DAY TWO Transfers of many unmarked adults, cocoons and larvae were observed, all coming from (A); six marked workers were involved in this (Table 7).

The second observation period in May was short and interrupted by heavy rains. There were 33 marked ants (ie. survivors from the previous observation period, when 214 ants were marked) active above ground during one morning (Table 8). No further ants were marked. Nests (A), (D), (E) and (F) were no longer occupied, and five new nests were active: (G), (H), (I), (J) and (K). Transfers of adults and brood occurred between (C), (G), (H) and (I). Transfers also occurred between (J) and (K). Nest (C) became inactive and its inhabitants were transported away, mainly to (G).

During the third observation period in July there was little activity generally. Ten marked ants were seen above ground (Table 8). There were only three active nests recognized in this complex (ie. nests which contained marked ants): (I), (M) and (L). Nest (L) was 87m away from the old nest (C). There was adult transfer between (I) and (M). Nests (I) and (M) were excavated at the end of this observation period. No further marked ants were found, which indicates that all the other ants marked in March-April had died. A large number of adults from nest (I) was dissected (Chapter 3, Table 14, 16.VII.1981).

The behavioural changes exhibited by workers as they age were documented over the few months during which this colony was monitored in the field. Some ants (33) were observed over a period of 50 days, and a few survivors (10) over a period of 100 days (Table 8). Of these 33 ants, 14 had not been active above ground in March- April (ie. they were "Innendienst" ants, having been marked either after being carried between nests or collected inside nests). By the middle of May all such ants were now involved in tasks outside the nests. A clear progression of above ground roles can be inferred from the change in the behaviour of particular ants with time, eg. #140, #201, #199, #70,.. (Table 8). Similar results are presented in Table 9 (eg. $175, $186, $230, $231,...). The general pathway appeared to be

Table 8 Ontogeny of individual workers' behaviour and movement within a complex of nests (colony two) over time. The activities of 33 marked ants were monitored during three separate field visits over a period of 12 weeks. The ants are grouped on the basis of occupancy of a particular set of nests in the nest complex, ie. (A),(C),(F) or (D), (E). Within the groups, the ants are arranged according to roles, ie. Innendienst workers, then above ground workers. (insi= confined inside nest, ie. Innendienst; exca= excavator; clea= cleaner; rec= carrier; fora= forager; abo= unknown above ground activity). Upper case letters between brackets refer to nests, see Figure 4; when two nests are separated by -, the direction of transfer was from the first to the second nest.

Ant	March/April data	May data	July data
#118	carried (A)-(C)	abo (H)	abo (I)
#140	carried (D)-(C)	abo (H)	rec (I)-(M)
#124	carried to (C)	exca (G)	abo (M),(I)
#201	carried to (A)	exca (G)	fora (M)
#114	carried (A)-(F)	exca (G)	---
#179	insi (C)	abo (G)	---
#155	insi (C)	abo (G)	---
#117	insi (C)	abo (H)	abo (M)
#113	insi (A)	exca (G)	---
#212	insi (A)	abo (H)	---
#62	rec (A)	abo (G)	---
#87	rec (F)	abo (G)	---
#58	clea,fora (C)	abo (G)	---
#150	fora (A), (C)	rec (G)	---
#152	fora (C)	abo (G)	---
#115	abo (C)	exca (G)	---
#56	abo (C)	exca,fora (G)	---
#208	abo (C)	fora (G)	---
#47	carried (C)-(D)	abo (G)	---
#40	carried (C)-(D)	abo (J),(K)	abo (L)
#90	carried to (D)	abo (J)	abo (L)
#186	insi (E)	abo (J),(K)	---
#128	exca (E)	fora (J)	abo (L)
#16	exca (D), (E)	fora (J)	---
#199	exca (D)	fora (J)	fora (L)
#4	rec (E)	fora (J)	fora (L)
#70	exca, rec (E)	fora (J)	---
#169	fora, rec (E)	abo (J)	---
#5	fora (D)	fora (J)	---
#9	abo (D)	abo (J)	---
#41	abo (D)	fora (J)	---
#65	abo (E)	abo (J)	---
#112	abo (E)	fora (J)	---

excavating, then cleaning, carrying and finally foraging. The data in Table 8 also reveal a substantial mortality of above ground workers. It must be noted that, of the ten marked ants seen in July, all had been seen in May also (they were all originally marked in March).

The results in Table 8 also show what the movement of ants within a colony complex (Figure 4) was over time. Nests disappeared (eg. (D),(E); (A),(F)), and other nests were initiated (eg. (J), (K),(L); (G),(H),(I),(M)). The nests active during the first observation period could be grouped on the basis of physical proximity, and the subsequent nests were located in either of the two sites. The movement between these two sets of nests stopped soon after the first observation period. The ants that were originally found in nests (C) and (D) were all later found (except #47) in (J), and then in (L). Similarly, ants in (A), (C) and (F) were exclusively found in (G) and (H), and later in (K) and (I). This is evidence that what was once a single colony (there were frequent two-way transfers of adults between (A,C) and (D)) had now become two disjunct groups of nests. There were no more transfers of marked ants between the two parts of the former colony, and colony fission had occurred.

2.4.4. Intensive study - colony three

This complex of nests was monitored over a period of four months. Observations were made during three separate visits: 13th- 27th October 1981, 30th- 5th December 1981, 27th January- 12th February 1982; these observation periods are referred to in Tables as (3a), (3b) and (3c) respectively. Ants were marked during each observation period.

Two hundred and thirty-five ants were marked during the first visit. Initially the colony consisted of three nests: (P), (Q) and (R) (Figure 5); (P) was the most active with much foraging originating from it. Nest (S) was started on 18th October. There were frequent transfers of brood and adults between all the nests, but no pattern of movement could be determined. The

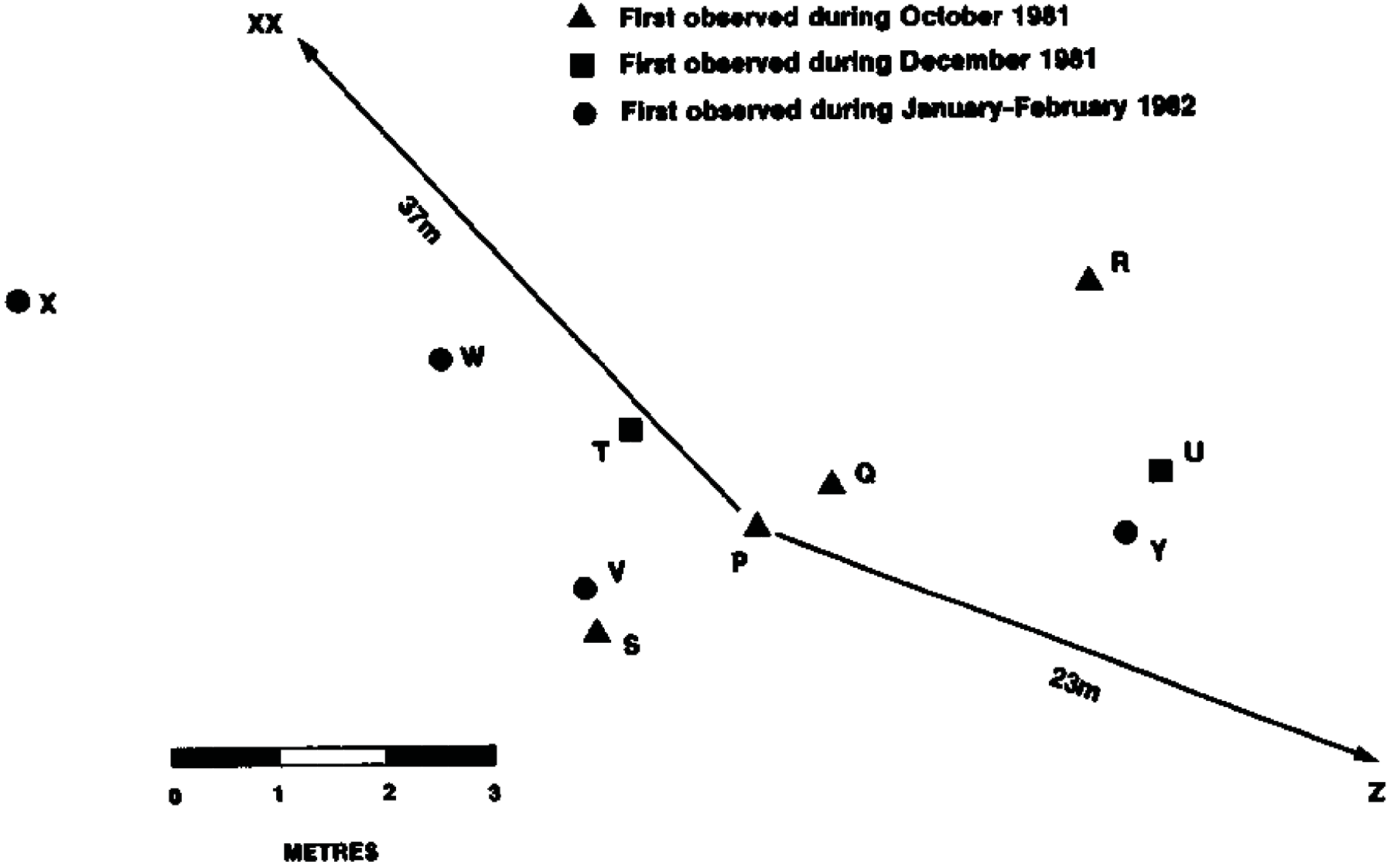

Figure 5 Plan of colony three. The nests were observed during different observation periods.

carrying behaviour did not appear to distribute adults and brood over the colony in any consistent fashion; even two-way transfers occurred simultaneously between some pairs of nests. Termites were frequently transported between nests. Nest (E) was excavated at the end of this observation period; it contained 81 adults, 34 of which marked, and brood (details can be found in Tables 1 and 14: 27.X.1981, first nest).

The following observations suggest that this nest complex had recently fragmented from an adjacent colony. Ant $110 was transferred (carrier ant was $78) from nest (P) to a distant nest, (XX); this nest was not known to belong to colony three, and was 37 m away from (P). The next day $64 and $120 (a forager) were active around (XX), which was part of a distinct nest complex with most of its ants unmarked. Another adult transfer was observed from (P) to (XX), and both ants were unmarked. Ant $110 (which had been carried here the previous day, see above) was seen active outside (XX). Later $120 carried a larva from (P) to (XX). An aggressive interaction was witnessed outside (XX): $62, an ant that had not been seen in this vicinity previously, was dragged for a small distance by an unmarked worker. A forager from (XX) was later released into (N). After 5 minutes it was dragged out of this nest. It seems clear that these two colonies were distinct from one another, yet a few individuals were still active between some of the new alien nests.

During the second observation period in December, nests (P) and (Q) were still active and (T) had been started. Nest (N) was initiated on 5/12 with transfers from (P) and (Q). Only 17 marked ants were seen above ground (Table 9); a further 112 ants were marked in this colony.

At the beginning of the third observation period in January-February there were six active nests; two had been seen before, ((P) with a new entrance, and (T)), and the others were new ie. (Y),(N),(X),(Z). Nest (Y) was started a few days later, and nest (X) then ceased to be active. Characteristically there were frequent adult and brood transfers between nests, including (Z) which was 23m away from nest (P). There were only six marked

Table 9 Ontogeny of workers' behaviour and movement within a complex of nests (colony three) over time. The ants are arranged according to roles, ie. Innendienst workers, then above ground workers. All the ants marked in October had disappeared 14 weeks later. (insi= confined inside nest, ie. Innendienst; exca= excavator; clea= cleaner; rec= adult carrier; lar= larva carrier; coc= cocoon carrier; fora= forager; abo= unknown above ground activity). Upper case letters between brackets refer to nests, see Figure 5; when two nests are separated by -, the direction of transfer was from the first to the second nest.

Ant	October data (3a)	December data (3b)	Jan/Febr data (3c)
$168	insi (P)	clea (P)	---
$175	insi (S)	clea (Q)	---
$186	insi (Q)	fora (P)	---
$220	insi (Q)	clea,fora (P),(T)	---
$221	insi (Q)	rec (P)	---
$224	insi (P)	clea,fora (P)	---
$228	insi (S)	abo (P),(Q),(U)	---
$235	insi (P)	fora (P),(Q),(T)	---
$232	exca (P)	abo (P)	---
$230	clea (Q)	fora (P)	---
$169	clea (P),(Q)	fora (P)	---
$231	lar,coc (S)-(P)	fora (P)	---
$63	clea,fora (P)	clea (P)	---
$70	rec,fora (P),(Q),(R)	abo (Q)	---
$159	abo (P),(Q)	clea,fora (P)	---
$166	abo (P)	fora (P)	---
$202	abo (P)	fora (Q)	---
$240	---	car (T)-(P)	fora (P),(Z)
$310	---	car (P)-(T)	clea (P)
$271	---	car (P)-(Q)	fora (Z)
$259	---	clea (P)	fora (V)
$348	---	abo (U)	fora (V)
$283	---	abo (T)	abo (W)

survivors walking around, and four of these were foragers (Table 9). Ninety-three ants associated with (Z) during the observation period were collected and marked; 46 of these were later seen in the other nests of the complex, which demonstrates the extensive movement of ants within a colony over a short period of time. However no marked foragers became associated with the main complex (ie. (P), (T), (V), (W), (X), (Z)); similarly, no unmarked foragers (ie. active in the main complex) became involved with (Z). Another 17 ants were marked around the main complex nests; these were involved in adult or brood transfer, and included ants that were themselves transported. A notable feature of this period of the year was that males were carried between the nests that belonged to one colony; the same workers that transferred adults and brood were involved in this. All the nests were excavated at the end of this visit; nests (P) and (W) contained very few adults and brood, and thus must have been in the process of being terminated. Large samples of adults were dissected in the other nests (Table 14).

The changes in behaviour exhibited by individual workers over time (Table 9) confirm the pattern of age polyethism suggested earlier on (2.4.3.). The data again indicate that there is a high mortality of above ground workers.

2.4.5. Intensive study - colony four

A part of this colony was observed from 16th - 18th April 1983. Nest (BX) had recently been started (ie. it was not there a few days earlier), and was 5 m away from established nest (BW). I marked thirty four above ground workers that were associated with (BX). Nest (BY) was started in the afternoon of day two. Many cocoons were transferred from (BX) to (BY), and five marked ants were involved in this, ie. %30, %27, %5, %31 and %15 (Table 10). A number of unmarked ants were also carried to this nest, including a callow ant. Fifteen marked ants were present in nest (BY) (Table 10), and most of these had previously been active above ground. There were also four unmarked excavators

Table 10 Activities of marked ants that were associated with the new nest (BY), colony four. On the morning of day three the inhabitants of this nest were evacuated to nests (BX) and (BZ). These two nests were excavated later on that day. Notation as in previous tables; upper case letters between brackets refer to nests, see text (2.4.5.).

Ant	Occupation on day one	Occupation in nest (BY) on day two	Occupation on day three
%2	exca (BX)	exca (BY)	(BX) *
%26	abo	exca (BY)	(BX) *
%18	abo (BX)	exca (BY)	(BX) *
%30	abo	coc (BX)-(BY)	coc (BX)-(BZ)
%27	abo	coc (BX)-(BY)	---
%5	rec (BW)-(BX),fora	coc (BX)-(BY)	rec (BY)-(BX)
%31	#	coc,rec (BX)-(BY)	abo (BZ)
%15	fora (BX)	coc (BX)-(BY),rec	coc (BX)-(BZ)
%7	rec (BW)-(BX)	rec (BX)-(BY)	(BX) *
%11	fora (BX)	fora (BY)	(BX) *
%33	car (BW)-(BX)	abo (BY)	coc (BY)-(BX)
%21	fora (BX)	abo (BY)	(BX) *
%20	abo (BX)	abo (BY)	---
%23	abo	abo (BY)	(BZ) *
%19	fora (BX)	abo (BY)	fora (BX)
%3	exca (BX)	abo (BY)	(BX) *
%9	#	abo (BY)	(BX) *
%28	abo (BX)	abo (BY)	(BX) *

* not seen above ground, but found during the nest excavations
\# marked subsequent to their transfer to (BY)

and an unmarked forager. Nest (BY) was attacked by myrmicine ants in the evening of day two, and the next morning (BY) was being evacuated. Parts of dead ant bodies were brought out by the invading myrmicines. Adults and cocoons were transferred from (BY) to (BX), eg. by %5 and %33. A new nest (BZ) had been started overnight (50 cm away from BX), and cocoons were brought to it from (BX), eg. by %30 and %15. When (BZ) was then

excavated, this showed that its five marked inhabitants had come from (BX) and (BY).

Nest (BX): 19 marked ants, 102 unmarked ants (70 were dissected, 14 egg-layers), 1 egg, 14 larvae, 63 cocoons.

Nest (BZ): 5 marked ants, 24 unmarked ants (of which 1 egg-layer, and 1 callow), 55 eggs, 13 larvae, 44 cocoons.

2.4.6. Individual histories of selected individuals

The activities of many marked workers were monitored in detail. A few observations of unusual behaviour by carrier ants are presented, each followed by some explanatory notes. The marked ants belonged to colony two (#) or three ($).

(a) Ant #7 was a carrier from (C) to (D). During the initiation of nest (E), it was itself carried from (D) to (E) by #4. Eighteen minutes later #7 was seen in the entrance of (C), which indicates that it walked directly from (E) to (C). On subsequent days #7 was never active in (E), but was now carrying adults from (D) to (C), ie. in the opposite direction to that which it had used, prior to being transferred to (E).
This account suggests that when experienced carrier ants are themselves transferred, their behaviour is not always altered in the same way as for other ants, ie. they do not remain active in the new nest. Ant $7 quickly left the nest into which it had been carried and returned to a nest in which it was previously active; such an ant appears to be very familiar with large parts of the home range of its colony.

(b) 09h39 $117 was carried from (R) to (P)
09h49 $117 came out of (P)
09h52 $117 entered nest (R)
09h53 $117 discarded a moist pellet out of (R)
10h18 $117 entered (P)
10h29 $117 discarded a moist pellet out of (P).

On previous days this ant had already been a cleaner in both (P) and (Q). It did not initially remain in (P) subsequent to being transferred there, but went back to its former nest.

(b) Day 52A 08h37 $412 entered (T) with its recruit from (Z)
08h42 $412 was now around (W), ie. it did not go immediately back to (Z)
09h22 $412 entered (T), coming from (Z)
10h24 to 11h05 $412 carried two cocoons and one adult from (T) to (W)
Day 53A 11h01, 12h04 $412 carried an adult, then a larva, from (W) to (Z)
12h50 $412 carried an adult from (Z) to (W)
13h28 $412 carried adults from (T) to (Z)
Day 54A $412 foraged and carried adults from (Z) to (P).

This is a carrier ant that was successively active in four nests. There was no pattern to its transfer of adults and brood, and this was unusual.

(c) 15h50 $70 was carried from (P) to (Q) by $97
16h16 $70 transferred $11 from (Q) to (P)
16h22 $70 entered (Q)
16h24 $70 discarded a food pellet out of (P)
16h26 $70 transferred $62 from (P) to (Q)

After being itself carried in one direction, this ant immediately carries a worker in the opposite direction; it resumed transfers in the expected direction after a short episode of nest sanitation.

2.4.7. Colony identity

A number of aggressive encounters were observed between ants belonging to different colonies. On the one occasion one ant dragged another by the antennae for 14 m; the dragged ant did not struggle. They stopped a few times and the aggressor attempted to sting the other. They later broke away and each walked in

opposite directions. In another instance four ants were involved in a conflict; the two ants in the middle held each other by the mandibles, while on the outside the other two ants each held a leg. . There was frequent antennation and at times stridulation, and the ants in the middle tried to sting each other. When they broke up the two parties ran away from each other.

Inter-colony encounters were predictably more common between the foragers, since many of these were active at the edge of their colony's home range. On one occasion, a marked forager from a colony under study was active some distance from its nest. It met an unmarked ant (ie. from a different colony) and there was a short-lived interaction. The unmarked forager found a termite, but this was then stolen by the marked forager. There was a short chase, but no subsequent aggression.

Some of the nests in colony two were separated by large distances. Another colony was present in this immediate area, and the axis of its nest complex lay perpendicularly across the corridor joining (A),(C) and (E),(F) (Figure 4). This approximates Brown's (1975) concept of "interdigitation of pathways"; parts of the colonies' home ranges consist of narrow pathways between the parts most used. During the transfers within these two colonies, there was a potential for encounters between alien ants, because the areas of recruitment activity (eg. between (A),(C) and (E),(F)) overlapped.

Aggressive interactions were sometimes observed between marked ants which belonged to the same colonies. The following descriptions are a representative sample of the encounters observed. In all instances the behavioural record of marked workers responsible for the evictions revealed that they were normally active above ground.

a) Ant #4 was held by its mandibles after being dragged out of nest (C) by #43. Apart from sporadic bouts of rapid antennation and attempts at stinging, the ants remained in this position for about 50 minutes. I separated them, #4 walked to (E) and #43 returned to (C). Ant #4 was at the time involved in carrying

adults from (D) to (E).
b) Ant $218 was carried from nest (R) into nest (Q). Seconds later $218 was dragged out of (Q) by $124 and released a few metres away. After having walked back into (Q), $218 was again evicted from this nest by two other marked ants, and was dragged to a distance of 15 m from the nest entrance. Ant $218 had never been seen above ground before; it had been marked after being collected inside (Q).
c) Ant $87, covered with fine dust, had just walked into nest (P), and was then dragged away by $85 (a forager). After being released it came back into (P), and was evicted by $84 and $206. Ant $161, which had earlier been excavating in (P), was also evicted from this nest by $84. A few minutes later $87 was again dragged out of (P), this time by $102.
d) Ant $15, which had just been marked, was released in its nest. It was dragged out and dropped 6 m away. $15 was back foraging in this nest later on in the day.

Colony recognition was explored further by confining workers belonging to different nests or colonies together, and then observing their interactions. All the tests were conducted in a plastic arena (27 x 35 cm) with the bottom covered with soil from the area. Foragers were always selected so that the workers involved were roughly of the same age. Pairs of workers from the same nests were used in each test, to contrast the interactions between nestmates and aliens.

As the ants were released into the arena they soon met up and there were distinct antennations; these encounters were timed and the subsequent behaviour monitored. A number of combinations of pairs of foragers were tested: (1) different nests from the same colony (n= 3); (2) nests from different, but adjacent colonies, where it was suspected that colony fission had recently taken place (n= 4); (3) nests from different, more distant colonies (n= 3).

The initial interactions between workers from different colonies were characterized by prolonged and vigorous antennal "boxing" (Ward 1983); in subsequent encounters between the same

individuals the duration of the antennations decreased. Some of the bouts of antennal boxing resulted in one of the ant adopting a submissive posture (see 2.3.6.). In contrast the interactions between nestmates were very short-lived (except for the first confrontation), and they were of a different nature. Thus it was clear that the ants can recognize individuals from other colonies. When ants from distant nests within the same colony were tested, their interactions were sometimes initially slightly longer than those between inhabitants of the same nest. After a few minutes however there were no more differences in the duration of the interactions.

At the beginning of the tests there was no overt aggression between foreign ants, and they would walk away after their initial encounters; after about five minutes however these repeated confrontations became more physical. There was momentary contact of the mandibles, the ants nudged heads or stood over one another's thoraces. Fighting sometimes followed; one ant held the other's mandibles (or their antennae or petiole) and lifted it off the ground, and other ants were dragged around. Tests involving adjacent and distant colonies gave similar results.

2.5. Discussion

2.5.1. Age polyethism

Age polyethism is a well-known phenomenon in the higher ants (Wilson 1971, 1976; Sudd 1982), but it has never been clearly demonstrated in the Ponerinae. Bonavita and Poveda (1970) showed that, in Mesoponera caffraria, some ants are foragers while others care for the brood. This division of labour is not a function of worker size (workers are monomorphic in almost all ponerines), and they suggested that the behaviour of the workers changes with age. In contrast, Traniello (1978) revealed that particular kinds of worker behaviour are independent of age in

the primitive Amblyopone pallipes. The reason for this is that a single brood per year is reared to maturity, making behavioural flexibility necessary. The uniform age distribution in the colony makes age polyethism impossible. In contrast, age differences occur among the workers of O. berthoudi (eggs are laid throughout the year, see 3.3.1.), and this allows for a different form of division of labour.

Age polyethism occurs in O. berthoudi. As the sterile workers age they change their roles according to a predictable sequence. The younger individuals are confined inside the nests for an unknown period of time; even after they have been carried between nests they remain underground. Over a period of a few weeks, marked workers in colonies two and three (2.4.3. and 2.4.4.) progressed from Innendienst duties or excavating to foraging. Almost every day, one or a few unmarked ants appeared outside the nests of colonies under intensive investigation. Their first above ground exposure often occurred while they worked as excavators; alternatively such ants repeatedly explored the immediate vicinity of the nest entrance and went in again. It is suggested that a consequence of the succession of roles is a progressive gain in above ground experience. As a cleaner walks some distance away from its nest to drop pellets, it orientates to the surroundings. Carrier ants are able to locate at least two nests in a colony, while foragers can venture away to sometimes distant hunting grounds.

The ants that were collected inside the nests with a blade of grass (Table 2) were not of uniform age. This was evident for two reasons: (1) when brought out of their nests, some unmarked ants ran straight back inside while others remained outside and behaved aggressively; (2) after having been marked some of these ants appeared above ground during the next few days, but others were never seen outside the nests during the rest of the same observation periods (Table 2). It seems clear that these behavioural differences are a reflection of varying ages. Bonavita and Poveda (1970) remarked that the emergence of new adults in the nests of Mesoponera caffraria may stimulate the older Innendienst workers to become active above ground.

Wilson (1976) studied age-correlated changes in behaviour and distinguished between two systems of organization: continuous (one age-group of workers for each task) and discretized (few age-groups, with workers from each group attending to more than one task). The age of the individual workers of O. berthoudi could not be accurately determined and thus it is not feasible at present to categorize this species' caste system in such detail. In addition, there were only four distinct above ground activities in this species. The following points may be noted however: (1) some ants appear to miss stages in the behavioural sequence, eg. they shift from cleaning to foraging; (2) certain ants perform more than one role, and this may be a stage along the behavioural progression; (3) there is considerable variability with respect to the levels of activity of different individuals. The marked workers that were observed over periods of a few weeks (colony two and three) took different lengths of time to change from one activity to another. The substantial mortality of workers active outside nests (see 2.4.3. and 2.4.4.) suggests that there is a need for a high replacement rate of older workers by Innendienst ones. Porter and Jorgensen (1981) suggested that the strong division of labour between "interior and exterior workers" in Pogonomyrmex owyheei may protect the colony by limiting predation to a small percentage of exterior workers; there is a high forager mortality in P. owyheei.

2.5.2. Recruitment between nests

Workers of O. berthoudi do not use chemical trails on the substrate for recruitment. Foraging and other above ground activities are undertaken independently, and the ants appear to rely largely on visual orientation cues. Canopy orientation (which has been documented in the ponerine Paltothyreus tarsatus; Holldobler 1980), and time-compensated sun orientation may also be involved in the ants' navigation. Individuals may spot-mark while they are unfamiliar with an area; while the exact

significance of this behaviour is unknown, it does not seem to be involved in the recruitment of other individuals. This lack of specialization contrasts with the well-developed trail-laying ability of other members of the same tribe (eg. Megaponera foetens, Leptogenys; eg. Fletcher 1973), as well as their use of tandem-running (eg. Pachycondyla tesserinoda, Maschwitz et al. 1974; Dinoponera gigantea, Overal 1980).

There are no semi-permanent trails between the nests of a colony in O. berthoudi, and this is in contrast with the situation in many polydomous species of higher ants (eg. Lasius sakagamii, Yamauchi et al. 1981), in which nests are connected by above ground paths. Contact within the colonies of O. berthoudi is maintained by carrying adults and brood between the nests. The physical transfer of ants is a form of recruitment. An ant that is transferred to a new nest remains in it; if it is a worker active outside, it will return to this new nest after performance of its above ground task. In addition the ant being carried is provided with visual orientation information; older workers were able to walk back to the nests from which they had originated, and thus additional knowledge is gained of the colony's home range. Harkness (1977) interpreted one of the functions of the carrying of individual ants in the formicine Cataglyphis to be an "educational procedure", enabling workers to learn their foraging territories. Haskins (1970) reported that "deportation" of adults is a common and stereotyped behaviour in many higher ponerines.

Moglich and Holldobler (1974) described social carrying behaviour in ants as a primitive recruitment technique used during nest emigration. In O. berthoudi, social carrying has additional functions. We may differentiate between three types of inter-nest transfers: (1) evacuation of workers and brood during occasional nest emigration - this is a mass event usually initiated by a number of carrier ants (see 4.3.3.); (2) settlement of new nests, which is part of a normal process of colony expansion (see 2.5.3.) - in this case recruitment of particular categories of workers seems adaptive; (3) normal, almost routine, transfers of adults and brood between existing

nests. The last event is not yet completely understood. Eggs are laid in every nest (see Chapter 3), and thus there is no obvious need for brood transfer. There might be differences between the nests with respect to (1) microclimatic conditions for cocoon development; (2) food availability for larval growth, due to local termite availability and numbers of foragers; (3) ratio of nurse ants to brood. However, the distribution of the colony workforce and brood in the established sub-units cannot be optimized in relation to these properties, because the recruitment activities are not coordinated. With the exception of occasional mass effects the carrier ants work independently, and the regulation of their transfers at the level of the colony is impossible. The recruiters seem to behave selfishly by trying to enlarge the population of their own nests, apparently at the expense of other nests. In this context the category of worker that is imported is thus not important. Furthermore it is suggested that the transfer of brood, and also termites, occurs when suitable adults are not available. Ultimately the only concrete effect of carrying is that the older ants orientate to the various nests of the colony. Through this recruitment process many older workers become active in more than one nest (Table 5), and this in turn generates more inter-nest transfers. There is a large movement of workers between the nests of one colony (eg. 2.4.4.; colony three, third observation period), and this maintains colony cohesiveness.

Recruiters can select individuals of a similar age during bouts of carrying, but the basis for this selection is not always clear. As said above, selective recruitment seems to be adaptive only in the context of the colonization of new nests. There may be a pheromonal basis for the recognition of age and reproductive status. The mandibular gland secretions of queenless honeybee workers vary according to social position, and the different characteristic blends of components produce distinct signals (Crewe and Velthuis 1980). A gas chromatographic analysis of the mandibular gland secretions of O. berthoudi was conducted using sterile workers of different ages as well as functionally-reproductive ants (see Appendix D). The

relative proportions of the various pheromonal components in individual extracts were found to vary between the different categories of nestmates. The chromatograms in Figure 6 reveal that components with identical retention times occurred in different quantities (quantity is determined by integrating the area under a peak). However the components of the mandibular gland secretions were not chemically identified, and thus these conclusions must remain tentative. It is suggested that the carrier ants can recognize the different pheromonal blends, and that a particular signal is remembered from one transfer to the next.

Carrying is preceded by a distinct antennal interaction which seems to determine whether the intended recruit will take a submissive posture and allow itself to be carried. The intensity of the antennation may be related to the respective ages of the participants (perceived pheromonally), as well as to the motivation of the recruiters. When a recruiter interacts with an above ground worker, they would both release a very similar exocrine signal. Additional information about the functional identity of the recruiter, its eagerness to carry, and its experience would need to be transmitted by tactile signals. Not all initiation interactions are followed by recruitment. In a number of instances one recruiter could not initiate carrying with a particular individual, yet the latter was successfully carried by another recruiter minutes later. Behavioural observations of ants with known occupations revealed that foragers and other recruiters were usually carried only by experienced recruiters. Very few foragers were carried between nests. Thus it seems that both chemical and tactile communication are involved in the initiation of carrying.

In O. berthoudi nestmates and brood are physically transported within complexes of nests. The importance of this social carrying lies in the recruitment of individuals to new nests as well as between existing nests. Colony integrity is maintained through this frequent inter-nest movement, and failure of the latter may lead to colony fission (see 2.5.5.).

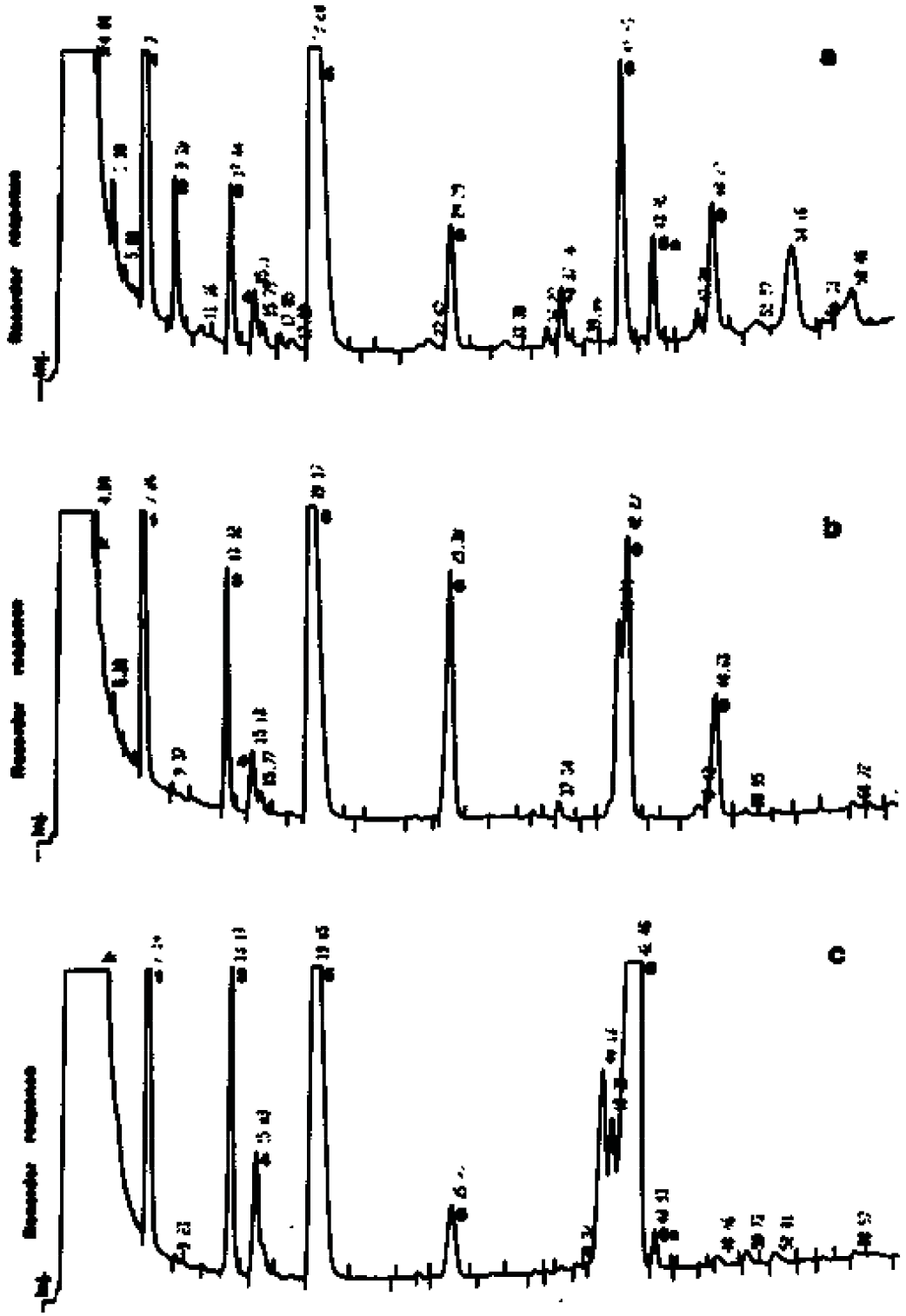

Figure 6 Gas chromatograms of the mandibular gland secretions of three functional categories of workers of Ophthalmopone berthoudi (a) Innendienst workers, (b) foragers, (c) breeding workers. Numbers printed above the peaks represent retention times (in min. and hundreds of min.). Comparison of the three traces shows differences in the relative proportions of the components. Methods are detailed in Appendix D.

2.5.3. Polydomy and colony expansion

This study represents the first published record of the existence of polydomy in the ponerines. What is the origin and adaptive significance of the polydomous organization of this species? These ants occur in hard clayey soil, and they do not seem capable of excavating their own nests because of inadequately-shaped mandibles. It appears that they inhabit the abandoned parts of termite galleries, and only perform minor alterations to these. When the population of a nest has grown to a stage where space becomes restricting, the workers cannot enlarge the underground chambers and they have to expand into an additional nest. I propose that this is the driving force for distributing the colony unit into a number of nests. This is supported by the excavation in July 1981 of a nest which yielded over 840 adult ants; this was almost twice as many as in the next biggest nest found, and it roughly equalled the population of a multi-nest colony. The particular architecture of this nest may have made growth possible without the need for extra nests; this nest did not seem to be part of an existing complex.

How is a new nest started? Foragers are the oldest and most experienced workers in a colony, and they are familiar with their hunting grounds. They often enter holes or cavities in the ground while searching for termites; it is likely that some of these holes lead into unoccupied networks of galleries. A younger forager with an appropriate motivation to recruit could find a suitable site, return to its nest, and start carrying other workers to this new site. Above ground workers are transferred first, and some of these in turn proceed to carry other workers to the new nest. It is only at a later stage that Innnendienst ants and brood are carried. Different sets of recruiters were involved during the initiation of nests (E) and (F) (colony two; see 2.4.3.), and this is a reflection of their simultaneous association with different parts of the same colony. The transfer of reproductive individuals (Chapter 3) is

an important part of the settlement of newly-established nests; one egg-layer was recruited soon after the start of nest (AF) in colony one (see 2.4.2.). In colony four (see 2.4.5.), nest (BZ) was excavated a few hours after it had been established; there was only one egg-layer among its 29 inhabitants. Another new nest was examined during April 1983, and many ants recruited to it after one day were egg-layers; after excavation 28 of the 74 inhabitants were dissected, and this yielded 9 egg-layers. Thus the transfer of reproductive individuals seems to occur at various stages of nest colonization, provided that there are already a certain number of labourers in the nest.

On a number of occasions workers that were recruited to a new nest changed their role (Tables 6 and 7); this may be a response to the new contingencies that arise in an incipient nest with an inadequate labour force, eg. some Innendienst workers become active outside the nests. The organization of activities seemed to be based on mass effects, eg. five workers suddenly started to excavate in nest (E).

The composition of nest complexes changes frequently; during the intensive study of all four colonies new nests were started and others abandoned. The dynamic turn-over of nests over a period of time was accompanied by the movement of adults and brood from unit to unit. It is suggested that ants evacuate an existing nest when it becomes unsuitable or after it has been attacked (eg. underground invasion of myrmicine or doryline ants). An advantage of polydomy is that, in the event of a nest being destroyed, other nests in the colony are permanently available to accomodate fleeing ants and their brood. Polydomy in this species does not always seem to be associated with a strategy for the efficient exploitation of termite resources in the area around the nests. Foragers did not always hunt in the vicinity of their own nests; they sometimes travelled long distances to hunt in areas where they were previously active, nearer other nests in the colony.

In this study, detailed data were obtained on the behaviour of the individuals that move between the nests of a polydomous colony; such data have not been available in previous Formicid

works. Little is generally known about the organization of polydomous colonies. Different functional categories of nests can be recognized on the basis of the direction and intensity of food, brood and workers exchange (Pisarski 1973). Cherix (1980) reports that the large "super-colonies" of Formica lugubris are arranged into sectors. Sectors are made up of a main nest connected with smaller nests as well as seasonal nests; these are produced from the main nest by budding. The sectors are linked by permanent paths, and thus the hundreds of nests in the "super-colony" remain part of an integral unit. In contrast, there is no movement of ants between the sectors in Formica exsecta (Cherix et al. 1980). In Lasius sakagamii the smaller number of nests are arranged according to a similar pattern, and there are weak territorial boundaries among colonies (Yamauchi et al. 1981). Pisarski (1973) reports that there is also an inter-nest movement of Innendienst workers in Formica, and this has seldom been documented in other studies.

Polydomy has often been assumed to be a strategy to colonize certain harsh habitats or to withstand interspecific competition (eg. Holldobler and Wilson 1977, Werner et al. 1979). Wilson (1971) thought that "unicolonialism" permits the rapid growth of populations in habitats that are relatively free of competitors but sparsely distributed. No such claims are made for O. berthoudi, where polydomy seems to have evolved as a consequence of restrictions on nest growth. In this phylogenetically primitive ant species, a complex organization has evolved which links sometimes-distant nests into one distinct colony unit. Significant differences with the polydomous colonies of formicines include the dynamic pattern of nest establishment, the smaller number of nests and the extensive interchange of nest members as opposed to that of food. As will be seen in the next section, polydomy in O. berthoudi is not associated with a reduction in inter-colony hostility.

2.5.4. Nestmate recognition

While there is amicable cooperation between the members of the same colony in eusocial insects, this altruism does not carry over to intercolony interactions. Nestmates must be distinguished from aliens, and this is usually achieved through the recognition of a colony odour (Crozier 1979). Greenberg (1979) demonstrated that odours are genetically specified in Lasioglossum zephyrum, and thus the similarity of the odours of nestmates is correlated with their degree of relatedness. However individuals do not use their own odour as a reference for nestmate recognition (Buckle and Greenberg 1981). They learn the odours of their nestmates and this leads to a "nestmate recognition memory" which stores the odours of all the bees in a nest. There is no transfer of odours among nestmates (Buckle and Greenberg 1981). It is not yet clear whether nestmate recognition in the ants is regulated in a similar way, because their colonies are much more populous.

The members of a nest complex in O. berthoudi share a distinct colony identity and they can recognize workers from foreign colonies. As a consequence there are also distinct home ranges; these can be described as that area around the nests in which the foragers are active. The exclusivity of the home ranges varies with the distance from the nests. The density of above ground workers is higher in the proximity of the nests, and thus a foreign intruder is less likely to go unchallenged. This small part of the home range approximates a territory. The edges of the home range are less well defined and thus there may be overlap with other colonies; this is where interactions between foreign ants often occur. Areas of great termite activity, eg. disused rhinoceros middens, can be exploited by foragers from different colonies.

The recognition tests (see 2.4.7.) were used to simulate foreign interactions and study the discriminating ability in this species. Haskins and Haskins (1979) used a similar methodology

to study colony identification among workers in different populations of Rhytidoponera metallica. In O. berthoudi, initial encounters between alien foragers were often followed by avoidance rather than aggression, but this is no measure of the distinctness of the ants' identities. When aliens meet away from their respective nests they probably run away from each other. Confined in the test arena the repeated encounters often led to aggression. Hermann and Young (1980) found a similar lack of conspecific aggression in natural populations of Paraponera clavata, and attributed this to territorial partitioning during foraging.

How can the existence of a polydomous organization be reconciled with the notion of colony integrity? The significant amount of adult exchange between the nests maintains the cohesiveness of the colonies. Many of the older workers are active in more than one nest, and this means that they are able to learn the total range of odour variation in the colony. Intra-colony aggression was observed (see 2.4.7.), revealing that individuals from another nest in the same colony were sometimes mistaken as foreign. These were rare events, but they indicate that variations in the odours of colony members do occur. Those individuals that evicted visitors from elsewhere in the colony may have been workers that were not yet active in more than one nest. The fact that it has been suggested that young adults do not recognize foreign odours (eg. Holldobler and Michener 1980, Crozier 1979) may explain why Innendienst workers of O. berthoudi, which are often confined to a single nest, do not evict visitors from other nests.

The physical isolation of nests that is associated with the event of colony fission (see 2.5.5.) gradually leads to their inhabitants becoming unfamiliar with the odours of the members of other nests. The divergence in "colony odours" is accentuated with time. As new generations of workers appear, novel individual odour variants are introduced into their respective colonies. The development of new colony identities following fragmentation was confirmed by testing the recognition abilities of ants collected from adjacent colonies. Three months after

the occurrence of the events mentioned in 2.4.4. (p. 40), inhabitants from colony three and nest (XX) recognized each other as foreign.

If there is a genetic basis for the production of nest odours, then the relatedness between nestmates must be considered. This will be done in 7.1., after the data on the reproductive structure of these colonies have been presented (Chapter 3).

2.5.5. Colony genesis

Most queenright ponerines found colonies according to a partially-claustral pattern (Haskins 1970); the lone foundress forages from her nest at periodic intervals in order to feed the first generation of larvae. This is thought to be the ancestral Formicid mode of colony multiplication. This pattern of progressive provisioning has given rise to a more specialized behaviour in the majority of higher ants; the queen isolates herself completely and feeds her brood by breaking down her wing muscles. This fully-claustral pattern of colony foundation is only found in one ponerine species, Brachyponera lutea (Haskins and Haskins 1950). By contrast, in those species which lack true queens, there is the detachment of small parties of workers, including one or more fertilized individuals, from older colonies (Haskins 1970). Colony fission has been documented in very few ponerine species. Overal (1980) observed an incomplete emigration to a new nest site in Dinoponera gigantea. Some of the brood from the original nest were carried across the short distance (5,5 m) between the two nests, while pairs of adults walked in tandem. This migration was completed in one day. However, Overal did not investigate the reproductive structure of the two resulting nests, and his study was too short to establish whether there was any further contact between the two units. In the Rhytidoponera impressa group, Ward (1981b) reported that there is little information on the frequency of colony fission in

worker-reproductive colonies, and on the size of newly-budded daughter colonies. The process of colony fission is difficult to distinguish from the movement of a colony from one nest site to another. Ward observed small, isolated clusters of workers and brood in the field; in each of the two cases where workers were dissected, only one individual was inseminated.

Polydomous colonies generally rely on budding as the principal means of reproduction (Wilson 1971). This is especially so when queens are present in every nest that belongs to a colony. The actual mechanism of this fragmentation has seldom been detailed in any formicids. Zakharov (1972; in Werner et al. 1979) described colony fission in Formica as follows: the exchanges of workers and food between main and secondary nests decrease and stop entirely after a while, leading to the creation of colonies hostile to one another and possessing their own territories. Colony fission occurred similarly in O. berthoudi. A previously-cohesive complex of nests (ie. colony two, see 2.4.3.) split and developed into two distinct colonies over a number of months.

The characteristic feature of colony two was the occurrence of nest (D) 75 m away from the other nests (Figure 4). At the beginning of the March-April 1981 observation period there were frequent transfers from nests (A),(B),(C) to nest (D). After a few days nest (E) was initiated and its inhabitants originated from nest (D). As a result, many of the workers which had been recruiting adults and brood from (A),(C) to (D) now became active between (D) and (E). Some two-way transfers between (D) and (A),(C) still occurred however. No recruitment was observed between (E) and (A),(C). A month later transfers were no longer observed between these two distant sites (now made up of nests (K),(J) and (C),(G),(H),(I) respectively); all the marked inhabitants (except one) of these two groups of nests had belonged to previous nests that were in the same sites (in April). Similar results were again obtained in July. One can hypothesize that the transfer of adults and brood between the two groups of nests gradually stopped soon after the March-April observation period. The two groups of nests became isolated

from one another because the above ground workers remained associated with their own nests or others in their more immediate vicinity.

The fragmentation of nest complexes occurs accidentally in this species, but it can be promoted by several factors. The establishment of a relatively distant nest increases the chances that social carrying will eventually stop. There are no permanent paths between individual nests and the latter are linked together exclusively through the activities of individual carrier ants. As recruiters die or become foragers more transfers occur less frequently, unless younger recruiters have also become active between the same nests. If recruiters that inhabit an outlying nest become involved in the settlement of yet another nest (eg. nest (E) in colony two), this will also result in fewer contacts with the parental units. With large distances, the probability is small that wandering recruiters or foragers will independently locate other nests of the same colony. Furthermore, distant foraging areas will not overlap and as older foragers (which were used to foraging in the proximity of their previous nests) disappear, contacts between parts of the original home range will cease. Distant nests do not always become isolated however; nest (Z) remained an integral part of colony three (see 2.4.4. and Figure 5) despite being 23 m away. The eventuality of colony fission is ultimately determined by the amount of unoccupied space around an existing nest complex. Empty sites that resulted from the excavation of entire colonies were occupied by nests from adjacent colonies within 10-15 months.

Colony fission will be discussed further in 7.1., when the results on the reproductive structure of the colonies of this species are integrated with these ecological and behavioural data.

CHAPTER THREE MULTIPLE BREEDING WORKERS, INSEMINATION AND THE CONTROL OF REPRODUCTION

3.1. Introduction

The replacement of the queen caste by worker-like individuals in many ponerine species (see Chapter 1) changes the nature of the significance of worker fertility. Worker laying is observed in many formicid species and usually has a dual function. In some cases workers are the major source of males (in Myrmica, Smeeton 1981). Some workers in other species also lay trophic eggs which are used in the nutrition of the queen or of the larvae (Wilson 1971). Thus the workers can only increase their classical fitness (as opposed to their inclusive fitness, where their labour benefits the queen) through the production of haploid eggs. A reason for this is that workers in most higher ants either lack a functional spermatheca or are behaviourally unable to become mated (Brian 1979). Non-mated workers can produce other workers (and queens) through thelytokous parthenogenesis, but this only occurs in very few species; it is well-documented in Cataglyphis cursor (Cagniant 1979), while the data for Oecophylla longinoda are equivocal (Ledoux 1950, Holldobler and Wilson 1983).

An understanding of the regulation of worker laying is necessary to interpret the queenless reproductive pattern. Queens of eusocial insects are usually able to modify the physiology and behaviour of the workers, and an important manifestation of this is the control of both the production of males by workers and the development of brood into female sexuals, through the differential treatment by workers (eg. Brian 1979). In the formicids the nature of the queen influence is still under debate; recent work reveals that it is often a non-volatile pheromone produced from exocrine glands located in the

head or abdomen (eg. Monomorium pharaonis, Berndt and Nitschmann 1979; Plagiolepis pygmaea, Passera 1980). The power of the inhibitory effect of the queen's pheromone is seen in Oecophylla (Holldobler and Wilson 1983), where colonies are exceptionally large and distributed in many nests spread over extensive arboreal territories. Here, the monogynous queen suppresses the production of males by any of the workers. The queen is very attractive to major workers, and her dense retinue of workers frequently lick the surface of her body as well as regurgitate to her; this creates the essential circumstance for the spread of primer pheromones. Holldobler and Wilson (1983) found that the presence of the queen does not actually inhibit ovarian activity, but rather restricts development to trophic eggs. The inhibition of the reproductive activity of workers is also evident in various queenright ponerines. In Rhytidoponera purpurea, worker-laid eggs give rise to males, but these are only produced when queens are absent (Haskins and Whelden 1965). In Odontomachus haematodes (= O. troglodytes, Brown 1976), queens inhibit egg-laying in the workers. Thus there is an apparent reproductive conflict (Trivers and Hare 1976) between the two castes. Much of the data available lend support to the idea that the reproductive altruism of the workers is maintained by parental manipulation (Michener and Brothers 1974).

The reproductive biology of very few queenless ponerine species has been studied in detail. Haskins and Whelden (1965) showed that in those species of Rhytidoponera lacking a queen, workers and males are produced by "workers"; this was also found in Dinoponera grandis (Haskins and Zahl 1971). The approach in both these studies was to isolate groups of ants (with broods of larvae and cocoons) and to monitor oviposition as well as the sex of the new brood produced. These authors did not dissect any ants and thus could not determine the exact pattern of male and worker production. Whelden (1957, 1960) was the first to demonstrate conclusively that a normal fertilization mechanism is involved in the production of female eggs by workers. He examined the spermathecae of workers in three species of Rhytidoponera and found that a small percentage of these were

inseminated in each sample. A further advance has been Ward's (1981b, 1983) detailed study of the Rhytidoponera impressa group. He made exhaustive dissections of all non-aggressive workers in a number of queenless colonies and determined that there were a number of mated reproductive workers per colony. Allozyme studies showed that there was a low level of relatedness between nestmates in such colonies. Ward (1983) did not however elucidate the factors that limit "mating and diploid reproduction" to a small proportion of the workers in a colony.

It is as a result of the lack of external differences between egg-laying individuals and sterile workers that many features of queenless breeding biology are unknown. Some of the critical questions that need to be answered are: Can all the workers become reproductive and if so, what controls the differentiation within the worker caste? Are all the mated workers active, or is oviposition by some inhibited through the behavioural domination of others? Who lays the unfertilized eggs that develop into haploid males? Are there seasonal fluctuations in the number of mated workers in a colony? In this study, the organization of a breeding system was inferred from the pattern of seasonal change in the reproductive population, that was determined by the dissection of individual workers.

3.2. Methods

3.2.1. Fieldwork

Colonies of O. berthoudi were collected from Mkuzi Reserve at different times of the year. Thirty four nests were excavated; over a third of these had been monitored for at least a few days and contained marked ants with a behavioural record (see Chapter 2). Care was taken to collect every ant and most of the brood. In the majority of excavations digging only stopped some time after the last ant had been found; a few nests were left open overnight to check for ants escaping from concealed chambers. Additional ants that were performing particular activities above

ground were also collected and included in the samples for dissection.

3.2.2. Laboratory study

Cocoons were opened throughout the year and pharate adults sexed and examined for wing buds. Abdomens were dissected under Ringer's solution (Bradbury 1973); the ovaries were examined and categorized as being developed or undeveloped. Differences in activity between the ovaries of fertile ants were documented by recording the occurrence of mature eggs in the ovarioles. Spermathecae were scored for the presence of sperm with a compound microscope; later this could be done under the dissecting microscope, because the clump of sperm gives the spermatheca a distinctive appearance. The following body measurements were taken using a micrometer eye-piece (these partly follow Bolton 1974): (1) head width, the maximum width of the head measured anterior to the eyes in full-face view; (2) head length, the straight-line length of the head in perfect full-face view, measured from the mid-point of the anterior clypeal margin to the mid-point of the occipital margin; (3) length of the first gaster segment, the length of the dorsal end, measured from the anterior to the posterior edge in side view; (4) height of the first gaster segment, the maximum height measured from the dorsal to the ventral edge in side view.

Large samples of adults from 20 nests were dissected in order to obtain an estimate of the numbers of fertile and inseminated ants; these nests belonged to different colonies and had been excavated at different times of the year. The entire adult population of each nest could not be dissected because of time restrictions. In addition there was sometimes a substantial mortality during the excavation and transport of the nests. Ants were kept alive in their social units until the time of dissection; the fatalities which occurred over time were more numerous among the older ants which had become active above ground. The samples of individuals dissected were biased

towards including those ants which always remained inside the observation nests. Towards the middle of the study the structure of the nest population became clear, and homogeneous groups in the nests subsequently analysed could be recognized. Groups such as the above-ground workers and callow ants (identified by their soft exoskeletons) were no longer dissected extensively. These sub-groups were classed as being sterile, and the ants in them were added to the ants dissected to make up an extrapolated number of ants examined (see Table 14); this procedure may lead to a slight underestimate of the proportion of egg-laying individuals in a nest.

3.3. Results

3.3.1. Dynamics of brood production

Eggs, larvae and cocoons were generally found throughout the year in all the colonies that were sampled (Table 1). Male pupae were found in the nests from January to April only (Table 11). There was no relationship between the size of the worker population in individual nests and the amount of brood found in them (Table 1); it is known that brood is transferred between the nests of a colony (see 2.3.6.). When a few nests all belonging to the same colony were examined at one point in time, marked variations in the proportion of the different brood stages in nests were evident (Table 1). Different colonies were sampled during the same period of the year over three successive years, and there was much variability in the amounts of brood present (Table 1).

There were some patterns in the relative abundance of the various stages of brood, but these did not seem to represent seasonal peaks in egg-laying. While numbers of eggs were found in the nests (n= 7) excavated in July, only a few larvae and almost no cocoons were collected. Those larvae present were predominantly small. This indicated a high larval mortality during winter, and thus adult workers may not emerge for these few months of the year.

Table 11 Stage of development and sex of the pupae in cocoons (n=588) collected in nests that were excavated at various times of the year in three successive years (1981-1983).

Time of year	No. of nests sampled	No. of cocoons opened	No. of prepupae	No. of worker pupae	No. of winged female pupae	No. of winged male pupae
January '81	3	81	28	50	0	3
January '82	2	41	7	10	0	24
January '83	3	65	41	8	0	16
February '82	5	54	7	44	0	3
March '81	2	77	15	57	0	5
March '82	2	30	3	27	0	0
April '83	3	91	20	52	0	19
July '81	5	2	1	1	0	0
July '83	2	11	0	11	0	0
October '81	1	48	12	36	0	0
October '82	2	44	2	42	0	0
December '81	2	44	21	23	0	0

3.3.2. Characteristics of the egg-laying ants

It was soon established that there was usually more than one egg-layer in each nest, and these could not be recognized by simple visual examination. Data from Table 11 confirm the absence of winged queens in this species.

The ovaries of the fertile ants had an unexpectedly simple appearance (Figure 7). Each ovary consisted of 3 ovarioles, and this structure was invariant in all the individuals examined. The eggs were found to be unusually large; they were yolky and elongate with a mean length of 1,64 mm $\pm$ 0,08 s.d. (n = 50); the range was 1,5-2,0 mm. The egg width was constant and measured 0,8 mm; this is presumably regulated by the width of the individual ovarioles. The strings of developing oocytes were short, with few reaching maturity simultaneously. There was seldom more than one mature egg inside the ovaries of fertile individuals, and three eggs was the most that was found (Table 12). This was clearly related to the large size of the eggs and to the short ovarioles, as seen in Figure 7. No individual laying rates could be determined in the laboratory, but data from isolated groups of ants (Table 13) support the idea that there was a slow rate of egg production.

The number of mature eggs found in the ovarioles was used as a criterion to assess the ovarian activity of fertile individuals. The large majority of reproductive ants examined were found without even one mature egg that was ready to be laid (Figure 8). These data also reveal a distinct variability in the degree of ovarian activity among the reproductive ants. Since there is a slow rate of egg production, the laying of just one egg prior to dissection will affect the ovarian activity score of an individual. As a result fecundity was underestimated in a number of individuals because eggs were laid continuously in the laboratory while large groups of ants awaited dissection (Table 12 and Figure 8). However, too few eggs were laid for this underestimate to affect all the ants in the "no egg category". Thus a significant proportion of the reproductive ants seem to be very poor egg-layers; the ovaries of some did not even contain any well-developed opaque oocytes.

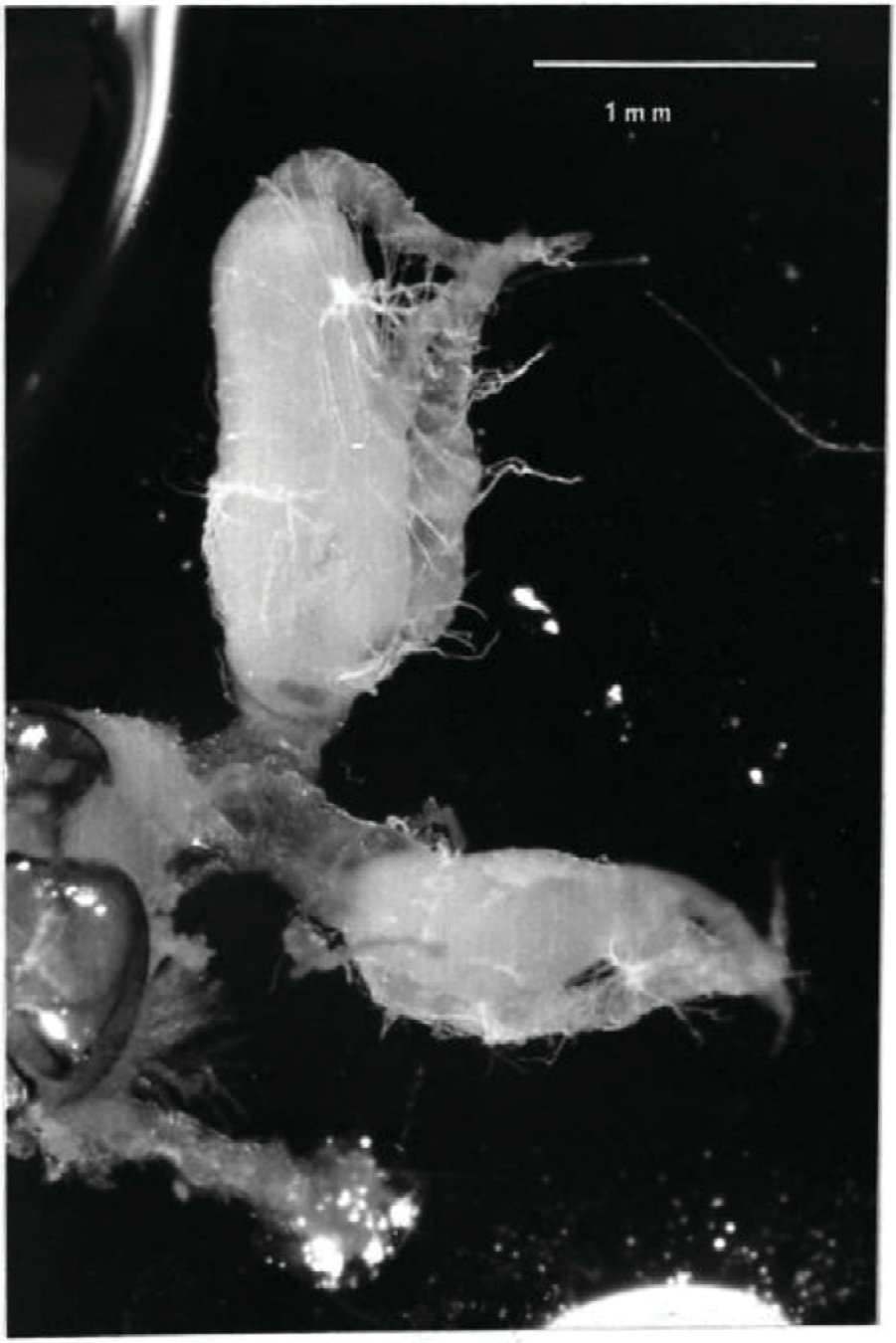

Figure 7 Developed ovaries of a breeding worker of Ophthalmopone berthoudi. A mature egg can be seen in one of the ovarioles at the top.

Table 12 Ovarian activity in breeding workers, samples of which were dissected in detail (ie. the numbers of mature eggs were recorded). Nests were excavated at different times of the year. All the breeding workers in the sample (n= 293) had 3 ovarioles/ovary. Eggs ranged from 1,5 - 2,0 mm in length.

	Time of the year when nests were excavated				
	December 1981	February 1982	March 1982	October 1982	April 1983
No. of breeding workers dissected on which detailed notes were made	99	11	85	64	34
No. found without a mature egg	75	5	79	14	26
No. found with one egg	21	3	6	43	5
No. found with two eggs	3	2	0	7	3
No. found with three eggs	0	1	0	0	0
No. of eggs laid while waiting to be dissected *	41 (9 days)	69 (14 days)	37 (8 days)	28 (4 days)	79 (11 days)

* other breeding workers that were not dissected could have contributed to these numbers

Table 13 Rate of egg production in various isolated groups of ants. The ants were dissected after a number of days. The rate takes into account the number of eggs found in the ovaries, but excludes eggs that may have been laid immediately prior to the period of isolation.

Time of the year	No. of ants in the group	No. of days in isolation	No. of eggs laid	No. of ants dissected	No. of breeding workers	No. of mature eggs found in ovaries	Rate of egg production
October '81	52	5	6	38	2	4	1/day
December '81	251	9	41	230	108	18	0,06/day
February '82	20	6	8	19	1	2	1,7/day
April '83	25	4	2	25	1	1	0,75/day
	106	7	21	56	10	8	0,4/day
	97	11	56	71	14	0	0,4/day

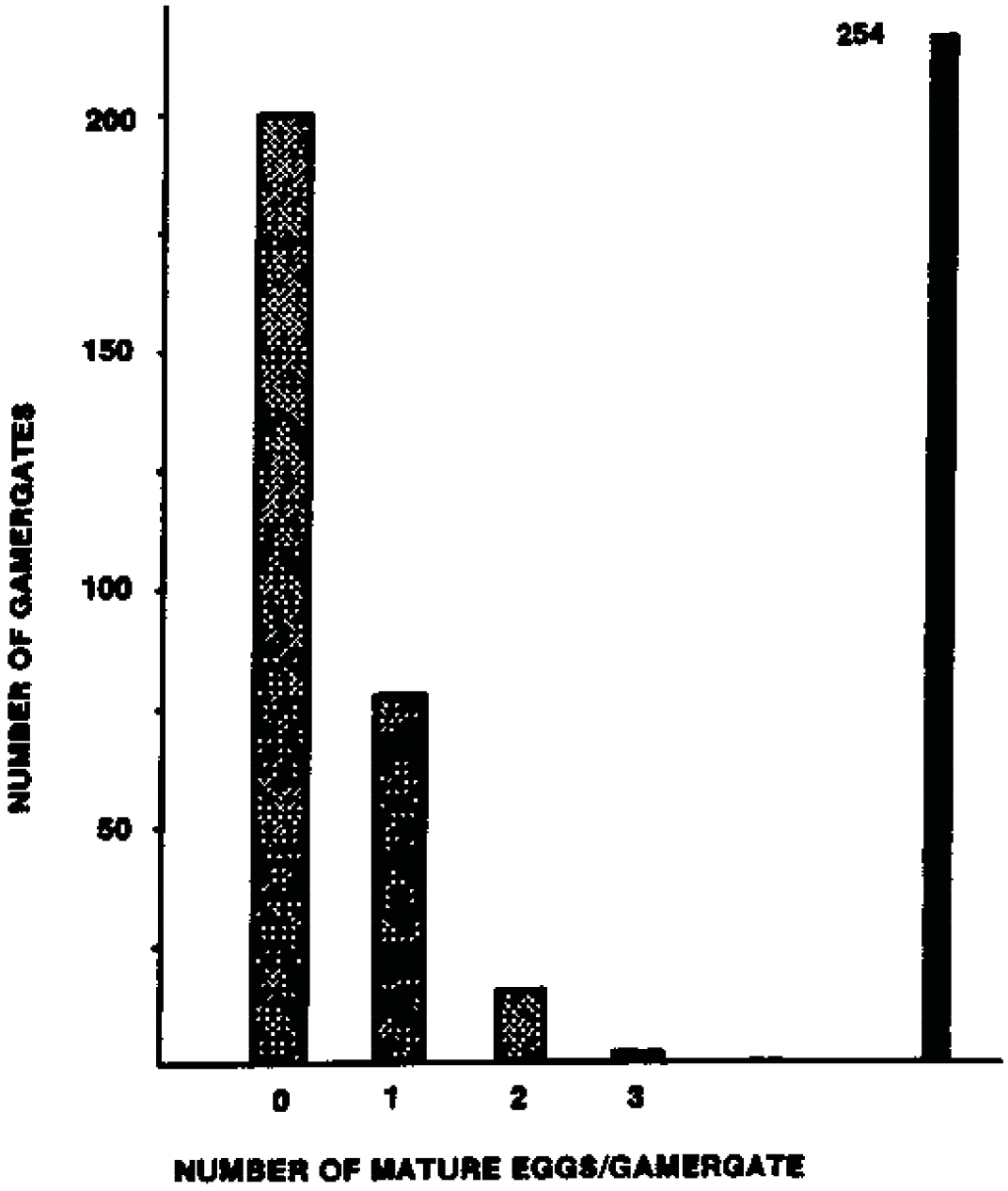

Figure 8 Frequency distribution of the number of mature eggs found in the ovarioles of breeding workers (=gamergates) collected from five colonies (see Table 12). The number of eggs laid by the combined populations of breeding workers is also indicated (solid bar); this occurred over various numbers of days.

The sterile workers were identified by their characteristically undeveloped ovaries; there were also three ovarioles in each ovary (Figure 9). A number of young "Innendienst" workers exhibited a limited amount of ovarian activity and oogenesis, but this was distinct (with a few exceptions) from the developed ovaries of fertile ants. Thirty-five callow ants were dissected and all were found to have undeveloped ovaries. The ovaries of 110 above ground workers (cleaners, excavators, recruiters and foragers) were examined and found to be undeveloped; the ovaries of foragers especially showed definite regression, with stringy ovarioles. All sterile workers possessed a spermatheca and a pair of accessory glands.

The spermathecae in a sample (n= 549) of the individuals dissected were examined in order to determine which of the ants were inseminated (Table 14). The spermathecal state was difficult to determine in 11 out of 227 fertile workers that were examined for insemination. Nine of these belonged to part of a nest which had been deep-frozen. All the other ants with egg-producing ovaries had sperm in their spermathecae, while none of the sterile workers were found to have been fecundated. However, evidence of non-mated workers with well-developed ovaries, or of mated workers with distinctly undeveloped ovaries, was not obtained. The strict association between insemination and ovarian activity was true at all times of the year.

Variations in the size of the ants' abdomens were noticed during the dissections, and ants with bigger abdomens were often fertile. Various measurements were initially taken to obtain an estimate of abdominal size, but they were inaccurate and could not be duplicated, eg. total abdomen length was unreliable because of intersegmental stretching. After dissecting ants from a number of colonies, it was found that not all the ants with bigger abdomens were fertile, and that many with smaller abdomens were fertile. However the association between "bigger" abdomens and fertility was a regular one and could not be ignored. Towards the end of the study the head capsule and the

Figure 9 Undeveloped ovaries of an unmated, sterile worker of Ophthalmopone berthoudi.

Table 14 Reproductive status of ants from a number of colonies. Sampled nests were excavated at different times of the year. The procedure for extrapolation of the dissection results is explained in the text (3.2.2.). All fertile individuals examined (n= 227) were inseminated, and similarly, all sterile workers (n= 322) had empty spermathecae.

Date of excavation	No. of ants collected in the nest	No. of ants dissected	Extrapolated no. of ants examined	No. of gamergates * found	% of gamergates * in the sample examined	No. of ants checked for insemination	
						gamergates *	sterile workers
31.I.1982 (1 nest)	140	69	74	1 #	1	-	26
27.I.1983 (1 nest)	222	42	64	1	2	0	6
11.II.1982 (5 nests, 1 colony)	72	70	71	1	1	1	34
	80	40	49	4	8	3	15
	100	99	100	2	2	2	20
	124	80	99	3	3	3	32
	27	23	23	1	5	1	13
23.III.1982 (2 nests, 1 colony)	119	74	70	46	59	32	10
	77	67	74	49	66	46	5
18.IV.1983 (3 nests, 1 colony)	106	66	66	10	15	6	16
	29	25	-	1	4	1	8
	121	71	84	14	17	1	9
(1 nest)	74	28	-	9	32	9	4
16.VII.1981 (1 nest)	247	122	-	11	9	11	57
15.VII.1983 (1 nest)	262	43	49	15	30	13	2
27.X.1981 (2 nests, 2 colonies)	81	26	47	1 #	2	-	9
	190	38	-	2	5	2	17
17.X.1982 (1 nest)	340	144	266	65	25	16	14
5.XII.1981 (2 nests, 1 colony)	261	230	242	108	45	64	15
	90	36	39	17	44	16	3

* gamergates = breeding workers (see 3.3.2.).
\# this is a minimum figure, deduced because eggs were laid in the isolated group of ants.

Table 15 Measurements (in mm) of the head and gaster of breeding and sterile workers. Significance levels (*=p < 0,05; **=p < 0,01; ***=p < 0,001) computed for the means of two samples by t-test.

	sterile workers	breeding workers	t value & significance
Sample size	29	10	
Mean length of head capsule	2,59	2,58	0,58
Mean width of head capsule	1,76	1,78	0,88
Mean height of 1st gaster segment	2,22	2,35	3,03 **
Mean length of 1st gaster segment	1,56	1,73	4,38 ***

first segment of the gaster were measured in a small sample of fertile and sterile ants. The morphometric data obtained (Table 15) revealed a marked uniformity in the head size but confirmed that there were significant differences in the size of abdomens. A certain margin of error must be expected in the measurements of the gaster segment because of its three-dimensional nature; in addition the sample of fertile ants measured was small.

Fertile and sterile workers exhibited differences in their behaviour, because the former were never active above ground. Insemination (or its subsequent physiological effects) thus inhibits adults from following the normal pattern of age polyethism shown by sterile workers (2.5.1.); egg-laying workers remain active inside the nests for the rest of their lives, even as they age. However a number of reproductive workers were collected (n= 10) while they were being carried above ground from

one nest to another within colony complexes; this type of transfer occurred throughout the year.

Peeters and Crewe (1984) have suggested the use of the term gamergate (ie. "married workers") to describe unambiguously those mated, reproductive individuals of worker origin which occur in a significant number of ponerine species. This term (W.L. Brown *Jr. pers. comm.) makes the clear nomenclatorial distinction* between ergatoid queens and breeding workers; it will be used interchangeably with breeding workers in the text.

3.3.3. Reproductive structure of colonies

The data in Table 14 indicate that there can be a very large number of breeding workers in a nest (eg. December 1981). *Breeding workers were distributed in all the nests of a colony.* Both the total numbers of gamergates and the proportion of gamergates in the adult population of nests varied considerably over the year and between nests. When nests from one colony were collected at the same time, the individual nests contained different numbers of gamergates (eg. December 1981, February 1982, March 1982). Nests collected in the same month of succeeding years showed variations in their frequencies of gamergates (eg. July 1981 & 1983, October 1981 & 1982). A seasonal pattern *of fluctuation in gamergate frequency can be* seen in Figure 10. Nests sampled in January-February contained *very few or no* breeding workers and a large number of sterile workers, ie. the percentage of breeding workers in the nests was low. In March-April there were large numbers of breeding workers in the nests and, significantly, these represented a large percentage of the individuals in the nests. The sharp increase in the proportion of gamergates in nests can be associated with the pattern of male activity (see Discussion).

Two nests were *excavated in March 1982 (Table 16). Most of* the ants confined to the inside of the nests were inseminated; *some of the unmated ants were callow.* These nests contained

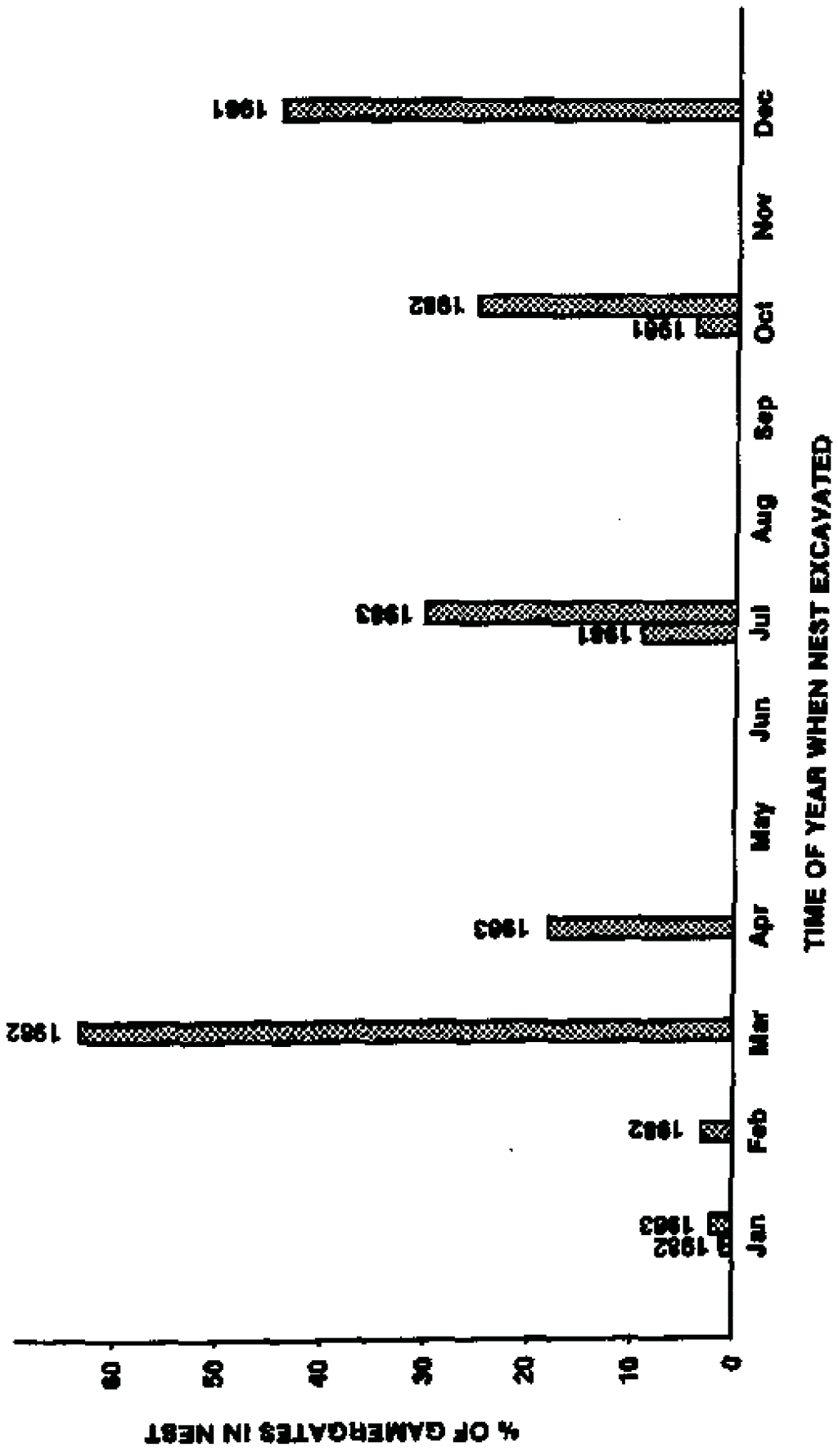

Figure 10 Frequency distribution of the percentages of gamergates in various nests collected at different times of the year. When more than one nest was excavated on a particular date, the percentage of gamergates in the combined population is plotted.

marked foragers and above ground workers; dissection revealed that none of these were inseminated or reproductively active *(Table 16). More marked ants could not be dissected because* they died soon after being brought to the laboratory.

Table 16 Reproductive status (determined by dissection) of individuals from two nests excavated on 23.III.1982 (ie. after the mating season). All the ants that were active above ground prior to excavation had been marked.

Total no. of ants found in nest	No. of above ground ants	No. of above ground ants dissected	No. of above ground ants with active ovaries	No. of unmarked ants dissected	No. of unmarked ants with active ovaries
77	24	7	0	50	49
119	13	7	0	67	46

3.4. Discussion

3.4.1. Reproductive differentiation among workers

The data obtained from the dissection of many individuals reveal that there are no distinct worker and reproductive castes in this species. All the ants have identical reproductive organs, and the functional egg-layers have a true worker origin. The queen caste has disappeared and has been replaced by multiple breeding workers. These results concur with those of Whelden (1957), who made a detailed anatomical study of the queenless

ponerine Rhytidoponera convexa. Whelden found that each ovary in this ant normally contained three ovarioles; in reproductively-active workers, any eggs nearing maturity occurred only in the very lowest part of the ovarioles. These mature or maturing eggs were invariably few in numbers, usually one or two in each ovariole; the maximum number found in any worker was six. The eggs measured 0,45 x 0,16 mm; this is substantially smaller than in O. berthoudi, which may explain the higher number of eggs inside the ovaries of R. convexa. A spermatheca was found in every ant, and sperm was present in 22 of the 274 individuals examined. Whelden did not indicate how many workers were reproductively-active, but he reported that there was no correlation whatever between the presence of sperm and the occurrence of mature or maturing eggs in the ovarioles. This contrasts with the definite association between insemination and ovarian activity in O. berthoudi. Thus, while the same lack of caste differentiation is evident among the workers of queenless Rhytidoponera species as in O. berthoudi, there appear to be differences in their reproductive physiology. The two genera belong to distantly-related tribes (Ectatommini and Ponerini), and this may account for some of the differences exhibited in the control of worker reproduction. Ward (1983) found that, in Rhytidoponera confusa and R. chalybaea, a few (8/139) reproductively-active workers were not inseminated. Non-mated fertile workers are not surprising in these species since, in other representatives of the genus (eg. R. purpurea, Haskins and Whelden 1965), non-inseminated workers produce male eggs in orphaned colonies. This worker-laying also occurs in the Ponerini however (eg. Odontomachus, Colombel 1972a).

In O. berthoudi, there is particularly strong evidence that insemination triggers reproductive activity in the workers. This hypothesis would be falsified if either inseminated workers with undeveloped ovaries, or unmated workers with developed ovaries, occurred. Such ants were not found at any time of the year, with the very few exceptions mentioned in 3.3.2. An alternative hypothesis would have that ovarian activity produces changes in the sexual behaviour of workers, which leads to their mating. If

workers became fertile at times of the year when males are absent, they could only become inseminated during the next mating season; since unmated workers with clearly active ovaries have never been found, this suggestion can be rejected. If there was the seasonal production of a cryptic caste of reproductive ants, and it was synchronized with the period of male activity, we would expect to find a few unmated ants with developed ovaries during January- April; again this is not the case. There is in this species firm evidence that the haploid eggs which develop into males are exclusively laid by the inseminated breeding workers.

Insemination thus plays a crucial role in the control of the division of labour in Ophthalmopone berthoudi; this is the first time that such an effect has been demonstrated in the Formicidae. Buschinger and Winter (1978) showed that, in Harpagoxenus sublaevis, non-mated ergatoids are sterile and a few of the non-inseminated workers are fertile. This species is a parasitic myrmicine with ergatoid reproductives which are morphologically intermediate between winged queens and workers. While insemination may trigger reproductive activity in the ergatoids, it is not involved in the differentiation into ergatoid and worker castes. There are distinct female castes in H. sublaevis, and the workers lack a spermatheca. In O. berthoudi there is a uniform worker caste with all the individuals possessing a spermatheca, and mating determines which workers become fertile. The ovarian stimulation could be caused by substances from the accessory gland in the male, which are transferred during copulation; these have been shown to have an indirect or augmentative effect on egg production in dipterans (Leopold 1976). In cockroaches the physical presence of a sperm mass in the spermatheca seems to be the stimulus which leads to egg maturation (Leopold 1976). It is not known which of these two explanations applies to O. berthoudi. Insemination has been reported to influence a number of aspects of the biology of Formicid queens. Berndt and Nitschmann (1979) demonstrated that mated queens in Monomorium pharaonis secrete a pheromone over the surface of their body which inhibits the production of new

sexuals; in contrast, unmated queens are unable to suppress the development of larvae into sexuals. In Odontomachus affinis, Ferreira Brandao (1983) showed that only mated females exercise effective control on worker oviposition. The unmated females in the nest do not lay eggs, but they take care of the brood as well as excavate and forage actively.

The breeding workers of O. berthoudi lack specialized ovaries. A mean rate of egg laying (0,7 egg/day) was estimated by isolating small groups of breeding workers (Table 13). Such a rate is not uniform throughout the reproductive population however and this estimate blurs the natural variability between gamergates. Individual rates of egg production are difficult to obtain. Ideally a gamergate should be isolated with a small number of sterile workers in order to monitor the number of eggs it lays over a few days. This was not feasible however since the reproductive status of individuals can only be ascertained by ovarian dissection.

A low rate of egg production was also inferred from an examination of ovarian development in different gamergates (Figure 8). Most of the gamergates exhibited very small numbers of large oocytes and mature eggs in their ovarioles. Real variations in ovarian activity between individual gamergates are difficult to assess. The criterion used to assess this activity, the number of mature eggs in the ovaries, is not a continuous measure and thus lacks sensitivity; the advantages are that it is clear-cut and easy to determine when dissecting large numbers of ants. If there is a slow rate of egg production and few ovarioles, differences in the number of mature eggs present at any particular time must be expected among the gamergates; their ovarian development will be scored differently before and after one of the large eggs has been laid. Many of the gamergates placed in the "no egg" category (Figure 8) at the time of dissection could have belonged to a different category at another point in time. Evidence of this dynamic process is the substantial number of eggs that were laid by the gamergates awaiting to be dissected (Table 12). The persistent occurrence of a large group of individuals with few medium or large oocytes

and no mature eggs does suggest that at least some of them are relatively inefficient breeding workers which lay eggs inconsistently. This is thought to be an effect of individual variability in the ability to obtain sufficient food or to produce eggs; this is discussed in 7.5. M.-C. Cammaerts-Tricot (pers. comm.) dissected Myrmica workers in whole colonies from which queens had been removed; many of the workers showed ovarian activity, but she found marked variations in the extent of ovarian development between individuals. As will be seen in chapter 4, an inhibition effect from other gamergates need not be invoked to account for the differences in reproductive performance between workers.

Why do gamergates often have larger than average abdomens? Only young workers become mated (see Chapter 6). The complete hardening of the cuticle takes a number of days; an adult that was dissected 18 days after its emergence still had a soft exoskeleton. It is suggested that if insemination occurs within a certain period after pupal emergence, the neuro-endocrine changes brought about by the stimulation of reproductive activity interfere with the further secretion of bursicon. Bursicon is an hormone released from the central nervous system which controls the biosynthesis of the cuticular tanning compound (Fraenkel et al. 1966, Andersen 1974). The softness of the cuticle depends on the degree of sclerotization, ie. the amount of cross-linking. The increase in volume of the growing ovaries may produce internal pressures in the abdomen which could cause the unsclerotized cuticle to stretch slightly. The casual observation that many gamergates had softer exoskeletons is further evidence that their cuticle has not tanned completely; in contrast, above ground workers with a soft exoskeleton were never found. Thus the occurrence of significant size differences between the abdomens but not the heads of sterile and breeding workers (Table 15) seems to be a consequence of reproductive activity; it is not true that workers with bigger abdomens are those more likely to become mated. The gamergates with smaller abdomens may be older "Innendienst" workers that become mated after the hardening of their exoskeletons has been

sterile workers with "bigger" abdomens seems anomalous; further work might reveal that these are generally bigger workers produced as an effect of favourable trophic conditions during their larval development.

3.4.2. Colony breeding patterns

Males of Ophthalmopone berthoudi are present in the nests from January to April, and most of the mating activity occurs in February-March (see Chapter 5). Individuals were observed entering foreign nests and copulation is inferred to occur underground. Since insemination is the trigger for ovarian activity, every worker that becomes mated during this period differentiates into a reproductive. The data in Table 16 indicate that only ants below a certain age, ie. non-callow "Innendienst" workers, can become mated. The older workers which have become active above ground no longer seem to be sexually attractive, and this could be related to a change in the signal produced by their pygidial gland (see Chapter 6).

The differentiation of workers into gamergates can only occur during the period of male activity. Many workers become mated in February-March (Table 16) and this results in a high ratio of gamergates to sterile workers in the nests. A theoretical expectation of the "insemination hypothesis" is that this ratio should then decrease until the next breeding season, because new generations of workers appear; these cannot become mated and remain sterile. The numbers of gamergates thus stay constant (save for mortality) from one period of male activity to the next. However the dynamics of the reproductive structure also depend on the possible overlap of successive cohorts of gamergates.

How long do the gamergates live? The age of the "Innendienst" workers which become mated is not known exactly. The results in Table 16 show that almost all the workers confined inside the nests became mated, and this represents a substantial range of ages. The longevity of gamergates and "Innendienst"