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Lipid storage by major workers and starvation resistance in the ant *Pheidole pallidula* (Hymenoptera, Formicidae)

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Abstract - *In* Pheidole pallidula, *a myrmicine ant with completely discrete major and minor worker sub-castes, there are functionally two types of major workers. The first type with a non-distended abdomen and a lower weight (about 68% of the major workers population) are involved in colony defence and constitute the real soldier subcaste while the second type ("repletes"), exhibiting a distended abdomen, rarely go out of the nest. In laboratory starvation experiments, the presence of repletes in groups of minor workers or soldiers significantly enhances their survivorship in comparison with those reared alone or with a soldier. The repletes, which are some of the largest major workers in the colony, act as storage vessels of lipids but, surprisingly, do not store carbohydrates or water. The high survivorship of the major workers subcaste and the role of the repletes in the colony are discussed.*

Keywords : Repletes, *Pheidole*, colony defence, survivorship, lipid storage, starvation.

Introduction

Most species of the genus *Pheidole* are characterized by a completely dimorphic worker caste consisting of minor workers and majors also called soldiers since they are postulated to serve in colony defence and as seed millers. Nevertheless, their role as soldiers is under question as certain authors pointed out that their participation in this task is very limited (Detrain et al., 1987 ; Detrain, 1989 ; Aarab et al., 1990) and that, accordingly to their age, they can be involved in other tasks more related with brood-care or nest maintenance (Aarab et al., 1988, 1989). Moreover, like for other species, namely *Camponotus (Colobopsis) fraxinicola* (Wilson,

1974), their function as a nutrient storage caste ("repletes") has also been suggested by Wheeler (1910) and Wilson (1984) and recently proved by Tsuji (1990) in the species *P. ryukyuensis*.

In *P. pallidula*, we noticed a similar phenomenon with an apparent behavioral difference between major workers with a normal abdomen and those with a distended one. Here, we studied if the majors with a distended abdomen function as "repletes" and examined their capacity in lipid and carbohydrate storage

Material and Methods

Defence experiment

Ten marked major workers from an alien nest were introduced in the foraging area of a colony containing 95 major workers. After 180 minutes of experiment, all the resident major workers present in the foraging area were picked up separately and the weight of all the major workers of the colony was determined with a Mettler ME 30 electric microbalance (precision of 10^{-6}g).

Starvation experiments

A colony of *P. pallidula* was collected near Verfeil (28 km North from Toulouse, South-West France) on 8th December 1990. This colony was kept in the laboratory (23 ± 2°C, 12L-12D) in an artificial nest for 1.5 month before the experiments. From this source colony, 5 types of experimental treatments were set up (see Tsuji, 1990) by installing the ants in nests floored with plaster and with a water reserve available in their center. The five treatments consisted of : (I) 20 minor workers only, (II) 20 minor workers and one major worker with a normal abdomen, (III) 20 minor workers and one major worker with a distended abdomen, (IV) 20 major workers with a normal abdomen only, and (V) 20 major workers with a normal abdomen together with a major worker with a distended abdomen. Each type of treatment consisted of 10 replications. No food was supplied during experiments. Number of surviving workers was recorded everyday until all workers died.

Determination of lipids and carbohydrates content

a. Lipids. For each type of worker, 15 samples, each containing 1 individual for major workers and 5 individuals for minor workers, were analyzed. All individuals, randomly picked up from the same source colony as in the previous experiment, were killed with ethyl acetate vapour and then subjected to the same experimental procedure as used by Peakin (1972). Fresh weights were determined (Mettler ME 30 electric microbalance). Samples were dried for 24 hours at 70°C and the dry weight of each sample was determined. Fat was extracted with petroleum ether (boiling point 40-60°C) in a Soxhlet apparatus for 24 hours and then the samples were dried again for 24 hr and re-weighed.

b. Carbohydrates. For each type of worker, 10 samples, each containing 10 individuals, were randomly picked up as previously reported, killed and dried at 90°C for 45 min in order to inactivate the enzymes. They were then analyzed following the procedure used by Van Handel (1985) employing anthrone as the color reagent which permits the determination of carbohydrates (glycogen and free sugars). Percentages and proportions were square-root arcsine transformed to obtain normal distributions (Sokal and Rohlf, 1969) and means were compared by t-test (significance for $P > 0.05$).

Results

Behavioural differentiation among major workers

The major workers which are involved in colony defence are always the individuals with non-distended abdomen, those with enlarged crop remaining in the nest in such a situation.

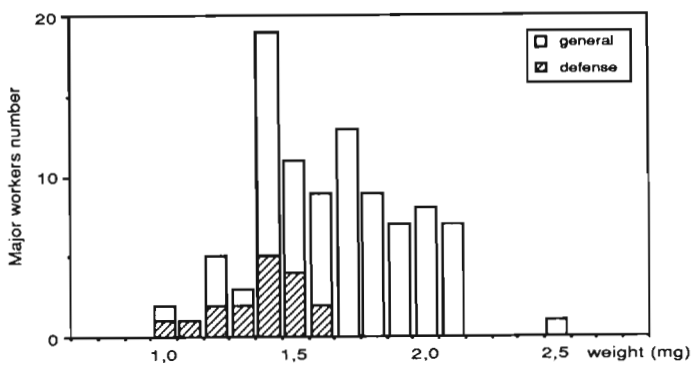

Fig. 1. - *Weight distribution of major workers of* Pheidole pallidula *involved in colony defence compared with the whole major workers population (general).*

Relative to the whole major worker population of the colony, the weight distribution of the attacking major workers (Fig. 1) is clearly shifted toward the lower values (with a maximum of 1.7 mg). These values essentially correspond to the major workers with a normal abdomen, which thus function as the real "soldiers" of the colony and will be named under this label subsequently.

Determination of the nutrient storage function

In all treatments, the survival rates decreased slowly (Fig. 2) during the first week of experiment and thereafter mortality is essentially correlated with the experimental set up. Thus, in treatments I and II, the survival rates dropped abruptly while the survivorship declined more gradually in treatment III. Surprisingly, the survivorship in treatments IV an V is very high indicating a very important resistance to starvation in soldiers, this resistance being enhanced when a major with a distended abdomen is present (treatment V).

The average survival period in each treatment (Fig. 3) was non significant between I and II but highly significant for all other comparisons. In treatments III and V, the presence of a major worker with a distended abdomen leaded to enhance the survival rates both of minor workers and of soldiers while the presence of a soldier seems to have no effect in this respect (treatment II).

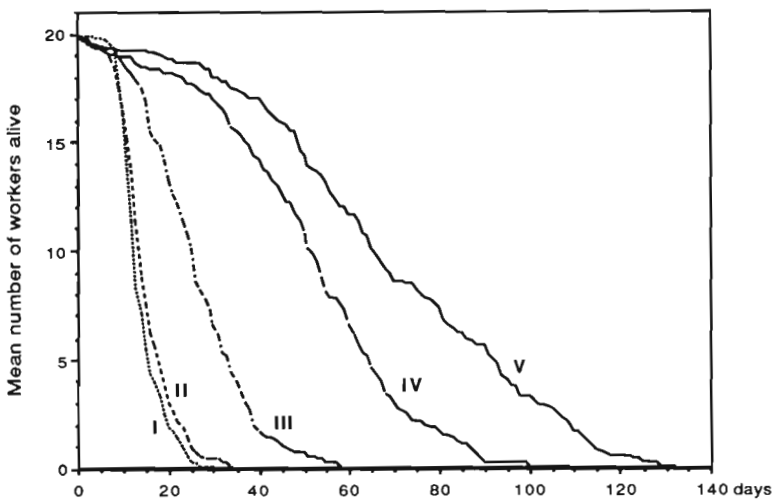

Fig. 2. - *Mean survivorship curves for 20 minor workers (I, II, III) and 20 soldiers (IV, V) of* Pheidole pallidula*: (I) no major worker present, (II) one soldier present, (III) one replete present, (IV) no replete present, (V) one replete present (10 replicates for each tratment).*

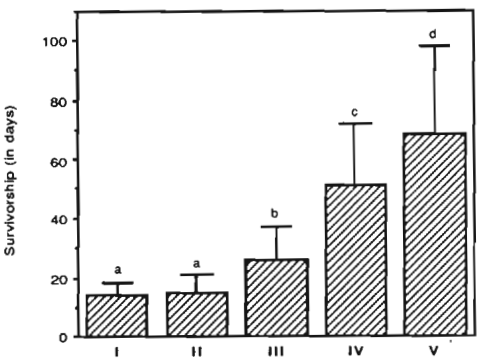

Fig. 3. - *Mean survivorship (n = 200 for each treatment). Means followed by different lower case letters are significantly different (two-tailed Mann-Whitney U test, $p < 0,05$).*

The average survival period of major workers (Fig. 4) was also significantly different between the three treatments (II, III and V), indicating that the major workers with distended abdomen are much more resistant to starvation than soldiers. This resistance varies accordingly to the context : it is much greater in the presence of soldiers than with minor workers which behave aggressively toward major workers whereas this aggressivity is very rare between major workers.

In conclusion it appears from these laboratory starvation experiments that the major workers of *P. pallidula* with a distended abdomen function as "repletes" in the same way as they do in *P. ryukyuensis* (Tsuji, 1990)

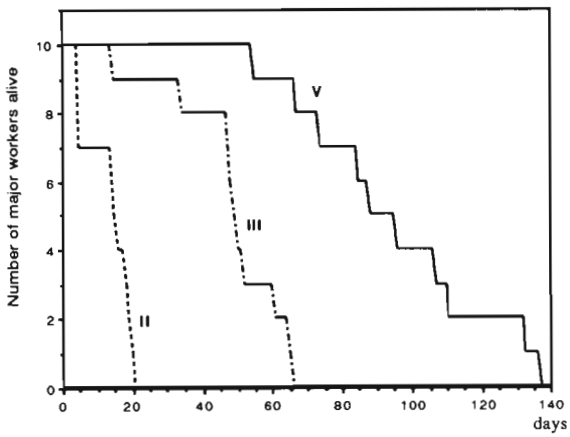

Fig. 4. - *Major worker survivorship : (II) one soldier with minor workers, (III) one replete with minor workers, (V) one replete with soldiers (10 replicates for each treatment).*

Determination of lipids and carbohydrates content

The comparison between fresh and dry weight (Fig. 5) confirms the differences reported for the three worker sub-castes, the repletes being the heaviest workers of the colony. Moreover, this comparison indicates that the differences cannot be simply explained by a greater storage of water by repletes since the loss of water is comparatively higher for both minor workers and soldiers (respectively 65.1% and 61.8% of fresh weight) than for repletes (44.9%).

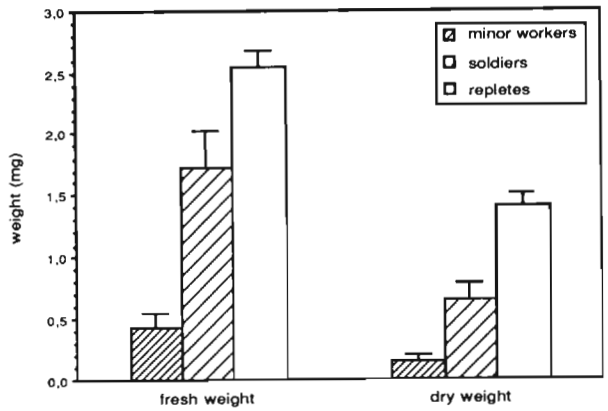

Fig. 5. - *Mean fresh and dry weight (mg) of minor workers, soldiers and repletes of* Pheidole pallidula.

The analysis of fat and carbohydrates contents (Fig. 6) shows that fat content is considerable in repletes, the ratio fat/dry weight reaching 60% compared with 20 to 22% for minor workers and soldiers respectively.

On the other hand, carbohydrate storage (glycogen + free sugars) is very low in repletes and more important in soldiers and minor workers (respectively 3 and 8 times higher).

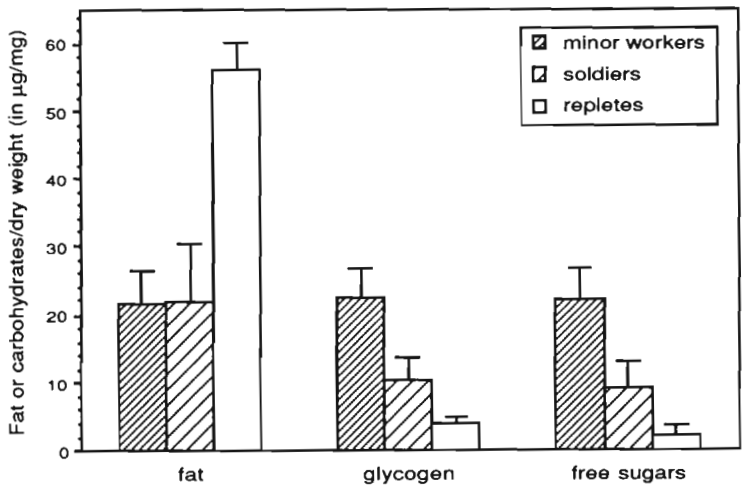

Fig. 6. - *Fat and carbohydrates (glycogen + free sugars) content for the three worker sub-castes of* Pheidole pallidula.

Discussion

The three worker sub-castes (minors, soldiers and repletes) display strong differences in behavior and fat-carbohydrates storage. The major workers with a distended abdomen act as repletes. These repletes exhibite a high ratio fat/dry weight and a low ratio carbohydrates/dry weight. By contrast, minor workers show a low ratio for fat and a high one for carbohydrates while soldiers have intermediary ratios for both fat and carbohydrates.

As for the very large repletes encountered in *Myrmecocystus* (Wheeler, 1910), the existence of repletes in *P. pallidula* could be inferred as an adaptation for highly fluctuating temperature in the environment (Creighton, 1950 ; Wilson, 1971). Fat is slowly metabolized and it is likely that fat storage by repletes which have not yet undertook a real hibernation may provide a long term energy reserve which can be progressively re-distributed during winter starvation.

Recently, evidence from Passera et al. (1989) who studied nuptial flight by *Formica lugubris* sexuals, demonstrates that glycogen is the main energy source involved in muscular effort. Since repletes do not defend the nest nor forage outside the nest, their need for carbohydrates is very limited. On the contrary, a reasonable explanation for the low storage of fat and the high storage of carbohydrates by minor workers is their need of a form of energy much more rapidly released (glycogen) as they perform the essential part of foraging, provisioning and colony defence (even more in this case than soldiers themselves, see Detrain, 1989). In the same way, the intermediary value obtained for carbohydrate storage by soldiers would correspond to their lower participation in foraging and colony defence.

Carbohydrates are known to serve as energy source for nuptial flight in ant sexuals and fat reserves are used to nurture the queen and the brood during the time of colony founding (see Passera and Keller, 1990). In workers, the highest fat content occurs at the time of emergence and it is likely that these reserves allow the callows to act quickly as nurses and to lay eggs (Passera and Keller, 1987). In *P. pallidula*, the workers are completely sterile and fat storage by repletes cannot be involved in worker egg laying. Nevertheless, fat reserves may be metabolized and could serve as food source for larvae feeding, which would explain the gathering of repletes near brood (Lachaud, unpubl.).

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