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Social organization, breeding biology and the process of reproductive differentiation in Ophthalmopone berthoudi forel, a ponerine ant

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workers should be comparable while they lead a sheltered underground existence. However as the older sterile workers engage in above ground activities, their mortality increases drastically ; observations of marked ants (see 2.4.3. and 2.4.4.) revealed that they disappeared a few weeks after becoming active outside the nests. Thus by December-January there is a very characteristic age distribution in the colonies of this species; the reproductive ants make up the older age class, and all the younger ants are sterile. It is inferred from data obtained on three separate colonies that the gamergate population in the nests suddenly decreases during the few weeks that precede the next period of male activity (Figure 10). The two nests excavated in December 1981 contained a large number of gamergates (125/281), in sharp contrast to the very low numbers of gamergates in the nests sampled in January (1/64; 1/74) and the beginning of February 1982 (11/350) (Table 14). All these nests were well-sampled, with the numbers of individuals examined representing an average of 67% of the nest populations. It is suggested that the gamergates had all come to the end of their adult life-span by this time of the year. Since the ants in a reproductive cohort are of roughly equivalent age they should die contemporaneously. There seems to be little overlap between succeeding populations of breeding workers, although 7/11 of the gamergates in the February 1982 nests (Table 16) did not seem recently mated (they had many eggs and large oocytes in their ovaries) and may still have belonged to the old reproductive cohort.

These longevity data provide additional evidence for the worker origin of the egg-layers in this species. Haskins and Haskins (1980) have shown that the queens in such ponerine genera as Odontomachus and Rhytidoponera have longevities that far exceed that of workers; thus even in species at this relatively primitive grade of Formicid social evolution, queens and workers show very different life spans. This is not the case for the gamergates of Ophthalmopone berthoudi.

The fluctuations in the percentages of gamergates in the various nests examined (Figure 10) do not explicitly support the

pattern of a decreasing ratio from one mating season to another that was outlined earlier. The reasons for this are two-fold. Firstly there are methodological problems inherent in the study of seasonal variations in the size of the cohort of gamergates. This relies on destructive sampling, and thus the same nest cannot be monitored over time. Different nests excavated at various times of the year must be used to make up a composite sample. This introduces an extra dimension of natural variability. Different nests with separate individual histories are compared, and these may have had widely-differing numbers of breeding workers at the end of a mating season. There were substantial differences between nests excavated at the same time of different years (eg. July, October). It would be simplistic to expect that the yearly pattern thus obtained (Figure 10) should approximate the changes inside any particular nest or colony.

Secondly, there is a set of reasons that are natural *historical*. Various factors affect the characteristics of the reproductive population of individual colonies. Each male is capable of inseminating more than one worker, but as suggested in Chapter 5, a sufficient number of foreign males may not always reach certain nests. Some suitably-aged workers then remain sterile. The size of the gamergate population in a nest is thus directly related to the number of male visits and to its number of sexually-attractive workers during the mating season. Thus the large number of gamergates in the December 1981 colony (Table 14) may have resulted from an above-average number of foreign males entering the nests. The percentage of gamergates in a nest is also affected by the stochastic effects of nest founding and colony fission.

An hypothesis on the control of worker reproduction based on insemination generates various predictions, and the latter are dependent on the timing of male activity. The observed natural pattern however is influenced by many additional factors, and therefore does not represent a test of the process itself (the insemination effect). Furthermore, there is the possibility that the number of workers which emerge after the mating season

is sometimes smaller than expected (see 3.4.3.). It must be noted that, while many nests were very well-sampled, the examined sample size in other instances was below 50% (eg. July 1981, October 1981, January 1983, July 1983). Since the samples were not strictly random, caution must be exercised before such results are extrapolated to the whole nest populations.

The existence of marked variations in the numbers and percentages of gamergates in the nest population (Table 14) is evidence of a lack of social control. A fixed ratio between gamergate numbers and the sterile worker population could be achieved in this species through the inter-nest transfer of adults, but there is no evidence that this occurs (see 2.4.2.). In addition there are no interactions among the breeding workers that result in a regulation of their numbers within the nests (see Chapter 4).

3.4.3. Ecological consequences of worker breeding

The labour force in colonies of Ophthalmopone berthoudi is seasonally depleted through the differentiation of breeding workers. When large numbers of workers in a nest are affected (as in the nests sampled in Table 16), an abnormal age distribution in the sterile workforce results. It is suggested that this may have notable consequences on the flow of food into the nests, and ultimately on the numbers of workers produced over the year.

Gamergates do not progress to above ground activities as they age. Since the older workers do not become mated, they carry on as excavators, cleaners and foragers. When this generation dies out there may be a scarcity of younger sterile workers that can replace them; this is evident from the data in Table 16. Some time elapses before a new generation of sterile workers (those which were callow during the mating season or which eclosed subsequently) reaches a suitable age and is able to leave the confines of the nest. A small number of foragers during these few weeks of the year would result in a decreased flow of

resources into the nests. The egg-laying rate of gamergates may decrease as a result of receiving less food; as shown in Chapter 4 they are not treated preferentially during termite meals and trophallaxis does not occur. Thus the low ratio of foragers to gamergates may lead to a temporary drop in the frequency of egg-laying. In addition the poor food supply into the nests may affect brood mortality; larvae perish during their development, or they are used as food by the adults.

The results in Table 1 show that there is an increased larval mortality during the winter months. Nests excavated in July 1981 contained eggs almost exclusively. It was first thought that the lack of larvae and cocoons represented a hiatus in egg production a few weeks earlier. However, further sampling at this time of the year (July 1983) yielded other stages of brood. A few larvae, mainly young ones, were found, together with 11 cocoons containing 2 unpigmented and 9 pigmented worker pupae. This is a significant gap in the age distribution of the brood; the pharates may have been survivors of earlier, more favourable conditions. Larval survival appeared to revert to normal by September; the nests sampled in October 1981 and 1982 contained brood with an expected age distribution. The end of September is the onset of the first summer rains which, together with the higher temperatures, signal an improvement in termite availability. Furthermore, the age distribution in the sterile worker population may have become normal again in September, and more foragers in the nests improve the trophic conditions for the larvae and the gamergates.

The differentiation of many workers into functional reproductives thus results in a trophic stress that is translated into a smaller population of sterile adults later on in the year. The data from the nests excavated at the beginning of December 1981 support this contention. These nests contained not only a large number of gamergates, but also a surprisingly high ratio of gamergates to sterile workers (125:156) that is not expected at that time of the year (90% of the total population of the two nests were dissected). This colony was unusual in that it consisted of only two nests (they were situated 6 m apart);

during the few days prior to the excavation there were frequent transfers of adult workers and gamergates from the large to the small nest. A large number of brood was collected in the larger nest (Table 1), which reflects the recent activity of the many egg-layers. As seen in Table 11, a sample of 44 cocoons that were opened consisted only of prepupae and unpigmented worker pupae; there were no pigmented pupae, and no callow workers were recognized during the dissections. There seemed to have been a significant decline in the egg-laying rate or in the larval survival rate earlier on in the year. After a hiatus of unknown duration it seems that pupae were beginning to eclose again; with the additional adults the restriction in nest space caused expansion into the other nest (see 2.5.3.). Thus in this colony there was still a high proportion of gamergates because only a few generations of workers had appeared over the nine months since the last mating season. The sterile workforce however was divided up into "Innendienst" workers (109) and above ground workers (47).

3.4.4. Gamergates in the Ponerinae

The results of this study indicate that the functional reproductives in Ophthalmopone berthoudi are true workers, and that they become differentiated from their sterile nestmates after being mated. Insemination stimulates ovarian activity in this species, and this is a novel explanation for the control of reproductive differentiation among nestmates. This category of mated egg-layers, which do not belong to a distinct reproductive caste, should be termed gamergates. Alternatively, the term "breeding worker" (Whelden 1957) may be used since it denotes that diploid eggs are produced; "fertile workers" merely refers to the ability to lay male or trophic eggs. Some of the studies done on other queenless ponerine species should be re-examined in the light of this work. It is clear that the functional egg-layers in Diacamma rugosum (Wheeler and Chapman, 1922), Ponera eduardi (Le Masne, 1956), Dinoponera grandis (Haskins and Zahl,

1971) and many species in Rhytidoponera (Whelden, 1957, 1960; Ward, 1983) are gamergates.

Worker mating has been a known phenomenon since 1922 (Wheeler and Chapman, Diacamma), but many authors have subsequently considered the morphologically indistinguishable egg-layers to be developmentally distinct from the sterile workers. Haskins' (1970) suggestion (quoted in Chapter 1, p. 4) that there has been an evolutionary convergence of queen and worker morphology can be discounted in general terms. Such a convergence would be distinct from the ergatoid modification, because the latter reproductives retain specialized ovaries (see 7.3.). The selective pressures that would cause the queen to revert completely to the worker condition are difficult to conceptualize. A more parsimonious alternative is that the queen caste has been lost altogether in many ponerine species. Holldobler and Haskins' (1977) implied suggestion of a caste polymorphism in Rhytidoponera metallica is contradicted by Whelden's (1960) detailed anatomical study of the workers of that species. Whelden found that all the individuals, some of which mated, possessed similar ovaries as well as a spermatheca. Reproductives in this species would fit the description of gamergates.

Ward (1983) suggested that the limited number of mated workers in "Type B" colonies of Rhytidoponera confusa and R. chalybaea is a consequence of subtle dominance interactions, which result in the inhibition of sexual calling behaviour in most workers. In O. berthoudi, the sexual attractiveness of workers depends on their age (see Chapters 5 and 6). Although there is only one brood of workers a year in R. confusa and R. chalybaea (Ward 1981b), these are born over a sufficiently long time period for there to be age differences between them; this may also result in the males being attracted to some of the workers only. Is ovarian activity also triggered by insemination in the R. impressa group? The small number (8/139) of unmated workers with well-developed ovaries (Ward 1983) may merely represent aberrant individuals; a few of these might also have been found in O. berthoudi. In the worker-reproductive

colonies which he examined (in which he dissected all the timid workers only), Ward found a mean number of mated workers per nest of four, with a range from zero to fifteen. These are very small numbers compared to those found in O. berthoudi, and it may indicate that the gamergates in the R. impressa group possess more specialized ovaries. However Ward (1983) collected his nests in autumn and in winter only, and his samples were lumped together. Thus he may not have detected higher numbers of mated workers, eg. after mating seasons.

The restriction of ovarian development to mated workers in O. berthoudi is an unusual situation in the Formicids. It is not understood why the unmated workers do not produce males since there would be strong selective pressures on them to do so. The possibility that breeding workers inhibit the ovarian activity of their unmated nestmates can be rejected. There are times of the year when few or no gamergates are present in the colonies, and yet the non-inseminated workers remain reproductively-inactive.

CHAPTER FOUR WITHIN-NEST INTERACTIONS BETWEEN BREEDING AND *STERILE WORKERS*

4.1. Introduction

4.1.1. Selected literature survey

The members of the queen and worker castes are generally involved in a complex social interplay within the nests, and this regulates the colonial structure. As seen in 3.1., the queen controls worker laying and the differentiation of brood into workers or sexuals. The mated queen may also prevent sexually mature virgin females from becoming functional egg-layers, as shown by Fletcher and Blum (1981) in Solenopsis invicta.

There are additional classes of interactions besides the "queen effect". Queens usually show hostility to one another in a nest, and monogyny is often the outcome of such conflicts. When polygynous systems exist, interqueen affairs (Brian 1983) are organized in various ways. In Camponotus herculeanus, Holldobler (1962; in Brian 1983) found that each individual queen has a territory in the nest; in a colony of Lasius sakagamii, each of the queens occupies a separate chamber in the nest (Yamauchi et al. 1981). Queen number can also be under the control of the workers. Fletcher and Blum (1983) demonstrated that, in Solenopsis invicta, workers execute supernumerary queens in order to restore the level of queen pheromone to within optimal limits in the colony. Workers select queens according to the quantity of pheromone that they release, and this amount is correlated with individual fecundity. Another class of within-nest interactions involves workers only, in the presence of the queen. In Leptothorax allardycei, Cole (1981) reported the existence of dominance hierarchies which usually involved agonistic encounters and avoidance between workers. The high-

ranking workers are favoured in liquid food exchange, and as a result have greater ovarian development and produce many of the male progeny in the nest. A similar behaviour has been shown among the slave-maker workers of Harpagoxenus americanus (Franks and Scovell 1983). Interactions ranging from avoidance to ritualized fighting lead to the establishment of dominance hierarchies, and these determine which of the workers are more often fed by the slaves. In addition the queen recognizes the dominant workers (which also show greater ovarian development) and solicits food from them more frequently than she does from the low-ranking workers or the slaves.

Little work has been done on the social interactions inside the nests of ponerines. Trophallaxis does not occur commonly between adults, except in Rhytidoponera (where exchange of nectar takes place) (Haskins 1970). In other species where trophallaxis has been observed (eg. Ponera eduardi, Le Masne 1952), the behaviour is crude and unspecialized; it is not known whether the queen benefits from it. The most detailed work on the interactions between female and worker caste is that of Colombel (1971, 1972a, 1972b, 1978). Queens of Odontomachus haematodes (=O. troglodytes Santschi, Brown 1976) partially inhibit egg-laying in the workers through the action of a pheromone (Colombel 1971). Queen extracts are also reported to reduce worker oophagy in orphaned colonies, (Colombel 1972b). Colombel (1978) showed that the queens influence the differentiation of larvae into workers or gynes, through the behaviour of the brood workers. In the absence of queens in a colony, brood workers respond more positively to the begging of third instar larvae, and they even stimulate these larvae to feed more often. Some larvae (about 30%) are naturally more sensitive to such stimulations, and these develop into gynes as a consequence of receiving more food. Colombel (1972b) also discovered a definite ratio between queens and workers (1:80-90) in nests of Odontomachus troglodytes; it is not clear whether this is controlled by the workers or the queens themselves.

Ward's (1981b, 1983) data on the Rhytidoponera impressa group also reveal the existence of mechanisms of social regulation

involving the queen. "Type A" colonies are strictly monogynous. More than one dealate queen was found in a few colonies, but the supernumerary queens had empty spermathecae and poorly developed ovaries. In addition, Ward (1981b) studied a colony which had been founded by two queens. These cohabited peacefully for three months until the first workers emerged, then one of the queens was aggressively ousted from the nest; in this instance, both queens had contributed worker offspring to the incipient colony. Thus, even in the phylogenetically-primitive ponerines a queen effect is evident.

The social interplay inside queenless colonies remains largely a mystery; an exception is Ward's (1983) study of "Type B" colonies in the R. impressa group, and this will be discussed at the end of this Chapter. In O. berthoudi, there can be many gamergates (= breeding workers) among the ants confined inside the nests. These egg-layers are normal workers that have been inseminated, and it was important to determine whether they behaved differently from their non-reproductive nestmates.

4.1.2. Aims of this investigation

Excavation of nests revealed that there were large numbers of ants which were confined inside the nests. Nests which had been studied previously in the field (Chapter 2) yielded unexpectedly large numbers of unmarked workers, showing that the older ants active above ground make up a small proportion of the total population of a nest.

A number of nests of O. berthoudi were kept in the laboratory so as to investigate the activities that occur inside the nests. Are there distinct interactions between sterile workers and gamergates? Are the gamergates recognized and treated differently by the other ants? How do the many gamergates coexist within a nest? Do they take part in other colony activities besides egg-laying? What do the sterile Innendienst workers do in addition to brood care?

4.2. Methodology

A number of nests were maintained in the laboratory for periods of a few weeks. Shallow depressions were made in moist soil collected from the excavation sites, and a sheet of red perspex was placed on top; this prompted the building of interconnected chambers. The ants were given access to arenas of various sizes, and fed on live termites (Trinervitermes sp. workers). Colours that could be distinguished through a red filter were used to mark different ants. The within-nest activities and the interactions between various classes of ants (marked and unmarked) were monitored through the perspex roof. Some of the ants were dissected at the end of the observations.

Three nest emigrations were induced by allowing the soil floor of existing nests to dry out and physically disturbing their structures; alternative nest sites with moist soil were provided about 1m away.

Towards the end of the study a small nest (143 adults) was excavated and chosen for a detailed investigation of gamergate-worker interactions. As shown in Table 15, the gamergates were often distinguished by bigger abdomens, and all such ants which remained under cover were marked with identical colour codes. As there was not a strict association between abdominal size and fertility (there were gamergates with smaller abdomens, and sterile workers with bigger abdomens; see 3.3.2.), the reproductive status of these "presumed gamergates" had to be verified at the end of the observations. A few individuals were also dissected during the observations. A number of other workers in the nest were marked with different codes, depending on whether they were active outside the nest or not. All the ants were then placed in an observation nest together with some brood, and the interactions within the group were studied. The inhabitants of the nest were usually starved for a few days and then supplied with live termites.

4.3. Results

4.3.1. Brood care

Eggs were laid singly and abandoned on the floor of the nest. They adhered to one another and were carried around in packets of 5-15. These packets were never left unattended; they were passed from one worker to another, and this was frequently accompanied by a struggle for possession.

The elongated eggs are large and very yolky. The early developmental stages could be seen through the chorion, and eventually the larvae occupied the whole egg capsule. A tiny larva was observed to wriggle out after rupturing the egg membrane at the head end. Very young larvae were often carried around in the nest, but older ones were found in piles. Larvae are naked in this species. They fed by extending their long necks into termite bodies which were held above or placed next to them. Prior to pupation the larvae were buried under a pile of soil by workers; this acted as scaffolding during the weaving of the silken cocoon. A few workers remained around the cocoons while pupation proceeded, and filled gaps that appeared with earth. The soil particles were removed before the spinning of the cocoon was completed. Many workers were then active around the cocoons and scraped them clean with their mandibles. Some cocoons had been isolated from the workers and were opened after some time. They contained fully-pigmented adults that immediately became active and walked hesitantly. This suggested that the callows are dependent on other workers for their emergence. On a few occasions 5-10 marked Innendienst workers chewed a cocoon open and removed the as-yet immobile callow worker. The workers proceeded to groom it and removed pieces of larval cuticle. Callows cannot be distinguished from other workers on the basis of pigmentation.

What is the spatial distribution of the various brood stages in the natural situation ? During the excavation of a nest in

October 1982, a number of very distinct chambers were found at increasing depths. The brood and adults collected from three different nest regions were kept in separate containers for later enumeration (Table 17). While most of the eggs and larvae were found in the uppermost chambers (samples B and C), almost all of the cocoons were collected from the deeper chamber (sample D). These data suggest that the various stages of brood are moved around the nest according to different patterns. In many other nests that were excavated, cocoons were found in surface chambers instead.

Table 17 Spatial distribution of the adult (n= 348) and brood populations within a nest excavated in October 1982. Various samples were recognized, according to where in the nest they had been collected: A= ants active above ground; B= one large chamber found 20 cm deep; C= another large chamber at depth of 40 cm; D= low-roofed chamber at depth of 50 cm.

	A	B	C	D
No. of adults	50	167	84	47
No. of callows	0	23	25	11
No. of non-callow ants dissected	0	70	44	32
No. of gamergates	0	16	26	23
No. of eggs	-	189	148	3
No. of larvae	-	131	37	7
No. of cocoons	-	2	2	45

The developmental period of brood reared in a laboratory nest was monitored. They were continually handled and moved around by the adult workers and thus could not be followed individually through their growth. Thus all the brood was removed from one

of the nests, and the approximate number of days taken by the first few eggs laid to reach adulthood was as follows: egg to larva= 22 days; larva to cocoon= 20 days; cocoon to adult= 32 days. This developmental period of about 74 days may be significantly affected by trophic conditions, and by temperature as Blomefield (1979) discovered in Leptogenys schwabi.

4.3.2. Feeding and other "Innendienst" activities

Returning foragers only entered the superficial areas of the observation nests. They dropped their termite loads close to the entrance, cleaned their antennae and left the nest again. Different workers then picked up these termites and took them to the deeper chambers; such ants sometimes attracted much attention.

Small aggregations of ants (3-6) were usually observed to feed around amalgamated termite bodies that were held off the ground. Once the soft tissues of the termites had been consumed, chitinous remains were packed into pellets and discarded outside (see 2.3.3.).

There was always a certain amount of allogrooming between individuals (including gamergates) in the nest. Mandibles, legs, the scapes of the antennae and the trunk were licked. It was difficult to determine whether some individuals were groomed more often than others.

Defecation occurred in remote corners of the nest. Oophagy was never observed, but larvae and unpigmented pupae were cannibalized on occasion.

As the marked workers which had been active above ground in the field died, some of the unmarked Innendienst workers started performing activities outside the laboratory nests.

4.3.3. Organization of nest emigration

This activity was difficult to distinguish from the normal

inter-nest transfers of adults and brood; its only characteristic is the high frequency and uni-directionality of the transfers. A nest was excavated in December 1980; half of its inhabitants were marked (n=150) and all the ants were then released. Over the next two days the ants moved into the other existing nests in the colony. One natural instance of emigration was observed in the field, when the ants vacated nest (BY) and moved into (BZ) (see 2.4.5.). O. berthoudi's emigrations are characterized by movements to pre-existing nest sites, eg. an abandoned set of termite galleries (see 2.5.3.). In contrast, groups of 9-20 workers in Pachycondyla commutata were observed to excavate a new nest site during the three days that preceded the concerted transfer of the whole colony (Mill 1982).

In the laboratory, there were always small numbers of workers scouting around the arena. Isolated instances of adult carrying were often seen, involving older workers. Carrying was initiated either inside or outside the nests. The various nest emigrations that were induced in the laboratory all followed a similar pattern, and only a typical sequence of events is described here. When an alternative nest site was provided a few ants investigated it independently. In the one experiment emigration was started 45 minutes after this initial exploration. A marked worker which had been observed visiting the new site was carried across by another marked worker. The former then also proceeded to recruit between the two sites. This sequence of events indicates that one particular ant had taken the initiative to move nests. Soon after the start there was a small group of workers involved in the recruitment (3-5), but this group seldom remained constant throughout the emigration.

As a recruiter entered the nest being evacuated, it went to an ant, antennated it, picked it up and carried it to the new nest. In some cases however, the recruiter went from one ant to another (antennating each one) before it eventually picked one up. It was clear that the recruiter led the carrying action because the recruit did not always have time to adopt the correct posture. Ants carried into the new nest were placed on the

ground and left there. A few seconds later they stood up and walked around. The recruiters departed soon after and returned to fetch another adult. Brood transfer usually started after the transfer of a number of adults. Recruiters took away packets of eggs or single larvae that other workers were holding. Later these nurse workers were themselves transferred across. Single eggs were also carried.

Older workers, Innendienst workers, brood and presumed gamergates appeared to be transferred in cycles. Termites were also carried between the two sites. By the end of the emigration the recruiters searched around the deserted nest site before they returned to the new nest with no load. Ants that came back from foraging trips were also picked up and carried across.

4.3.4. Behaviour of breeding workers

The gamergates never became active outside the nests, either in the field or in the laboratory (see 3.3.2.). Most of the behavioural data on the gamergates were obtained during the observation of the marked nest mentioned in 4.2. There were 119 surviving adults in this nest when the observations started, and 18 of these were classed as presumed gamergates. The status of only 15 of these was confirmed by ovarian dissection, and another two unrecognized breeding workers were found in the rest of the population. These misidentifications may have blurred slightly the distinctness of the activity patterns of gamergates and non-reproductive workers respectively.

The presumed gamergates generally remained together in one part of the nest, and this reduced the frequency of interactions with other workers. The location of these exclusive gamergate groupings (there were sometimes more than one) often changed, but occasionally they were found in the vicinity of the brood. There were few distinguishable interactions within these groups. Gamergates seldom performed any activities in the nest, although some appeared to be involved with the brood, eg. one was carrying

an egg. A few instances of egg-laying were observed; the individuals were by themselves and soon walked away leaving the single egg on the soil.

During the chaotic conditions surrounding nest emigrations, presumed gamergates behaved aggressively towards workers, especially those recruiting brood; vigorous antennations and mandible play were seen. Interactions between sterile and breeding workers were normally observed during termite feeds. These were facilitated by witholding food from the inhabitants of the nest for a few days, and by restricting foragers to bringing only a few termites into the nest at a time. This was to observe any conflicts that might arise between nestmates under a situation simulating a food shortage; under normal conditions many excess termites were piled on the floor of the nest.

When termites were brought in some presumed gamergates left their groups and moved closer to the feeding aggregations. Termites were never consumed in the remote corners of the nest. Gamergates attempted to gain access to termites being eaten, but on many occasions they were clearly forced to retreat by one or more of the workers present. One worker antennated rapidly and lunged in the direction of a gamergate. However such aggressive behaviour was also exhibited towards sterile workers trying to join one of these groups. At other times, a few gamergates were feeding together and no aggression was seen.

4.4. Discussion

The absence of a distinct queen caste in O. berthoudi has a dramatic effect on the nature of social control inside the colonies. The investigation of such a reproductive system requires a unique approach. Various manipulations of the reproductive structure of colonies were initially contemplated in order to investigate the existence of a queen effect. These would have involved combining nests belonging to one colony to

find out if there was a fixed ratio between workers to reproductives, and removing reproductives so as to release individuals from an hypothetical inhibition from these reproductives. However the relevance of these was questionable after (1) a nest was found (December 1981) which contained over 100 breeding workers; (2) ovarian development was shown to be associated with insemination.

The differences in the behaviour of breeding and sterile workers observed in O. berthoudi were generally similar to those reported in "Type B" colonies of the Rhytidoponera impressa group (Ward 1983). "Mated workers" of Rhytidoponera tended to remain close to batches of brood (eggs and larvae) and often several individuals were found together within a 1 cm radius. Ward noted that while mated workers were extensively groomed by non-reproductive workers, such grooming was not reciprocated. Ward (1983) also suggested that there may be subtle dominance relationships associated with "antennal boxing" between mated workers. "Antennal boxing" was a conspicuous behaviour between various individuals in the nests of O. berthoudi, but it is not thought to be associated with more than individual recognition and the initiation of carrying behaviour (2.5.2.).

Various numbers of gamergates coexist inside a nest, and there are no aggressive interactions between them. The size of the reproductive population is not regulated but is rather a consequence of stochastic events such as the number of male visits to any particular nest, and the number of workers which are sexually attractive during the mating season (see Chapter 6). There is no consistent relationship between the number of sterile workers and the number of gamergates. This ratio fluctuates during the year with the foundation of new nests and the occurrence of colony fission. This system contrasts with the situation in queenright ponerines where the number of reproductives is under social control (eg. in the polygynous Odontomachus troglodytes, Colombel 1972b). In "Type B" colonies of Rhytidoponera confusa and R. chalybaea, Ward (1983) reported a highly significant statistical regression of oocyte count on number of mated workers per nest, and took this as evidence of

inhibitory effects on the reproductive output of cohabiting mated workers. Data on oocyte count are not available in O. berthoudi and thus no direct comparisons can be made. However, analysis of the number of mature eggs found during the dissection of individual gamergates (Table 12) does not suggest that there was such an inhibitory effect. The groups of individuals dissected were collected in nests with reproductive populations of various sizes, yet there were no systematic differences in the degree of reproductive activity of the gamergates in the different groups. Thus the number of eggs per mated worker does not decline with an increase in the number of mated workers in a nest. There may be an overall decrease in fecundity in nests with a large reproductive population, but it is then a consequence of a depletion of the above ground labour force (see 3.4.3.). Although Ward (1983) thought that there were subtle dominance relationships, he found no differences in reproductive activity between the mated workers in a same nest.

The reproductive system of Ophthalmopone berthoudi is indeed a very unspecialized one. Individual gamergates lay eggs at a relatively slow rate; this is partly related to the large yolky eggs characteristic of this species, to limited ovarian capacity and to energetic constraints. There must exist an upper limit to the rate of egg production, because the breeding workers have to feed themselves by acquiring termites from the stocks that are brought inside the nest. It was found that they are not given preferential treatment during termite meals. Workers are usually crowded around termites when they feed, and it is then difficult for additional nestmates to join this feeding party. Newcomers attempting to push their way in cause some of the ants present to stop feeding and to meet them aggressively. Gamergates were often seen waiting around the perimeter of a feeding aggregation. Their timid behaviour may be responsible for their inability to join in as easily as their sterile nestmates. Individual gamergates do not appear to receive more food than an average worker, which is what would be expected if there was an altruistic relationship between non-reproductive workers and gamergates.

While breeding and non-reproductive workers have different behaviours in the nest, the former have not re-evolved new queen attributes. There is evidence that they can recognize each other, but the non-reproductive workers do not enter into the kinds of trophallactic exchanges found in queenright species. In addition, there is no regulation of the reproductive function and all the workers that become mated seem to lay eggs.

CHAPTER FIVE MALE BEHAVIOUR : COPULATION WITH WORKERS INSIDE FOREIGN NESTS

5.1. Introduction

In the majority of formicids the males and virgin queens emerge from their nests and leave on a mass nuptial flight (Wilson 1971). Among those ponerine species with winged queens, synchronized dispersal and mating flights also occur and are undertaken in large coordinated groups (eg. in Ponera pennsylvanica, Haskins 1970) or individually (eg. in Odontomachus assiniensis, Ledoux 1952). In the primitive Amblyopone pallipes, the sexuals fly away from their nests, but the virgin queens quickly come to rest on prominent objects in the vicinity and adopt a "calling" posture. Low flying males then appear and land on vegetation near the females; copulation ensues (Haskins 1978). A modification of this pattern is seen in ponerines with wingless ergatoid queens. In Megaponera foetens, Longhurst and Howse (1979) found that no swarming occurs; after dispersing, males land and search on the ground. They orientate to foreign nests using the scent trails laid by the workers; the virgin ergatoids are fertilized inside the nests. In Leptogenys ocelliferа, Maschwitz and Muhlenberg (1975) observed the males running along the permanent recruitment trails of workers, and they suggested that ergatoids may be mated on these trails. Finally, in the queenless species of Rhytidoponera (eg. R. metallica, Holldobler and Haskins 1977; R. impressa group, Ward 1981b), workers are mated near the entrances of their own nests. Wheeler and Chapman (1922) found a male copulating with a worker inside a nest of Dlacamma rugosum. Thus the change in the nuptial flight behaviour of ponerine males is associated with the disappearance in many species of the alate female reproductives and their replacement by wingless ergatoids or by fertile

workers.

5.2. Methodology

The information presented in this chapter was collected in Mkuzi Reserve during the mating season (January-April). Observations were made throughout the day. A number of males were painted on the thorax and released, and some individuals had their wings clipped. Many of the observations on male activity were made around colonies under intensive study in which the location of the nests was known and all the above-ground workers had been individually colour-marked.

5.3. Results

5.3.1. Dates of male activity

Normal winged males are produced in this species and were found inside excavated nests during January-April (Table 18); this limited period of male production was confirmed by sexing pupae collected throughout the year (Table 11). There were only a few males active above ground in January; mating activity reached a peak in February-March and then decreased markedly in April.

Results from nests excavated during the mating season (Table 18) revealed that males were generally present in every nest of a colony; the numbers varied between nests collected in the same month. During January and February a few males were seen to be carried between the nests of a colony; the carrying posture was similar to that used in worker transfer, with the recruiter's mandibles around the male's wings. The recruitment of males within colonies did not follow any organized pattern (see 2.5.2.), and occurred together with the transfer of workers and brood. Many of the cocoons that were transferred between nests

during that period contained male pupae, some of them pigmented.

Table 18 Size of the male population in nests excavated at various times of the year during the period January 1981 to July 1983. These nests correspond to those in Table 1.

Time of year	No. of colonies sampled	No. of nests excavated	No. of males found in each nest
January '81	2	4	5,0,6,10
January '82	1	1	17
January '83	2	3	3,6,9
February '82	1	5	7,9,19,13,12
March '82	1	2	0,0
April '81	1	3	0,0,1
April '83	1	3	0,0,2
July '81	5	5	0,0,0,0,0
July '83	2	2	0,0
October '81	2	2	0,0
October '82	2	2	0,0
December '81	1	2	0,0

5.3.2. Exit from home nest and dispersal

Every day during a three-week observation period in January-February 1982, a few males (1-8) left from each of a number of nests under intensive observation. Departing males appeared in the entrance holes and then retreated; they repeated this a few times over the next 10-30 minutes. Eventually individuals walked out, often climbed up low vegetation and immediately flew off and disappeared; the initial flight took them well away from their home nests.

The time of departure from nests was quite consistent, ranging from 09h00 to 12h00; this was often a time when above

ground workers were no longer active. Male exit times did not appear to be affected by environmental conditions; on cool, cloudy or rainy days, males could come across workers as the latter remained outside the nests for longer periods in the morning (see 2.3.2.).

The dissection of males collected inside the nests (during excavations) revealed that there was little or no sperm in the vasa deferentia and ejaculatory ducts of many of them. An adult male that was painted while being carried between two nests remained inside that nest for nine days; then it left and flew off. Thus it was clear that males left their home nests only when physiologically ready, and this took a few days following emergence from the cocoons. During this time sperm was presumably maturing.

5.3.3. Orientation to foreign nests

Aspects of the searching behaviour of males were studied by observing individuals which had lost one wing naturally or had their wings clipped. Since these were unable to fly they could be followed with ease. The males appeared to search for nests in a random way while on the ground, moving between partially bare areas and the undergrowth at the edges of small bushes. They walked quickly with frequent changes of direction, and investigated little holes and depressions in the ground; they frequently cleaned their antennae while doing this. The males climbed up short grass or low vegetation, where they attempted to take off. Although they inevitably fell on the ground, the mutilated males repeated their attempts many times, interspersing this behaviour with ground searches. This suggested that, after the initial dispersal flight, the normal male tactic was to fly low while looking for suitable sites from the air; then they landed and explored on the ground, but they could take off again if unsuccessful. The movements of one flying male that was observed confirmed this. This male was walking on the ground, went past (1 m away) an occupied nest without locating it , then

climbed up a little plant and took off. It flew for one meter and landed on a grass stem, came down to the ground and resumed the search. In contrast some males landed very close to nest entrances, and others walked straight towards occupied nests shortly after landing; it appeared as if some nests were more easily located than others.

5.3.4. Behaviour around foreign nests

Males were often observed waiting immobile outside nest entrances, either on the ground or on top of short grass stems. The working assumption was that such males did not originate from these nests; as detailed in 5.3.2., the normal behaviour of males leaving their home nests was to fly away immediately. Some nests frequently had many males in their vicinity, while other nearby nests were seldom seen to have any males around them. At times some males investigated the entrance holes with their antennae and hesitantly walked in; some ran out immediately afterwards.

Individual males were repeatedly seen to be forcibly evicted from nests by workers. The males were held by their legs, wings or antennae, and they resisted fiercely (this is in contrast to the passive behaviour of workers being dragged out of nests, see 2.4.7.); many tried to hold on to ground features to prevent their eviction, and some managed to struggle free. A few males stridulated while being dragged. On a few occasions a number of workers cooperated in the eviction of foreign males; workers sometimes attempted to sting the males. After releasing the males in the vicinity of the nests (30 cm-1 m away), the workers ran back into the entrance holes. The uninjured males cleaned their antennae and then immediately attempted to enter the nests again. In one of the nests that was observed five males were trying to walk inside and were being repelled; there seemed to be a small number of workers guarding the entrance, and when these were out dragging some of the males away, the other males managed to enter. In between their attempts the males waited in

surrounding vegetation. When a male encountered a worker in an entrance hole he often turned around and ran away; some workers actually chased males when they came across them outside the nests. In such a situation a marked male escaped by flying away, but later returned to the same nest.

Five males that were present outside one nest on a particular day were marked with a spot of paint. One of these was found near the same nest on the next day, together with eight additional males (ie. unmarked). The latter were marked with a different colour; on the third day one male from the first day and five males from the second day were attempting to enter the same nest. There were also five unmarked males in the morning, and another four at noon.

Eviction did not always follow a male's entrance. Observation of a number of marked males revealed that some remained inside for at least 15 minutes. Males were never seen trying to mount workers outside nests.

5.4. Discussion

Copulation in Ophthalmopone berthoudi was never observed above ground and it is thought to take place exclusively inside foreign conspecific nests. This is a very unusual situation in the formicids. Mating usually occurs at some distance from the nests, and may take place on vegetation, on the ground or during nuptial flights in the air (Wilson 1971). In various ponerines as well as in socially parasitic myrmicines, fertilization occurs in the immediate vicinity of the nests. Holldobler and Haskins (1977) have described the mating of gamergates in Rhytidoponera metallica; what they termed "ergatoids" emerged from their nest (the stimulus for this is not mentioned) and grouped quietly near the entrance. These ants had their heads and thoraces lowered to the ground, the gasters raised and arched, and the intersegmental membranes between the last two

segments dorsally extended.

> Males flying out from other nests are attracted by these "calling" females. On drawing close to a female, the male first touches her with his antennae, then grasps the female's thorax with his mandibles. While riding on her back, he extends his copulatory apparatus in search of the female's genitalia. If she is ready to mate, she turns her abdomen slightly to the side, so that the male is able to couple. Then the male releases his mandibular grip on the female's thorax. With the pair in this position the copulation can last several minutes (Holldobler and Haskins, 1977).

In R. chalybaea, Ward (1981b) reported clusters of hundreds of males outside the nest entrances. There were also large numbers of workers milling around the entrances and most behaved aggressively towards the males, but this did not deter the latter from making repeated attempts to mate with workers. The "calling" behaviour was seen in laboratory colonies but not in the field. Successful male-worker matings outside the nests were observed by Ward (1981b), but males also entered nests and may have mated with workers within these. Males were occasionally dragged out of nests. There are thus a number of interesting parallels between O. berthoudi and certain species of *Rhytidoponera*, but a significant difference is that mating was never observed above ground in the former. This may be related to divergence in the pattern of male dispersal in the queenright ancestors.

Thus males of O. berthoudi need to enter foreign nests in order to mate with suitable workers. The colony units have distinct identities (see 2.5.4), and alien males are recognized as different by the workers, which attempt to remove them from their nest. This aggression exhibited towards alien males contrasts with their acceptance by workers in ponerine species with ergatoid queens. This was reviewed by Longhurst and Howse (1979), who reported that males of both Megaponera and Leptogenys were not attacked following their entry into foreign colonies. In addition, Overal (1980) observed a male of Dinoponera gigantea being carried into a nest by a forager; there seem to be

gamergate reproductives in this species. In O. berthoudi, it seems to be as a result of their persistence that many of the males manage to remain in a nest for long enough to mate. Two circumstances may ease a male's entry into a foreign nest. His colony odour can resemble that of the nest into which he wants to get; this possibility arises from Greenberg's (1979) results on kin recognition in bees. Alternatively, access might be easier during the hot hours of the day, when guards have retreated away from the surface chambers.

It appears that only the older ants (especially those active above ground) are responsible for the evictions; this is expected because workers in this age class also show aggression towards intruding workers (see 2.5.4.). The younger "Innendienst" workers are unlikely to behave aggressively towards the foreign males, and the data in Table 16 indicate that these are the workers that become inseminated. It was observed that males mounted and attempted to copulate with "Innendienst" workers in laboratory colonies. The sexual attractiveness of younger workers appears to be related to their release of an appropriate pygidial gland secretion; this is investigated in Chapter 6. Ward's (1981b) data are very comparable to these in that the males of Rhytidoponera also seem to be attracted by workers of a certain age only. Many of the workers milling around the entrances during male swarming were molested by the latter, but returning foragers were not. Significantly, timid workers collected near these nests were found to be inseminated. Le Masne (1956) also remarked that males of Euponera eduardi copulated with young workers, which he was able to distinguish by their lighter pigmentation.

The exit of males from their own nests is not coordinated; throughout a period of a few weeks individuals were seen to depart. This is in contrast to the situation in the queenright ponerines where the emergence of female and male reproductives appears to be synchronized, possibly through the release of a mandibular gland pheromone by the males (Buschinger 1975). The coordination of the activity of the sexes promotes outbreeding, and it is highly adaptive because dispersal and the initiation of

new nests must occur during optimal environmental conditions (eg. after rain). With the disappearance of the queen caste and the new mode of colony foundation (colony fission, see 2.5.5.), male dispersal no longer has to be timed with a particular environmental stimulus. A reason for this is that copulation is not followed by independent colony foundation by the mated workers. However males have changed their behaviour markedly in order to find their now wingless sexual partners, be they ergatoid queens or workers.

The exact strategy of nest location remains to be elucidated in O. berthoudi. In a study of Leptogenys ocellifera, it was shown that the dispersing males move around in short leaps until they eventually come across one of the chemical trails that lead from the nests into the surroundings (Maschwitz and Muhlenberg 1975). This is not possible in O. berthoudi since trails are not laid. Instead, as the males fly low they search an area; they cannot always use cues such as worker activity because the latter are not active above ground during certain hours on hot days (see 2.3.2.). Chemical attractants are likely to be involved, but this was not investigated in this study. D. Brothers (pers. comm.) observed a number of mutillid males congregated at one spot on the ground. He collected them and when he later returned to the same spot more males were present. Later still, after additional males had arrived, a female mutillid emerged out of the earth. A sex attractant which diffuses through the soil is likely to have been involved. Two exocrine secretions may have attractive qualities in O. berthoudi: (1) the scent marks which workers deposit outside the nests (2.3.5.); (2) the secretions from the pygidial gland (Chapter 6). These are thought to be produced underground by a certain proportion of the workers, thereby indicating their sexual receptiveness. This chemical signal may also be used inside the nest by males trying to find the chambers where the Innendienst workers are. In addition, the volatile secretions from many workers may diffuse out of the nest and the males may perceive this signal and use it as a cue for the presence of a nest.

Once they have located a suitable nest, the males remain near it and do not attempt to find further nests. The same individuals were seen around one particular nest for up to three days, and they may have spent the night in neighbouring vegetation. Some nests seem to be located by more males than others, possibly because they emit more pygidial gland signal. Visual cues, such as other males active around an entrance, can also be involved. During February 1982 many males were observed on successive days around one particular nest; when this nest was excavated five weeks later most of its inhabitants had been inseminated, except for the above ground workers (Table 16). In contrast some nests may not be visited by males or may be visited by very few males, and as a result suitable Innendienst workers do not always become mated. As mentioned in Chapter 3, the differential rate of success with which nests are found by males partly explains the variability in the numbers of gamergates per nest (Table 14). As a result of the polydomous organization of the colony, gamergates can be recruited between nests and hence, a colony should survive from year to year as long as one of its nests is visited by an adequate number of males.

Male visits are irregular, and thus sperm may be limiting in any particular nest. Sperm is however not thought to be limiting at the level of the populations. The reproductive investment in males appears to be no different from that of other formicids. There were never many males inside a nest at any one time, but because haploid eggs are produced for an extended period, substantial numbers appear to be produced every year. A colony (made up of five nests) excavated in February 1982 yielded sixty males; in addition there were those that had already left the nests as well as those that were still pupae.

Since copulation occurs underground, data on the average number of copulations per male and on the incidence of individual workers being mated by more than one male are not available. A subjective comparison between the amount of sperm present in the testis and that held in a spermatheca suggests that one male can inseminate a number of workers. Additional evidence for this is the existence of large numbers of gamergates in some nests (eg.

108 in the nest excavated on 5.XII.1981, Table 14); such nests would have to be visited by an equally large number of males if the latter each copulate with one worker only. This suggestion is unrealistic, even with an extended mating period such as in this species. The actual occurrence of male mating must be inferred from the change in the numbers of breeding workers found in nests. The five nests that were excavated in February 1982 contained only eleven gamergates (Table 14), and yet aggregations of males around nests in the area had been observed for a number of days previously. This can be explained in two ways: (1) by chance, these nests were not located by any males; (2) most of the matings take place later in the season when the numbers of males present above ground has increased, ie. at the peak of male activity (see 5.3.1.). If the latter suggestion is correct, the mating activity occurs for a shorter period than is suggested from the data on male presence.

Data on the extent of outbreeding between colonies of O. berthoudi were not obtained. The distances which the males cover before they land and proceed to search for nests are unknown. However there is no indication that inbreeding occurs; since male sexual behaviour is normally triggered only once they have left their nests, they should not mate with their nestmates. As Crozier (1980) remarked, inbreeding may be much less common in social insects than is often thought. It is frequently invoked on an ad hoc basis in order to hypothesize high levels of genetic relatedness between nestmates, a necessary prerequisite for the efficacy of kin selection (Hamilton 1972). Field observations in this species show that the initial flight of males bears them away from the other nests of their colony. When the colony consists of nests that are far apart (eg. 75 m), there is a remote possibility that males would disperse from one nest of their natal colony to another, but it is far more likely that they will encounter a foreign nest.

A knowledge of the reproductive structure in O. berthoudi reveals that haploid eggs are exclusively laid by inseminated egg-layers; unmated workers do not lay eggs (Chapter 3). This suggests that egg fertilization is a voluntary act by the

gamergates, which thus have the ability to determine the sex of their offspring; the control of sperm release from the spermatheca during oviposition has been demonstrated in various Hymenoptera, eg. in leaf-cutter bees (Gerber and Klostermeyer 1970). Sperm exhaustion is not thought to be an important factor in the production of haploid eggs in O. berthoudi, because individual gamergates do not produce very many eggs during their lifetime. In many formicid species unmated workers are partly or entirely responsible for the laying of haploid eggs. In the queenright Rhytidoponera purpurea, unmated workers can produce a prolific male brood in the absence of the parent female; it is not known whether the seasonal male brood prpduced in normal colonies is queen- or worker-derived (Haskins and Whelden 1965).

Males are only present for a relatively short period of time (no male pupae or adults were ever collected outside the period January-May), and this indicates that haploid eggs are only laid at a specific time of the year. Since the developmental period from egg to adult is roughly 75 days (4.3.1.), it is inferred that the gamergates begin to lay unfertilized eggs at the end of September-beginning of October. It is not known whether all the gamergates in a nest produce haploid eggs. The existence of nests in which male eggs are not laid would be obscured by the inter-nest transfer of adult males and pupae. What is the trigger for haploid egg production? The gamergates are confined inside their nests and this restricts the types of environmental cues available to them. Nkuzi Reserve is in a summer rainfall area and the period May to August is generally dry. The first summer rains occur in September and October and are characteristically heavy downpours which can last for a few days (Appendix E). As significant quantities of water seep through the soil, the humidity inside the nests would markedly increase, and this could be a stimulus recognized by the gamergates. An additional cue such as the seasonal changes in photoperiod might prevent mistiming due to the eventuality of freak off-season rains (eg. August 1981, Appendix E). Gamergates inside the nests could detect this by monitoring diurnal foraging activity, which changes from monophasic in winter to biphasic with the

rising day temperatures (see 2.3.2.). The validity of these speculations is reinforced by a correlation between the rainfall pattern at the end of 1981 and 1982, and the dates of male activity in 1982 and 1983. The end of 1982 was unusually dry, with the first rains occurring later in the year; little male activity was witnessed at the end of January 1983, in contrast to that observed at the same time the previous year. Later in the year (April 1983) many males were present outside the nests, and there was a substantial number of male pupae (19) in the nests that were sampled (Table 11). Thus the dates of male occurrence vary slightly from year to year. The extended period of male activity reflects the inefficient synchronization of haploid egg production by the unspecialized gamergates.

Originally, the ancestral reproductive castes would have been produced seasonally; however, since the queens have disappeared, the male reproductives are now restricted to mating with a sample of the workers, whose emergence coincides with theirs. The workers which are sexually attractive remain inside their nests, and as a result male activity no longer needs to be synchronized with their dispersal. In addition, the uncoupling of mating and colony foundation obviates the need for selecting appropriate environmental conditions during dispersal. This relaxation of the selective pressures on male behaviour has resulted in the latter leaving their home nests individually over a long time period. Ward (1981b) found a similar pattern in Rhytidoponera. The seasonal production of male reproductives is an evolutionary legacy dating back to queenright ancestors. In O. berthoudi it no longer seems to have any adaptive significance since workers that can become mated occur throughout the year.

CHAPTER SIX SEXUAL ATTRACTION AND THE PYGIDIAL GLAND

6.1. Introduction

Female sex attractants were first discovered in a ponerine ant by Holldobler and Haskins (1977); previously only myrmicine ants were known to possess sex pheromones (Holldobler 1971). Holldobler and Haskins showed that in Rhytidoponera metallica the chemical attractant was released from the hitherto unrecognized pygidial gland. This new gland has subsequently been found in a number of ponerine genera (Holldobler and Engel 1978). The secretions of the pygidial gland are involved in several aspects of chemical communication between workers in the Ponerinae. The pygidial gland in Bothroponera (=Pachycondyla) tesserinoda is the source of a tandem running pheromone (Holldobler and Traniello 1980a) which is released into the air to ensure that the second ant follows the leader. The gland is also used in other species to lay chemical trails on the substrate. In Pachycondyla laevigata it produces a trail pheromone which regulates recruitment to termite prey (Holldobler and Traniello 1980b). The workers of Leptogenys chinensis, a termite-raiding species with an ergatoid queen, use the pygidial gland secretions as a ground-laid recruitment signal for group predation and colony emigration (Maschwitz and Schonegge 1977). It is only in Rhytidoponera workers that this pheromone has been implicated in mate attraction. This function may be of particular importance in those species in which the female reproductive caste has been replaced by breeding workers.

Holldobler and Engel (1978) reported that the pygidial gland lies anterior to the seventh tergite (pygidium), near the intersegmental membrane. It consists of two clusters of rounded cells, each cell being connected to the intersegmental membrane

by a fine duct. The membrane is invaginated to different degrees to form a reservoir for the gland's secretions. If no reservoir is present, the glandular structures can easily be missed during dissection, and histological sectioning is therefore required to determine whether or not the pygidial gland is present (Holldobler and Engel 1978). In this chapter the occurrence of the pygidial gland in various categories of workers of O. berthoudi was investigated histologically. The Materials and Methods and the Results are modified from Villet, M., C. Peeters and R. Crewe (The occurrence of the pygidial gland in four genera of Ponerinae (Hymenoptera: Formicidae). In preparation).

6.2. Materials and Methods

O. berthoudi individuals were arranged into four categories:
(i) callows - recently emerged from the pupal cocoon and with poorly sclerotized cuticle (n= 3)
(ii) young workers ("innendienst") - with sclerotized cuticle and confined inside the nest; they are unmated and have undeveloped ovaries (n= 2)
(iii) older workers - differentiated from the above category on the basis of observed age polyethism (n= 5)
(iv) gamergates - identified by dissection and examination of ovarian development and spermathecal contents (n= 3).

After individuals had been immobilized by chilling, tergites six and seven were dissected from the abdomens and fixed in Pampel's solution for 24 hours. These were then dehydrated, cleared in propylene oxide and embedded in Epon-Araldite. The resin blocks were cut into 5 µm sections on a Porter-Blum ultramicrotome, stained with toluidine blue and mounted for light microscopy.

6.3. Results

All the individuals investigated were found to have pygidial glands with reservoirs. Thus the gland occurred in sterile workers of all ages as well as in the gamergates. The location of the glandular cells is indicated in Figure 11. In all the categories of individuals the gland consisted of approximately the same number of cells per section. The degree of cuticular sculpturing did not vary within the species.

6.4. Discussion

The results show that the pygidial gland is present in this ponerine. The gland was found in all the workers of Ophthalmopone berthoudi examined, irrespective of their age or reproductive status. This provides further evidence that there are no separate reproductive and worker castes in this species. Holldobler and Haskins (1977) reported that in Rhytidoponera metallica, some workers produce a sex attractant in the pygidial gland which they discharge during sexual calling. Holldobler and Haskins seemed to believe that the reproductive workers belong to a distinct female caste, differentiated from other workers partly on the basis of their behaviour during the mating season, ie. only the former come out of the nest and "call". In O. berthoudi, there are differences in the sexual attractiveness of workers, but they seem to be related to age. The results in Table 16 reveal that a proportion of the workers remained virgin during the period of male activity. The above ground workers did not become mated, which indicates that adults are not sexually attractive when they are above a certain age.

The response of males to the secretions of the pygidial gland was not tested in O. berthoudi. Holldobler and Haskins (1977) found that in Rhytidoponera metallica, males responded with agitated locomotion and attraction to the scent of the gland.

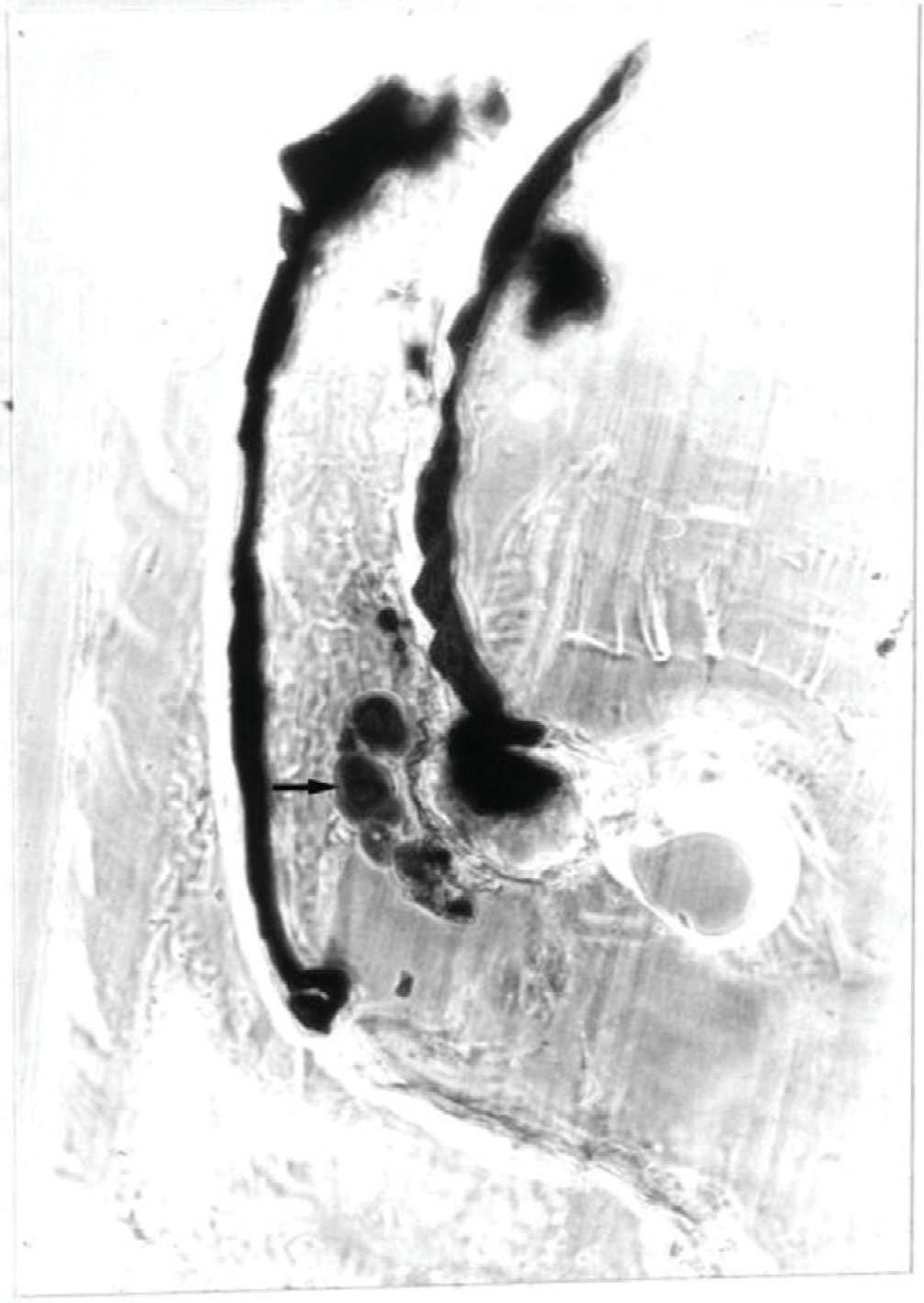


Figure 11 Longitudinal section through the sixth and seventh abdominal tergites of a gamergate of Ophthalmopone berthoudi, showing the cells of the pygidial gland (marked with an arrow) (x 860).

In O. berthoudi it is reasonable to assume that the function of the pygidial gland is sex attraction; there is no tandem-running or trail-laying behaviour, which is how workers of other ponerine species utilize these glandular signals (see 6.1.). (The discrete chemical marks deposited on the substrate, see 2.3.5., are laid using the ventral tip of the abdomen and are presumably produced in the Dufour's gland). It is not known whether the unmated workers of O. berthoudi adopt the calling posture inside the nests. It is suggested that the differential attractiveness of workers to males is an effect of differences in the signals produced by their pygidial glands. Meinwald et al. (1983) recently characterized the pygidial gland secretions of Rhytidoponera metallica and showed that there were a number of substances involved. Such a multicomponent pheromone can have differing information content depending on the relative proportions of the various components. As has been shown for the mandibular gland pheromones of worker honeybees (Crewe 1982a), blends of components can change with age. Such changes in biosynthetic activity would not be reflected in the glandular morphology of different age groups of workers, and would need to be investigated using gas chromatography and bioassays. The proximate cues for the emission of the pygidial gland signal by the workers remain to be discovered. Either it is released exclusively during male visits, or it is released continuously and only takes on a sexual meaning when it is perceived by the right audience, ie. when foreign males are present.

A description of the sexual "calling behaviour" of Rhytidoponera metallica (Holldobler and Haskins 1977) has been given in 5.4. A similar posture was observed not only in R. chalybaea workers (Ward 1981b), but also in the winged queens of Amblyopone pallipes (Haskins 1978). In the myrmicine Harpagoxenus sublaevis, the true ergatoids adopt a "calling posture" while releasing a sex pheromone from the poison gland (Buschinger 1975); this posture is essentially identical to a component of the tandem-calling behaviour of the recruiting workers in some species of Leptothorax (Moglich et al. 1974). This suggests that sexual calling in the myrmicines is derived

from the recruitment behaviour; a similar evolutionary transformation might have taken place in the ponerines that lost the queen caste. Ward (1983) suggested that "if the queens attract males by pheromonal calling, then similar behavior might well evolve in workers, making them liable to insemination". This worker behaviour in fact existed, but it had a different purpose (ie. recruitment, see 6.1.). How did the males come to recognize the chemical signals of the workers?

Holldobler (1971) showed that, in the myrmicine Xenomyrmex floridanus, the secretions from the poison gland function as a signal for two different audiences. The workers' secretions release a strong recruitment and trail following behaviour in workers, while the poison gland secretions of female reproductives are both attractive and sexually stimulating to males. The workers' poison gland secretions also attract males which reveals that the signal intended for one audience can also be recognized by another audience. Thus Holldobler (1971) hypothesized that sex attractants and recruitment pheromones may have a common evolutionary origin. More specifically, Holldobler and Haskins (1977) postulated that the primary function of the pygidial gland was not the secretion of sex pheromones, since it has been found in workers of a number of queenright ponerine species (Holldobler and Engel 1978). Recently Holldobler (1982) speculated that the pygidial gland pheromone in Rhytidoponera also functions as a recruitment signal, because workers are attracted to it. It is not clear however whether the pygidial gland is present in the abdomens of winged and ergatoid ponerine queens, because various authors (eg. Holldobler and Haskins 1977, Holldobler 1982) seem to use the terms "wingless virgin females" and "ergatoids" to describe breeding workers.

All the above evidence suggests how breeding workers may have taken the place of queens in the Ponerinae. When both female reproductives and workers of queenright species possess the pygidial gland, the same gland appears to serve different functions in the two female castes: released in the air the secretions are a sex pheromone, laid on the ground they are involved in recruitment. The presence of the pygidial gland in

the workers thus represents a preadaptation for a change in reproductive systems; with the disappearance of the queen caste the workers can become mated since they produce the appropriate signal for male attraction.

CHAPTER SEVEN GENERAL DISCUSSION : LOSS OF THE PONERINE QUEEN CASTE

7.1. Biology of Ophthalmopone berthoudi

The ponerine O. berthoudi has undergone a dramatic modification in its reproductive system. The queen caste has been permanently replaced by multiple breeding workers (=gamergates) which coexist in the nests. Gamergates are differentiated from their sterile nestmates during the period of male activity, because insemination stimulates ovarian development. Every worker in a colony is capable of becoming reproductively active. However, while workers are produced throughout the year, males are only produced during summer. The workers have a short life-span, and thus the lives of some of them do not coincide with the mating season. In addition, not all workers are sexually attractive when foreign males visit the nests. Since only the non-callow "Innendienst" workers become mated, these individuals are suspected of releasing an appropriate recognition signal from their pygidial gland, to which males are attracted. Although they are occasionally carried between the nests of their polydomous colonies, the gamergates remain confined underground instead of becoming active outside the nests as they age.

Nests generally contain a number of breeding workers, and these numbers are high in colonies that are located by many males. What is the adaptive significance of large reproductive populations? Holldobler and Wilson (1977) have interpreted polygyny in myrmicine and formicine ants as a trait which is selected at the level of the colony, ie. a strategy to colonize certain habitats. However O. berthoudi does not have a true polygynous system since the queen caste has been lost. The many egg-laying workers lack specialized ovaries, and they lay large

yolky eggs at a slow rate. In fact, the day-to-day laying is performed by a succession of different individuals, and one breeding worker probably contributes only a small number of eggs during its life span of a year. Thus cooperative breeding by many gamergates is necessary for colony growth. Worker breeding appears to be an evolutionary *ad hoc* response to the disappearance of the queen caste; this substitution is likely to have no survival value outside a specific ecological context (see 7.2.).

The gamergates have not evolved new queen attributes. There is only one caste in the nests and the interactions between individuals are not very elaborate. Hence, the cohort of breeding workers does not elicit preferential treatment from the non-reproductive workers, eg. during termite feeds. There is no regulation of the numbers of gamergates, either by the non-reproductive workers or by the gamergates themselves. Dominance hierarchies were not detected, and there does not seem to be any inhibition of laying or oophagy.

The presence of a non-reproductive group of individuals cannot be accounted for in *O. berthoudi* by arguments such as the genetic ones used in the kin selection and parental manipulation hypotheses. The effectiveness of kin selection (Hamilton 1972) as an evolutionary force that maintains reproductive division of labour is dependent on the degree of genetic relatedness between colony members. Although electrophoretically-detectable genetic variation was not studied, an understanding of the structure of the breeding system in this species leads to a prediction of low kinship between nestmates. A large number of breeding workers and a substantial number of males take part in sexual reproduction. There is no overlap between successive generations of gamergates and thus the relationship of the egg-layers in a colony ranges from sisters to cousins. Thus the degree of relatedness among the progeny is probably exceptionally low. This is in spite of the likelihood that breeding workers are singly-inseminated and that individual males mate with a number of workers in the nests. Both these factors would raise the relatedness between nestmates to a limited extent.

Furthermore, the frequent transfer of brood and adults between the nests of a colony leads to a decrease in the number of sisters in a nest. This has significant consequences when a new colony buds off from an existing one.

The complete loss of the queen caste undermines the notion of parental manipulation (Michener and Brothers 1974, Alexander 1974). Queen oppression cannot adequately explain the origin of the functional sterility of a class of nestmates in O. berthoudi. Furthermore this present social system is not maintained by an inhibitory effect of the gamergates on the workers.

The colonies of O. berthoudi are polydomous. The recruitment of adults and brood between the nests is a common occurrence, and this has the important effect of integrating all the workers as members of a distinct colony unit. Breeding workers are found in every nest of a colony, and this makes colony reproduction by fission possible. Colony fission is obligatory in this species because the worker nature of the egg-layers precludes partially-claustral colony foundation. Breeding workers, singly or in small groups, could not produce a first generation of workers quickly enough. Worker behaviour is totally inappropriate for the colony-founding task, and this dictated an irrevocable modification in the genesis of colonies.

Workers can discriminate between colony members and non-members. Colony fission is accompanied by the acquisition of a distinct new identity. The discovery of a marked ability to recognize nestmates in these societies is in sharp contrast with the finding by Holldobler and Michener (1980) that colony odour seems usually less distinct in polygynous (and often polydomous) ants than in monogynous ones. These authors assumed that the queen provides some of the essential ingredients of colony odour. A queen is absent in O. berthoudi, and the presence of multiple breeding workers introduces substantial genetic variation in the colonies, yet there is a strong colony integrity.

Colony multiplication occurs as a result of the chance fragmentation of nest complexes. It takes place throughout the year and need not follow the mating season. Crozier (1979) suggested that there may be a threshold value for intra-colony

relatedness below which colony fission should accelerate. There is strong evidence from the present work that colony fission is brought about by stochastic factors unconnected with either genetic relatedness between nestmates or colony odour. The ability of individuals to recognize members of their own colonies is not affected by the predicted low kinship between nestmates. This supports the idea (Buckle and Greenberg 1981) that individuals learn the range of odours which occur in a colony.

Low levels of genetic relatedness between nestmates have been reported in other ponerines. Ward (1983) estimated pedigree relatedness in "Type A" and "Type B" colonies of Rhytidoponera confusa and R. chalybaea. Assuming that a "Type B" colony had four breeding workers, the relatedness between nest-mates in the monogynous "Type A" colonies was more than twice as high as in "Type B" colonies (Ward 1983). These results were obtained from both a study of the pattern of genotypic diversity within colonies and from a population-wide regression analysis of the frequencies of marker alleles. In the primitive ant Nothomyrmecia macrops, Ward and Taylor (1981) found a low level of genetic relatedness among workers from the same nest. This finding was surprising since these queenright colonies are monogynous. It strongly suggests the possibility of additional reproduction by mated workers, but also emphasises that high kinship between colony members is not a sine qua non of eusociality.

7.2. Why lose the queen caste?

The assumption of the breeding role by mated workers has occurred repeatedly and independently in a number of groups of ponerines: the metallica complex and the larger species of Rhytidoponera (Haskins and Whelden 1965), Diacamma rugosum (Wheeler and Chapman 1922), Ponera eduardi (Le Masne 1956), Dinoponera grandis (Haskins and Zahl 1971), Pachycondyla ("Bothroponera") (Ward 1983), Ophthalmopone berthoudi,

Streblognathus aethiopicus (I. Bampton pers. comm., Peeters unpublished data), Platythyrea arnoldi, Hagensia marleyi, Plectroctena conjugata and Pachycondyla wroughtoni (Peeters unpublished data). The circumstances leading to the disappearance of the reproductive caste remain enigmatic. Loss of the queen is unexpected since the identifying characteristic of eusociality is a division of labour through reproductive differentiation and specialization. Ward (1983) has postulated that the evolutionary origin of worker-reproductive colonies "may be traced to selective pressures on worker reproduction in orphaned queenright colonies or in queenless colony fragments isolated during colony movement". I suggest instead that it is a result of selection against queen reproduction in particular ecological contexts. More specifically, the queen caste may have been lost in environments where there is a decline in food availability during certain periods of the year (eg. dry winter).

The members of the sub-family Ponerinae are distinguished by their highly-specific diets, ie. many of them hunt a single prey such as termites, beetles or isopods. While genera such as Leptogenys and Megaponera exhibit sophisticated mass recruitment systems which they use to coordinate their hunting activities, many other genera are unable to lay chemical trails on the substrate and must resort to solitary foraging. This lack of specialization in the mode of hunting may lead to trophic stress during the unfavourable times of the year. In semi-arid environments for example, the extended dry season is a period of reduced prey availability. The foraging activity of O. berthoudi drops to a low level during the winter months, when the low nocturnal temperatures and the dry soil cause a reduction in above-ground termite activity. The foragers of another queenless ponerine in Mkuzi, Plectroctena conjugata, only hunt their millipede prey after rain, and are thus inactive for many weeks in winter (Peeters unpublished data). Streblognathus aethiopicus occurs in high-altitude mountain habitats where the very cold winter temperatures and snow halt the hunting of crickets and beetles. Thus certain species of ponerines with restricted diets are likely to experience seasonal difficulties

in obtaining their food.

Is there an interaction between temporal trophic stress and reproductive behaviour? Many formicid species produce a large number of winged sexuals. These do not work in the nests and are a drain on a colony's resources since they need to be fed as both larvae and adults. Even after this substantial energetic investment, only a small proportion of the virgin queens mate and found colonies successfully. Colony foundation is generally characterized by a dealated female becoming cloistered with her first brood inside a cell; this is the dominant pattern among the higher ants. In contrast, ponerines which have retained queens exhibit a partially claustral mode of colony foundation (Haskins 1970), and this is more risky. The dealate queen must leave her nest at frequent intervals to obtain food, because the energy obtained from the resorption of her flight muscles is insufficient to raise a first generation of workers. This lone hunting by the ponerine foundress makes her vulnerable to predators and accidents, and her brood can be parasitized during her absence from the nest. Her success at founding colonies is linked to a particular set of biotic and abiotic conditions. Successful emergence, aerial dispersal, coupling of the sexuals and location of nest sites free from competition with other existing colonies, are all prerequisites to the process of colony foundation, and can also influence the mortality of founding queens. In spite of many perils, partially-claustral colony foundation persists in some extant species. In others, the foundation of new colonies by winged females may have been made much more precarious by a change in ecological conditions. Only an increase in the numbers of virgin queens produced could offset the lowered success rate. The mating activities of male and female reproductives are generally synchronized with the advent of summer; this appears to be an ancestral Formicid trait. In many higher ant species living in temperate latitudes, brood can overwinter and thus the eggs that turn into sexuals can be produced at the end of the previous summer. In ponerines where this does not occur, the larvae that turn into reproductives must be produced at the end of winter. Certain

ponerine species inhabiting semi-arid habitats cannot get adequate amounts of food at this time of the year, and this may prevent them from having a large sexual brood. These species would then be unable to produce sufficient virgin queens to achieve an adequate replacement rate. Colonies in which workers were able to replace the female reproductives may have been the only ones to survive.

Did the evolutionary substitution of the queens by workers occur slowly and gradually? Ward's data (1981b, 1983) on the conspecific occurrence of queenright and worker-reproductive colonies in the Rhytidoponera impressa group apparently suggest that it did. However, the dimorphism in colony structure is stable, and thus this situation does not represent a transition in an evolutionary sequence. It is not known whether the presence of occasional queens in these ants is selectively advantageous.

It is surmised here that reproductive systems changed during speciation events. These involve episodes of allopatric isolation, thought by many to be short-lived and to involve small numbers of individuals (Paterson 1984). A segment of an ancestral queenright population could have become restricted to a sub-optimal habitat in which partially-claustral colony foundation was no longer adaptive (as outlined above). This resulted in strong directional selection which led to the elimination of queens. Worker reproduction was viable in the ponerines because of a number of preadaptations (see 7.4.). The mechanisms which are responsible for the physical disappearance of an entire caste need to be investigated in greater detail.

Ward (1983) reported an association of xeric habitats with worker-reproductive colonies in the genus Rhytidoponera, which supports the hypothesis that a temporal food shortage is the driving force for the change in reproductive pattern. However Ward suggests that ".. xeric conditions favor polygyny and colony fission over monogyny and queen colony foundation". He thus interprets "polygyny" (an inappropriate use of the term when referring to multiple breeding workers, see 7.1.) and colony fission to be strategies that evolved because of their higher

selective advantages under xeric conditions. My explanation stands as a distinct alternative to Ward's, because it shifts the focus from the advantages of worker reproduction to the disadvantages of queen reproduction. The queen-based colonial organization with its associated energetic constraints was not flexible enough to accomodate to a new set of environmental conditions, and worker reproduction became the only feasible solution. In a reproductive system with breeding workers, those individuals that do not become fertile function as labourers, and there is thus little wastage of reproductive investment. However, ergonomic benefit (Oster and Wilson 1978) is thought not to have been the prime driving force for the elimination of queens; if it was, queen loss should have occurred more frequently throughout the Formicids.

Ward (1983) repeated Wheeler's idea (1928) that worker mating is primitive in ants and that a dimorphism of winged and wingless females predates the origin of eusociality. This is clearly not supported by the arguments presented in this section.

7.3. Ergatoids and breeding workers

The ponerine group has come to be characterized by the presence of worker-like egg-layers in many of its species, and these are often termed "ergatoids" or "ergatogynes". Wheeler and Chapman (1922) pointed out however that there is a distinction between ergatoid queens and breeding workers, and this is crucial to an understanding of the range of reproductive strategies exhibited by the Ponerinae.

Wheeler and Chapman (1922) suggested that "ergatoid forms may be derived from the typical winged queen through a loss of the wings and a progressive, degenerative simplification of other characters". The external appearance of ponerine ergatoids usually makes them distinctive from the workers. B. Bolton (pers. comm.) describes the ergatoid of Megaponera foetens as lacking ocelli, without a trace of flight sclerites but with a grossly swollen abdomen; it differs from the workers in size as

well as in various external details. Bolton considers that the major step in the transformation from the alate to the ergatoid condition in Plectroctena (in which both winged queens and ergatoids occur) was the reduction in size of the mesonotum and the suppression of the transverse suture (which divides the mesonotum into a large anterior scutum and a small posterior scutellum). However there are reports that, in both Plectroctena and Leptogenys, the ergatoid queens are not always very distinct. Haskins and Whelden (1965) note that in Leptogenys sens. str., "the laying female is no longer morphologically distinguishable from the worker". Similarly, L. schwabi Forel lacks a distinct ergatoid (Blomefield 1979). It is suggested that ergatoids may have been replaced by gamergates in species of both these genera (eg. in P. conjugata and in L. maxillosa (Smith), Peeters unpublished data).

Haskins and Whelden (1965) have pointed out that the ergatoid is as effective a reproductive as the winged female. However, whether the loss of wings was paralleled with a degeneration of the specialized reproductive organs has not been thoroughly investigated. The examination of ovaries in various classes of individuals from different species (Peeters unpublished data) demonstrates that the ergatoids are still part of a distinct reproductive caste. Two ergatoids and some workers of Leptogenys nitida (Smith) (collected from Knysna, Eastern Cape Province) (Figure 12a) were dissected. There were structural differences between the ovaries in these two castes; ergatoids (n=2) possessed 13-14 long ovarioles per ovary (Figure 12b), while workers only had 1 or 2 ovarioles (these were not very distinct). Similar differences in ovarian structure are also found in ponerine species with winged queens. In Paltothyreus tarsatus (collected from Mkuzi), virgin queens (n=5) (collected during their aerial dispersal) had two pairs of 12-14 ovarioles, and there were 7 undeveloped ovarioles per ovary in the workers. These data indicate that caste differentiation in some Ponerinae was accompanied by a marked elaboration of the ovarian apparatus, and this was not lost during the evolution of ergatoid queens. In addition it is thought that the workers still recognize and



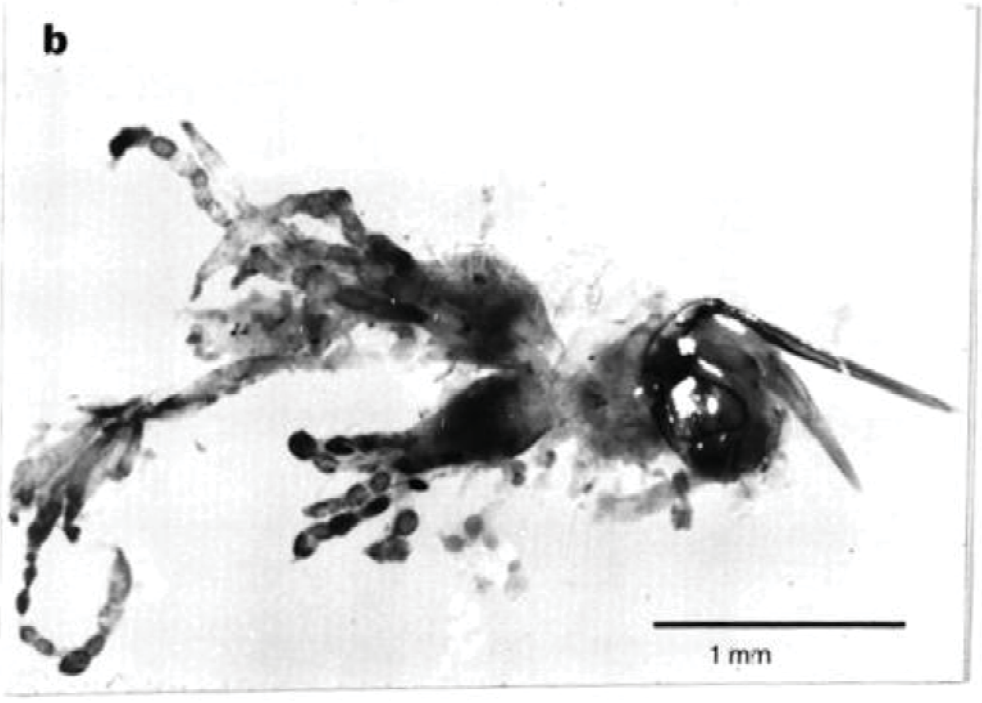


Figure 12 (a) Ergatoid queen (left) and worker (right) of Leptogenys nitida.
(b) Ovaries of inseminated ergatoid of L. nitida, showing two pairs of about 11 ovarioles, as well as the small size of the eggs.