cause of colony survival to that of species and colony reproduction (Richards, 1953).

The occurrence in the laboratory of apparently diapausing larvae may be a laboratory artifact due to sub-optimal conditions. If cultural conditions resembling those in nature can be achieved in the laboratory, they may show that there is no condition of endocrinal diapause as currently understood.

While no close comparisons in other social insects can be made with conditions in Myrmica, as here described, the occur-:rence both of seasonal food changes (Polistes, Deleurance [1950b]) with temperature, and of special worker produced food (Apis, Ribbands [1952]) are of fundamental importance in showing the possible occurrence of such mechanisms throughout a wide range of social Hymenoptera.

VII. General conclusions on the trophic control of female development, and its significance in other aspects of ant sociology.

It appears that the trophogenic control by workers of the time of metamorphosis of female larvae depends on the quantity of a special autochthonous food available in the colony. This special food is produced by all workers (though perhaps the quantity varies with worker ethal type) and the production of this food declines with seasonal advance.

It/

It is possible that worker egg input, some components of queen egg input, and larval metamorphosis via non-dormancy, are attributable to one and the same social food. If larval develop-:ment via non-dormancy is attributable to this special food, it is tempting to suggest that all larval development including caste potentiality is also caused by this food. It appears then that conditions in nature must be regulated by the alloca-:tion of this food by workers to the four possible outlets listed above. The relative distribution in nature of this material among these four aspects of colonial physiology is presently unknown and the factors controlling it have not been investigated. It seems likely that periodic egg production and the resultant brood batches are only explicable in these terms (Paper II of this thesis).

Finally, the possible socio-ecological significance of individual ontogenetic plasticity is here discussed briefly.

Schneirla (1949) has described cyclical activity in Eciton, under sub-tropical conditions. Such cyclical activity is large-:ly independent of seasonal climatic variation and is, apparently, inherent in the sociological mechanism of the colony. There is in Eciton no condition of larval dormancy, for succes-:sive brood groups produced are reared to pupation before produc-:tion of the next brood group (as discussed in Brian, 1951b), the times of egg hatching and of imaginal emergence coinciding. Schneirla (1949) favours causal linkage as the mechanism producing/

producing such cycles. He suggests that the queen obtains the benefit of the retarded demobilisation of foraging workers as the larval population declines through pupation. This improve-:ment in her nutritional condition stimulates ovarial develop-:ment and a cyclical causal system is thus established.

The periodic production of brood batches in Myrmica may represent a form of socio-ecological adaptation, to an adverse seasonal environment, of the inherent cyclical activity known to occur under sub-tropical conditions in Eciton (Schneirla, 1949). In sub-tropical conditions periodicity is a sociological attribute more or less uninfluenced by climatic conditions, and there is no such modification or temporal plasticity of individual ontogeny via dormancy and non-dormancy. Periodicity is imposed in Myrmica by the seasonal environment and the sociological mechanism of the colony has been adapted (via individual ontogenetic plasticity under social control) to allow maximal sociological plasticity to counteract this enforced physiological periodicity.

Larval dormancy in Myrmica may be regarded not as the eco-physiological adaptation of an individual to an unfavourable climate (as it often is, in insects), but as the socio-physiolo-:gical adaptation of the colony to facilitate colony survival and colony reproduction under these adverse conditions.

4. SUMMARY

1) Experimental observations on early larval growth and development in Myrmica are described.

2) Trophic differences have been demonstrated. These show that larvae are fed increased amounts of nitrogenous food throughout the season.

3) Worker condition is critical in controlling the composition of larval food at any one time.

4) The allocation of food by workers to the larvae is uneven, and some of the controlling factors have been investigated.

5) Experiments under laboratory conditions (which may be un-:natural) have shown that a condition of endocrinal diapause is unlikely.

6) A condition of trophogenic sociological diapause is postulated.

7) The significance of such sociological diapause to the colonial physiology of Myrmica is considered, and the ecological significance of this condition demonstrated, with reference to previous work of the present and other authors.

FIGURE 1

This shows, as a series of histograms, the length of the larvae at each census, of the group of 20 larvae used in experiment 5.

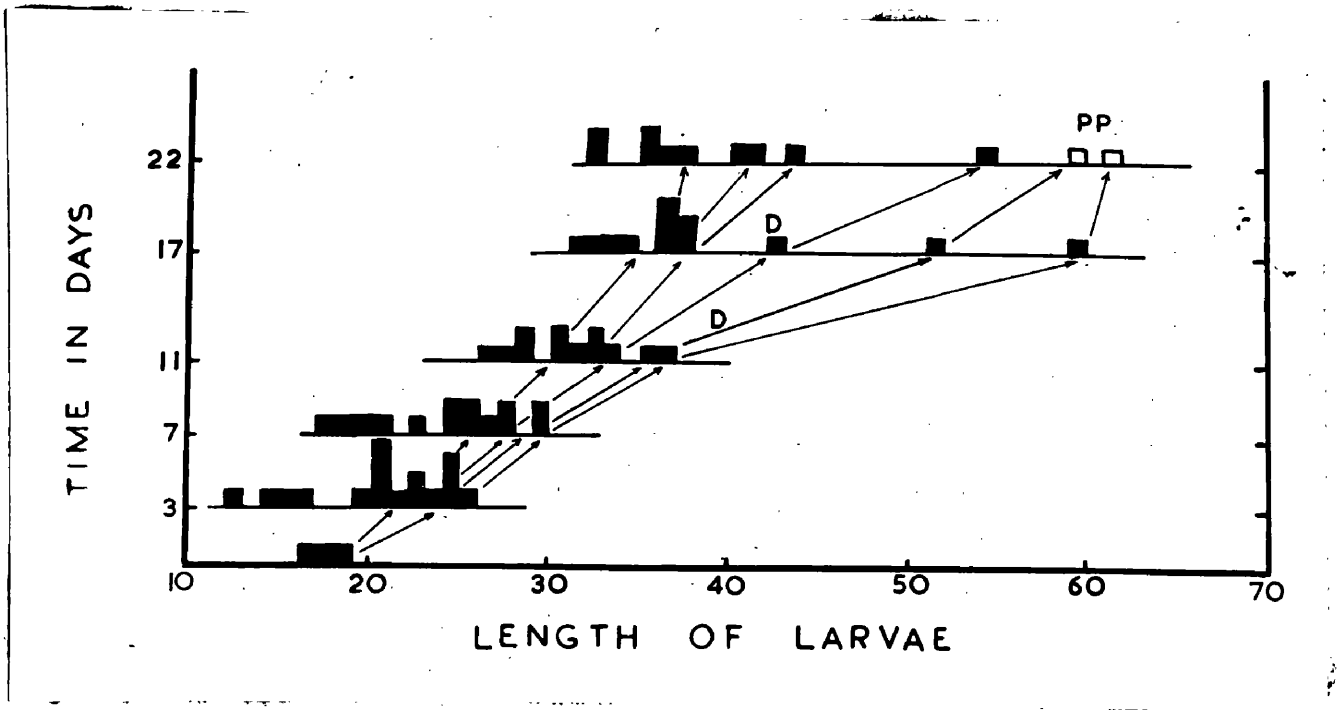
PP - Prepupae
D - Dye marking of individual larvae
1 unit of length = 0.0625 mm.

The initial frequency distribution is not shown completely but all larvae were between 16 and 19 units in length. The probable path of development of some of these larvae has been indicated by arrows.

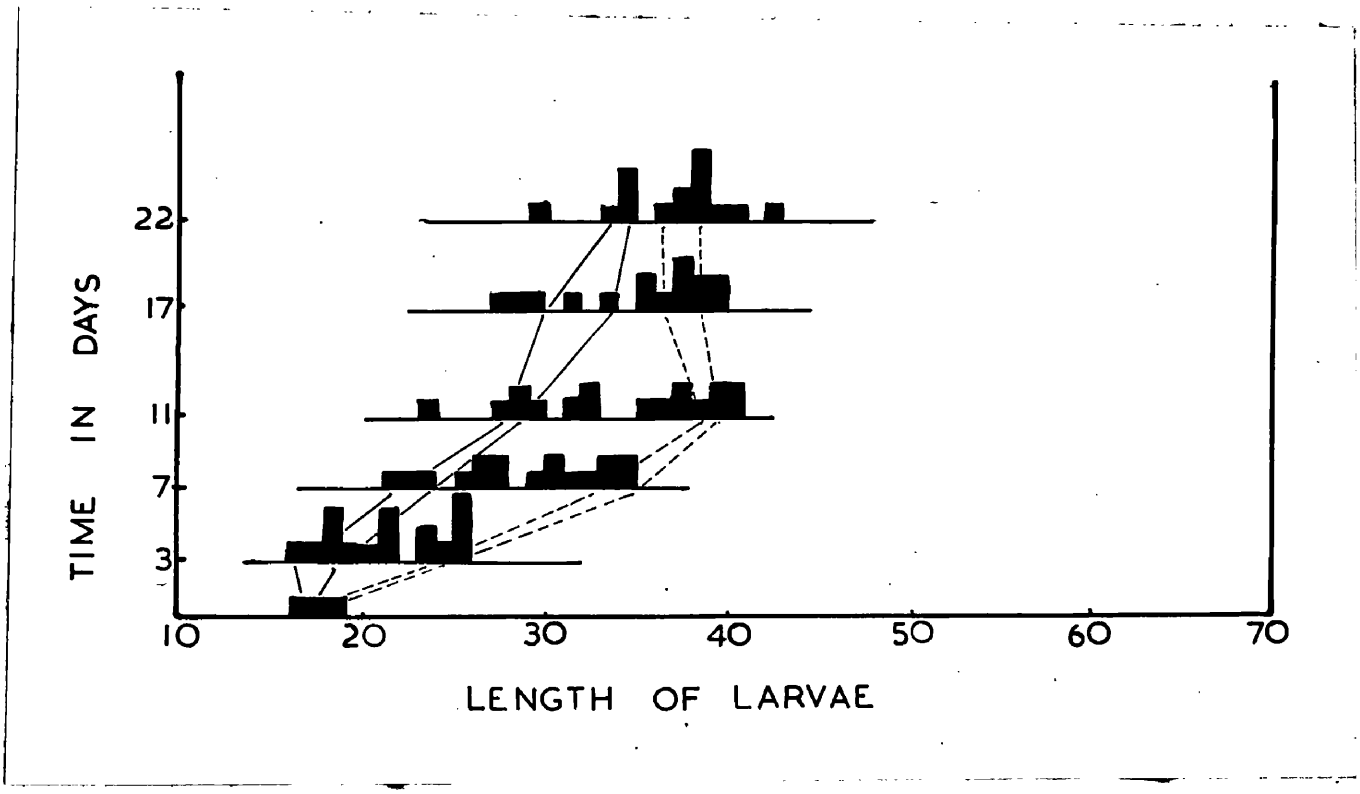
FIGURE 2

This shows as a series of histograms (on the same scale as figure 1), the length of the larvae at each census, in the 10 groups of two larvae used in experiment 5. The growth of the larvae in two typical cultures is indicated on the figure, those in one culture by the straight lines, and in a second culture by the dotted line.

1 unit of length = 0.0625 mm.


PP
D
D
22
17
11
7
3
TIME IN DAYS
10
20
30
40
50
60
70
LENGTH OF LARVAE

1.


22
17
11
7
3
TIME IN DAYS
10
20
30
40
50
60
70
LENGTH OF LARVAE

2

FIGURE 4

This shows diagrammatically the three developmental paths followed by larvae in experiment 7.

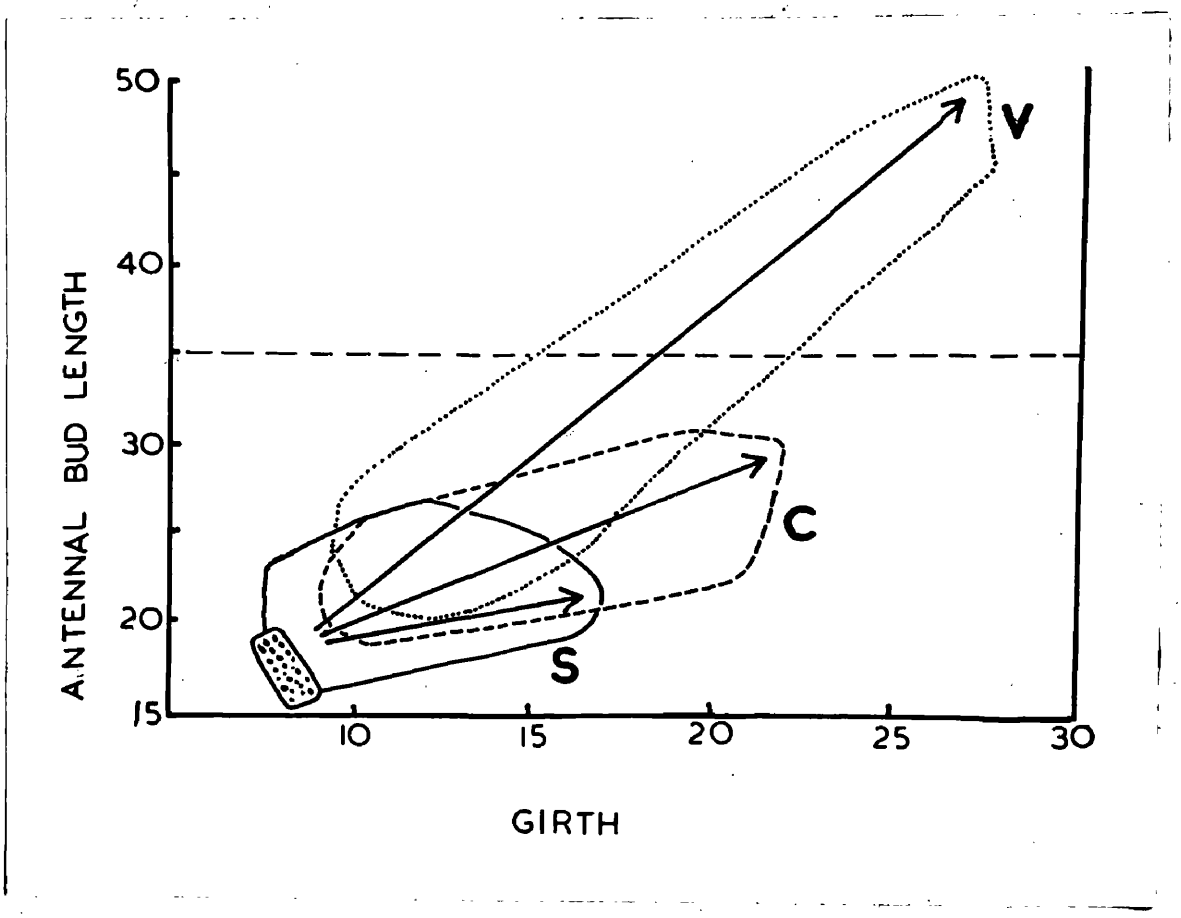
V - Vernal workers

C - Callow workers

S - Serotinal workers

The line round each arrow includes all the larvae in each group. The arrow indicates the maximal distance travelled along each path by one larva during the seven days of the experiment. All larvae started initially from the region which is shaded. The dotted line at antennal bud length 35 indicates that this is the critical developmental stage. The units of girth are derived by calculation and are of partly relative significance.

(1 unit of length = 0.00294 mm.)



4

FIGURE 5

This shows the average path development of the two larval groups in experiment 8 after 14 days. The final data of the two larval groups were averaged and this value corresponds to the tip of the arrow. All data were closely similar, justifying the use of average values. The initial value is also the averaged value. The dotted line represents the critical antennal bud and sheath length of 35+5 (where 1 unit = 0.00294), and the girth is a calculated value derived from the weight and the length.

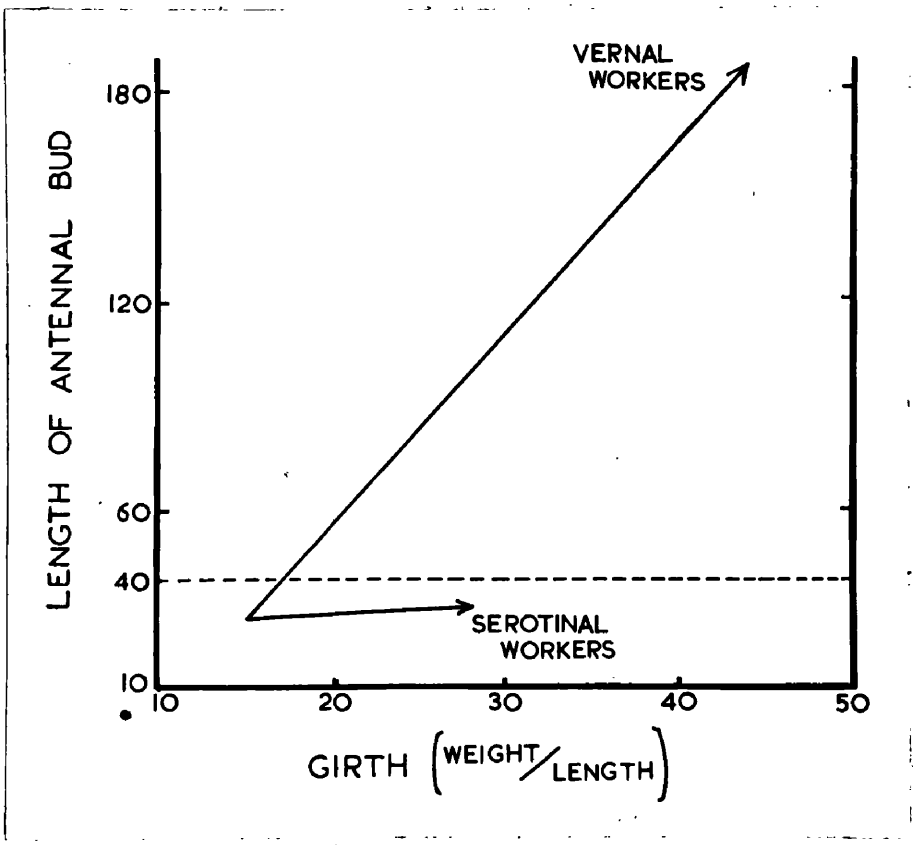

VERNAL
WORKERS
180
120
60
40
10
LENGTH OF ANTENNAL BUD
SEROTINAL
WORKERS
10
20
30
40
50
GIRTH (WEIGHT/LENGTH)

FIGURE 6

This shows the relationship of total larval area to gut area in the three groups of larvae removed at successive intervals of 8 days in experiment 9. The first group removed (after 8 days) is denoted by the dots and figure 1, the second (after 16 days) by the crosses and figure 2, and the third by the circles and figure 3. The arrows indicate the direction of larval progression from the initial polygonal area within which all larvae lie in the early third instar, to the three final conditions.

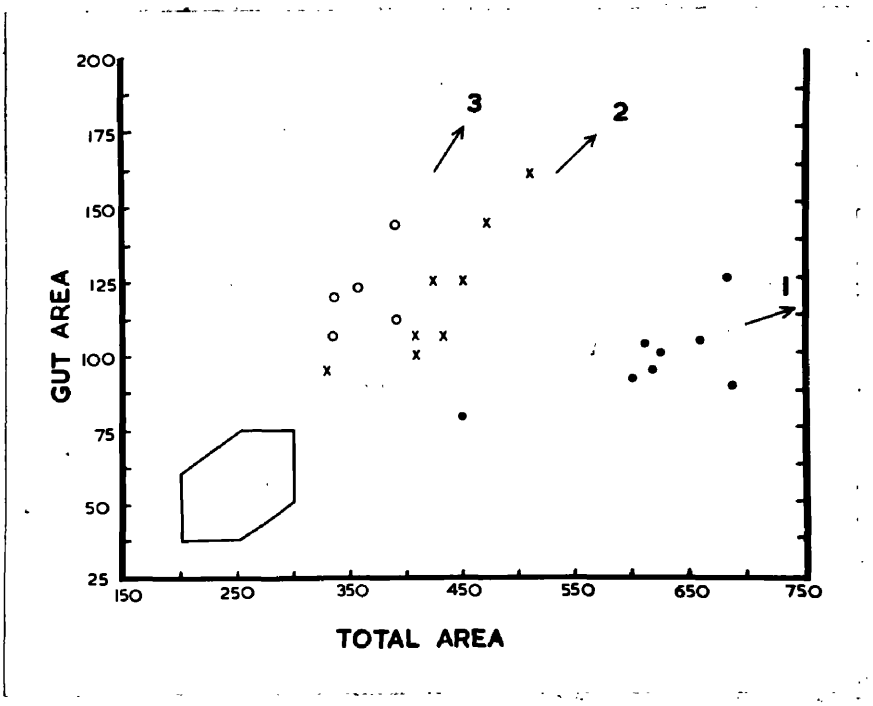

GUT AREA
TOTAL AREA
200
175
150
125
100
75
50
25
150
250
350
450
550
650
750
1
2
3

FIGURE 7.

This shows the relationship of the nitrogen content, dry weight and wet weight, of larvae of both seasonal types (experiment 11). All weights are in milligrams.

Of the dotted lines:-

N indicates a possible level of essential body nitrogen;

RN indicates a visually fitted regression line of nitrogen on wet weight;

PD-ND indicates the line above which all larvae are post-dormant (P.D.) and below which they are non-dormant or pre-dormant (N.D.)

White squares and circles indicate larvae; black squares and circles, prepupae.

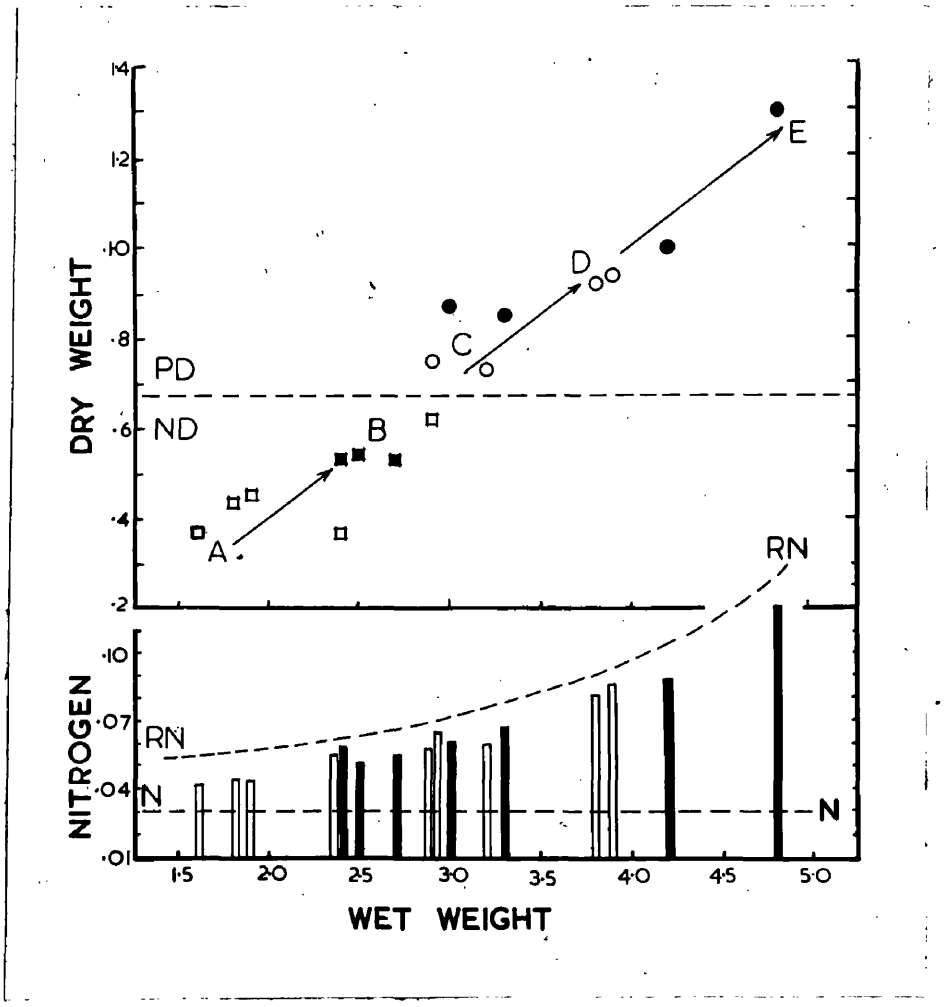
A - Non-dormant larvae

B - Non-dormant prepupae and possible pre-dormant larvae.

C - Post-dormant larvae and some prepupae producing workers.

D - Post-dormant larvae producing queens.

E - Post-dormant prepupae producing queens.


DRY WEIGHT
·14
·12
·10
·8
·6
·4
·2
PD
ND
A
B
C
D
E
RN
NITROGEN
·10
·07
·04
·01
RN
N
N
1·5
2·0
2·5
3·0
3·5
4·0
4·5
5·0
WET WEIGHT

4.

FIGURE 8.

This shows the results of experiment 12 as the average of the four developmental paths in a plot of the log of the antennal bud length against time. The value 5 has been deducted from the bud length.

1 unit of length = 0.00294 mm.

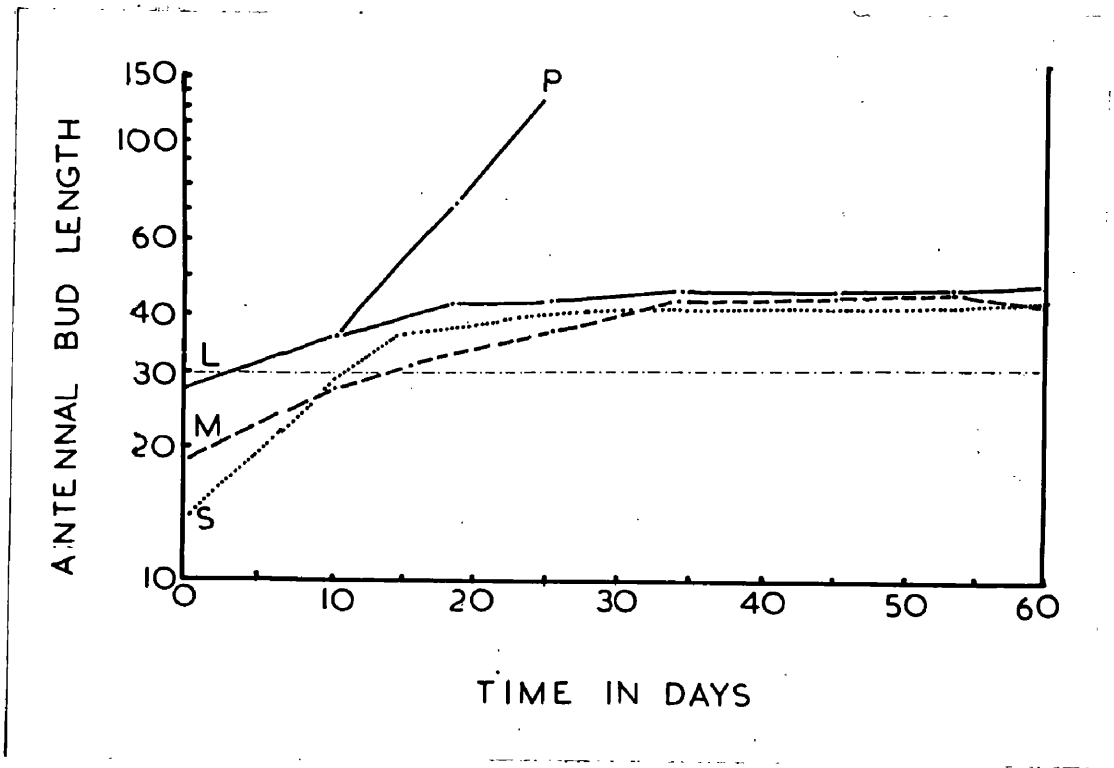
L - Large larvae

M - Medium sized larvae

S - Small larvae

P - Prepupae

The critical value 30 (35 - 5) of antennal bud length has been marked on the figure.


ANTENNAL BUD LENGTH
150
100
60
40
30
20
10
P
L
M
S
0
10
20
30
40
50
60
TIME IN DAYS

A P P E N D I X I

The usage of the following terms throughout this investigation is here defined:-

DORMANCY

NON-DORMANCY

DIAPAUSE

Usage is based on work on the physiological concepts involved, by the present and other authors, the reasons being outlined below.

The use of the term "diapause", as applied to insects, is attributed, in the first instance, to Wheeler (1893), when it had a purely embryological significance. Later, following Henneguy (1903), ecologists have applied this term to the state of physiological rest or "dormancy" observed in many insects. But the subsequent wide use of these terms in insect physiology and ecology, to describe any sort of delayed development, attracted the attention of Shelford (1929) who suggested that the use of these terms should be restricted. It is to be regretted that Shelford's suggestions have not been followed. Numerous authors have applied the term diapause to instances such as simple cold restriction where growth and/or development may be resumed immediately the temperature is raised. Andrewartha (1952) has shown the desirability of adherence to Shelford's/

Shelford's proposals. The use of the term "quiescence" however [as proposed by Shelford (1929)] is inconvenient and confusing in reference to the condition of an ant colony.

The terms non-dormancy, dormancy, diapause, have therefore been allotted certain strictly defined meanings based on observations on the larval developmental paths, larval growth experiments, and observations on the retrocerebral endocrine system. In brief, these show that when the early third instar larva reaches a critical developmental stage (Appendix II), it either ceases to grow and develop at this stage, irrespective of the environmental conditions, or it continues to develop and undergoes metamorphosis within a short period of time, but, in this latter case, only under suitable environmental conditions.

NON-DORMANCY

Throughout this investigation the term non-dormancy has been applied to the case where larval growth and development is continuous, and is not subject to a period of delay at the critical stage. There is, in this case, no question of any prolonged physiological or environmental restriction on the course of larval growth and development. Other possible designations which might be used (e.g. rapid brood), are liable to criticism.

DORMANCY/

DORMANCY

The term dormancy has been used to describe the case where prolonged growth and developmental restriction occurs in the third instar, either prior to, or during, the critical stage. Such restriction may be caused by either environmental or physiological factors.

ENVIRONMENTAL RESTRICTION

Use of this phrase has been confined to the case where it can be shown that a dormant larva can take advantage of favour-:able environmental conditions and, by growth and development in these conditions, pass into a post critical stage.

DIAPAUSE

Use of the term diapause has been confined to the case where it can be shown that the dormant larva is not environmental-:ly restricted, i.e. given suitable conditions for growth and development, the larva is unable to grow and develop beyond the critical stage.

PHYSIOLOGICAL DIAPAUSE

This term has been used to describe the case where growth and development in diapausing larvae is inhibited by changes of activity in the retrocerebral endocrine system and in the larval brain. Such physiological diapause is known in several insects. There are, however, many types and varieties of growth restriction/

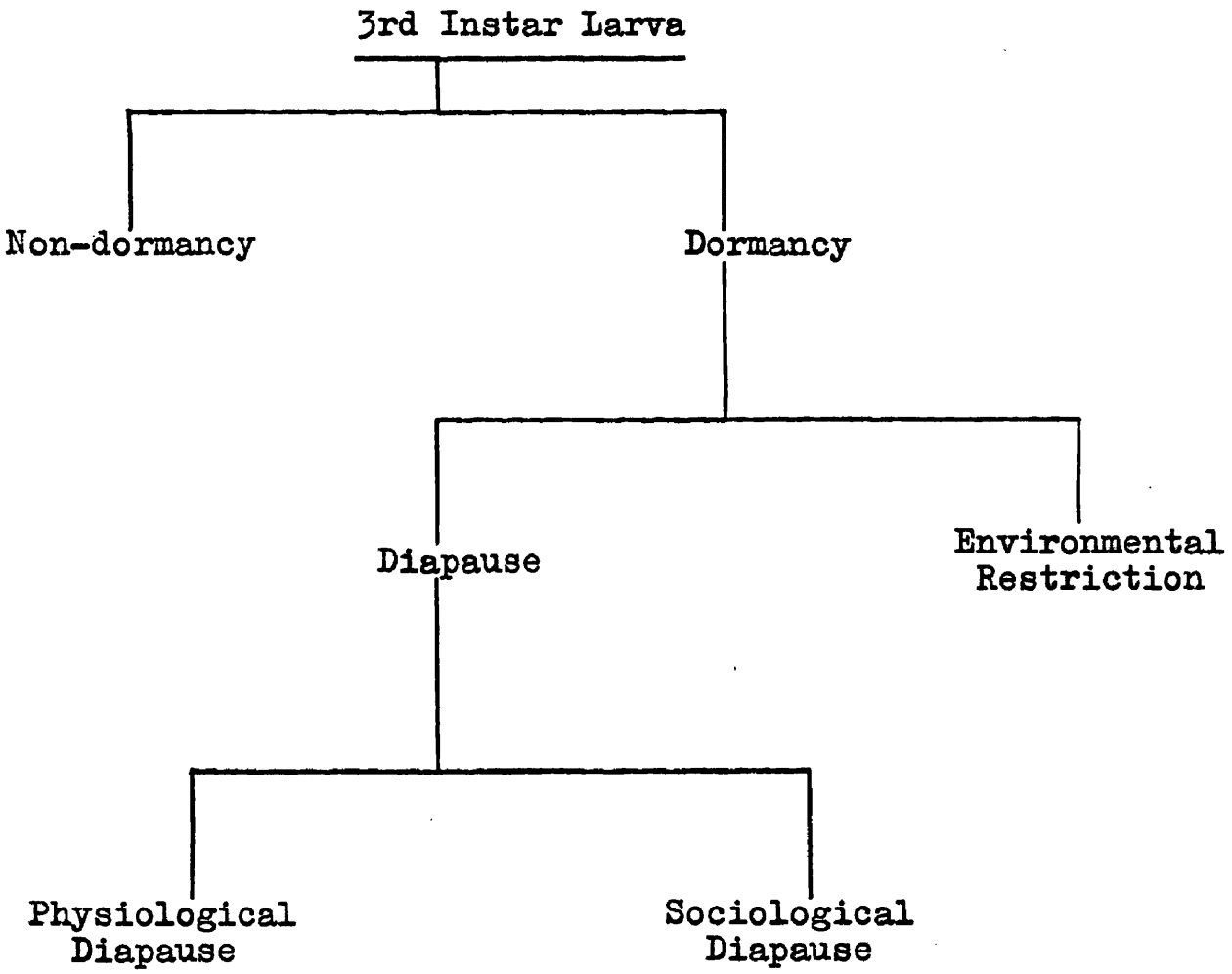
restriction, and diapause may conceivably occur without causative operation of the retrocerebral endocrine system. This is particularly so in the case of the ant community, where sociological factors may affect the course of development.

SOCIOLOGICAL DIAPAUSE

The phrase sociological diapause has been used to describe the case where larval growth and development at this critical stage is inhibited by sociological factors such as the inter-:action of larvae and workers.

The relationships of these terms are shown on the accompanying diagram (p.5).

TYPES OF LARVAL GROWTH AND DEVELOPMENT



A P P E N D I X I I

A CRITICAL DEVELOPMENTAL STAGE in larval ontogeny has been described by the present author and by Brian (1954). The significance of this stage may be outlined as follows.

When the early third instar larva reaches this stage it may either become dormant and overwinter in this condition or it may continue to develop and metamorphose to the imago within a short period (under favourable environmental conditions). In the section of this report describing the investigation of the retro-:cerebral endocrine system and larval brain, all 3rd instar larval conditions there described are of larvae which had been determined for this latter developmental path, except where otherwise stated. This critical stage is the earliest stage during individual ontogeny at which it becomes possible, by measurement of the imaginal rudiments, to determine which developmental path will be followed. However, at this early stage, the use of certain developmental markers is not infallible. Brian (1954) and the present author have shown independently that the degree of brain movement [a standard developmental marker in post-dormant larvae (Brian, 1954)], is influenced by the water content of the larvae. Use of the antennal bud as a develop-:mental marker(as proposed by the present author), is more efficient in view of the early growth and development of this bud, as reported also by Tiegs (1922) on Nasonia.

APPENDIX III

Summary and Synoptic Discussion of a Study of the Retrocerebral Endocrine System of Myrmicine Larvae

SUMMARY

1) The anatomy and histology of the retrocerebral endocrine system of female larvae of the ant Myrmica rubra microgyna Brian and Brian, 1949, have been described, and changes during development assessed from both aspects. These morphological and histological changes are comparable with those described by numerous workers on other groups of insects.

2) These anatomical and histological changes have been related to a critical developmental stage* in the larva, which can be defined by developmental markers.

3) In the female larvae of Myrmica at this critical stage, the structural anatomy of the brain has been described and compared with that of Pieris as described by Hanström (1925). Differential growth leading to the reorientation of the larval brain in the imaginal head capsule/

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* For definition of this term see Appendix II.

capsule during the prepupal stage has been described. The histology of the brain has also been described and compared superficially with the imaginal condition.

4) By the use of staining techniques, it has been possible to detect neurocolloid in the corpus paracardiacum, though any similar localisation in the brain is unreliable.

5) It appears that a "growth and development" hormone analogous to that described in other insects, and leading to the initiation of metamorphosis, can be first liberated at the critical stage. The liberation of this growth and development hormone is contemporaneous with the appearance of neurocolloid in the corpus paracardiacum.

6) There are no detectable qualitative differences in the retrocerebral complex in dormant* compared with non-dormant* larvae, or in diapausing* compared with non-diapausing* larvae.

SYNOPTIC DISCUSSION

This investigation was undertaken to reveal any cytological evidence bearing on the problem of larval growth in relation to non-dormancy, dormancy and diapause.

The/

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* For definitions of these terms see Appendix I.

The periodic production of neurocolloid has in fact been demonstrated. A correlation exists between the occurrence of neurocolloid in the corpus paracardiacum at the critical developmental stage, and larval development past this stage. Similarly, if no development past this stage is observed in certain larvae, examination shows that no neurocolloid is present in the corpus paracardiacum. The appearance or non-appearance of neurocolloid at this critical stage can then be related to the onset of non-dormancy or dormancy, always provided that the environmental conditions are suitable.

This production of neurocolloid at the critical stage is the earliest detectable point in individual larval ontogeny at which it may be said that the physiological mechanism control-:ling the onset of metamorphosis has been initiated. Also it may be said that larvae at this developmental stage which do not have neurocolloid are dormant, or, in some cases, in diapause.

If diapause and dormancy coexist, they will both be encountered in a series of larvae in late autumn, if the series includes the entire larval size range. No qualitative dif-:ferences in the retrocerebral complex or the brain have been detected in such series either in the late autumn, or subse-:quently, at the critical developmental stage, but the possibility of quantitative differences must not be overlooked.

APPENDIX IV

Summary and Synoptic Discussion of a Study of the Larval Gut

SUMMARY

The functional anatomy of the mid-gut of larvae of Myrmica rubra microgyna, Brian and Brian, 1949, has been described.

Observations have been made on the modes of formation of the two distinct types of peritrophic membrane, which may correspond to the primary and secondary peritrophic membranes of Wigglesworth (1953).

The secondary peritrophic membrane is a relatively thick sac-like structure suspended from the oesophageal valve. Residual material derived from the ingested food accumulates inside this secondary membrane. The numerous thin primary peritrophic membranes appear to function as separators for the successive layers of digestive secretions and, presumably, semi-digested food. These primary peritrophic membranes eventually fuse with the outer surface of the secondary peri-:trophic membrane. These two types of peritrophic membrane have separate origins, the secondary peritrophic membrane being produced by an annular peritrophogen of secretory cells round/

round the oesophageal valve, while the primary membranes are produced by successive delaminations of the inner surface of the cells of the mid-gut.

In view of the independent high development of these two types of membrane, the hypothesis is advanced that they have different functions. Further, it is suggested that the corresponding structures in adult Apis do not represent, as advanced by earlier workers, an intermediate stage in the evolution of the peritrophic membrane and its mode of formation.

A P P E N D I X V

Summary of observations on the growth of the antennal bud.

In first and second instar larvae, the antennal bud appears as a globular structure lying immediately below the cuticle of the head capsule. There is, at this point on the cuticle of the head capsule, a circularly sclerotised ring enclosing a number (two or three) sclerotised projections from the cuticular surface. This is the only evidence of any external antennal structure and has been designated the antennal rudiment. The appearance of the antennal bud at this stage, as seen in optical section, is shown in figure 1. The bud itself is surrounded by a sheath derived from the hypodermal cells.

In the early third instar larva the bud elongates as does also the bulk of the sheath which comes to lie posteriorly to the bud, on the anterior surface of the brain (figure 2). While the bud is in this condition the critical developmental stage (Appendix II) is reached, and is definable as the condition in which the length of the bud in optical section is 0.105 mm. and the length of the sheath in optical section is 0.015 mm.

Subsequent growth and development of the antennal bud towards the adult condition is of no relevance to the present investigation, and it is hoped to describe elsewhere the changes which/

which then occur. It is of interest to note that sections of the antennal bud at all developmental stages show the presence of a fine cuticular membrane lying between the sheath and the bud itself, and attached to the hypodermal cuticle (figure 3). The thickness of this membrane is in the order of .5μ - the limit of optical resolution. It is present also in the imaginal buds of the wing and the leg. Preliminary examination of the relevant literature has failed to reveal any previous description of such a structure in this or any other insect.

FIGURE 1

This shows the antennal bud (as seen in optical section in lateral view) of a first, second or early third instar larva.

FIGURE 2

This shows the antennal bud (as seen in optical section in lateral view) of a critical stage third instar larva.

FIGURE 3

This shows the antennal bud (as seen in transverse section) inside the sheath, and with the intervening cuticular membrane.

A - Anterior

B - Antennal bud

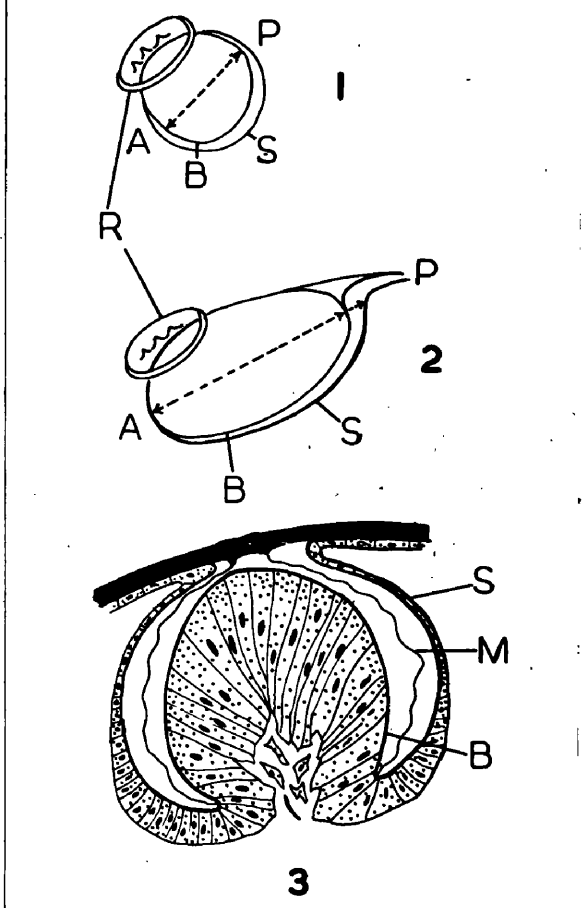
M - Membrane

P - Posterior

R - Antennal rudiment

S - Sheath

The arrow indicates the line of measurement of bud length.


P
A
S
B
1
R
P
2
A
S
B
S
M
B
3

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