

Effect of Orphaning On the Effectiveness of Queen Attraction and On Worker Behavioral Repertoire in *Cataglyphis cursor* (Hymenoptera: Formicidae)

By

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ABSTRACT

The effectiveness of queen attraction towards workers, foragers or nurses, was measured in *Cataglyphis cursor*. Effect of queenlessness was studied four months after the partition of the society, just before hibernation, when the reproductive activity had stopped. The results show first that the maternal queen is equally attractive to foragers and nurses. Secondly, that queenlessness induced an impairing of the effectiveness of queen attraction towards either foragers or nurses. The activity of the nurses and the foragers is greatly reduced when the queen is missing. This may be only partially explained by differences in queenright and queenless colony odor. The *Cataglyphis cursor* queen is not the only source of colonial odor. The queen is more important in the infinitely complex organization of the society. After the queen removal, the observed behavioral drift is an expression of the social flexibility which allows the normal society ergonomomy.

KEY-WORDS : Ants, *Cataglyphis cursor*, Queen Attractant effectiveness, Orphaning, Recognition, Queen Cues.

INTRODUCTION

In *Cataglyphis cursor* Fonscolombe, a monogynous Formicinae, when the single mated queen is missing, workers are able to produce males and females (winged females and workers) through deuterotokous parthenogenesis (Cagniant 1979). This phenomenon raises doubts about the queen's function. Observations of queenright and queenless societies usually lead one to assume that the queen is of central importance in worker kin discrimination. The amount of aggression among workers is dependant upon the presence of common cues. According to Carlin and Hölldobler (1986, 1987), the queen of *Camponotus* spp. is of main importance in the production of colonial odor. When the queen is missing, the eusocial structure is usually altered and this

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affects the ability of the workers to distinguish nestmates from non-nestmates.

In *Cataglyphis cursor*, interindividual discrimination does not seem to be influenced by the presence or absence of the queen (Berton, 1989), so that in this species, the queen does not seem to be the primary source of colony odor. Nevertheless, a behavioral drift is observed in queenless societies suggesting that the queen presence is important.

The attractiveness of the queen is a fundamental characteristic of social structure that could facilitate the achievement of principal tasks through individuals living together and possessing more effective communication systems. Previous works related to the closure of the society have shown that queen recognition and queen attractant effectiveness are connected [Keller & Passera (1989) in *Iridomyrmex humilis*; Berton *et al.* (1991), in *Cataglyphis cursor*]. When measuring the relative attractiveness of queens to workers in experimental colonies of *Iridomyrmex humilis*, Keller (1988) showed that queens in polygynous societies are less attractive than queens in monogynous societies. In *Plagiolepis pygmaea* (Mercier *et al.* 1985), *Solenopsis invicta* (Vargo & Fletcher 1990), and *Myrmica rubra* (Cammaerts & Scannu 1985), physiological factors could explain differences in the effectiveness of queen's attraction. The mechanisms that control the individual development could be altered by modifications in the social environment, particularly when the queen is missing. It is therefore of great interest to study ethograms and assess the role of the queen and the involved mechanisms.

In this work, we measured the effectiveness of queen attraction towards queenright and queenless groups of both foragers and nurses. This allowed us to consider quantitative and qualitative modifications of attractiveness as a function of particular experimental and environmental factors.

MATERIALS AND METHODS

Rearing

Colonies of *Cataglyphis cursor* were collected in the Mediterranean area in the South of France. They were kept in the laboratory, in an air-conditioned room at 25°C, at a constant light regime of 12hL:12hD from June to October. Hibernation was induced from October to March. Insects, honey and water were supplied every day.

Experimental set-up (Fig. 1)

Experiments were carried out in a cylindrical box (diameter: 8cm, height: 5cm). The upper 20mm of the vertical sides were covered with fluon which prevents the ants from escaping. The arena was divided into two equal areas in which a tube was glued. A piece of gauze isolated the tubes from the arena, preventing physical access but allowing the diffusion of any possible volatile pheromone compound. Each area thus consisted of two different parts, the

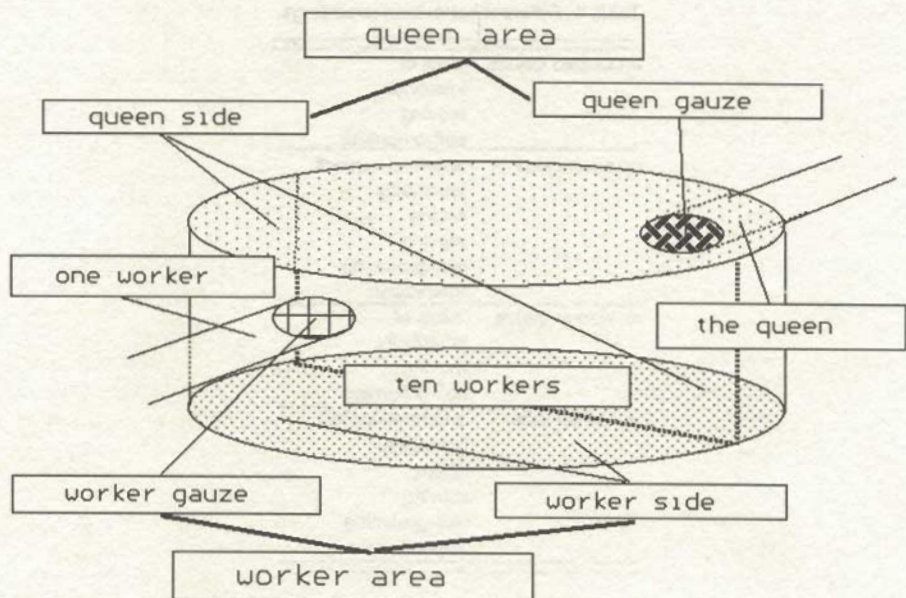


Fig.1. Experimental set-up.

"gauze" and the remaining area, the "side" (surface proportion 1:60).

Tests of attractiveness

Tests were conducted between 10 a.m. and 3 p.m. (U.T.) from late September to early October, just before hibernation, when ovarian activity was stopped. There was only a small quantity of brood in the colonies since *Cataglyphis cursor* development is annual, without winterbrood.

In experiment 1, workers were taken from queenright societies. They were either foragers (A) (15 tests from different societies), or nurses (B) (12 tests from different societies).

In experiment 2, workers were taken from queenless societies. They were either foragers (C) (15 tests from different societies), or nurses (D) (12 tests from different societies).

Queenright workers were tested with their maternal queen. Queenless workers were also tested with their maternal queen from whom they had been separated 4 months earlier.

For each test, eleven workers were taken from a single colony. Ten workers were placed at the center of the set-up and the eleventh confined in a tube. The

Table 1: Different behavioral recordings.

on Queen Gauze	bites of immobility moving self-grooming
on queen side	antennal contact immobility licking moving self-grooming trophallaxis
on worker gauze	bites of immobility moving self-grooming
on worker side	antennal contact immobility licking moving self-grooming trophallaxis

second tube contained the colony queen. The survey began 15 minutes later. Data were recorded every five minutes for an hour, thus 12 times, making a total of 120 records for the 10 ants. A repertoire of mutually exclusive behavioral acts was also recorded. Each behavior was defined by its location (queen or worker area, gauze or remaining area). A maximum of twenty different behavioral recordings were identified (Table 1).

Data Analysis (SPAD software, Lebart et al., 1988)

In order to take into account as much information related to experimental factors (foragers or nurses, queenright or queenless) and to establish any useful correlation with continuous variables, we used first a Factorial Multiple Correspondence Analysis (FMCA). The matrix was composed of 11 columns (11 active variables with 2 modalities) and 54 lines (54 tests). Second, we used two Discriminant Analyses (DA), one analysis according to experimental treatment (queenright or queenless), the other according to workers' age (foragers or nurses).

Statistical Analysis

Statistical analysis was carried out from raw data, using the Mann-Whitney test (one tailed or two-tailed according to the case, $P \leq 0.05$) for independent group comparisons and the Wilcoxon test for matched pairs.

RESULTS

Worker distribution in queen and worker areas (Wilcoxon test)**Experiment 1 (Queenright societies)**

Queenright workers in the queen area: Workers originating from queenright societies do not spread by chance, but more often visit the queen area that appears very attractive (A: 74.7%, $P < .0001$; B: 71.4%, $P < .001$).

Queenright workers on the queen gauze (Fig. 2): Considering the respective surfaces of the "gauze" and the remaining area, the "side" (1:60), frequentation of the queen gauze (A: 41.4%; B: 24.7%) represents more than 29 and 17 times respectively, the percentage expected if the workers were moving randomly on the area. This result is confirmed by a significant difference in the frequentation of queen and worker gauzes (respectively A and B, $P < .00001$).

These results show that queenright foragers and nurses easily discriminate between queen and worker gauzes.

Experiment 2 (Queenless societies)

Queenless workers in the queen area: The worker repartition in the

Queen and Worker Gauzes

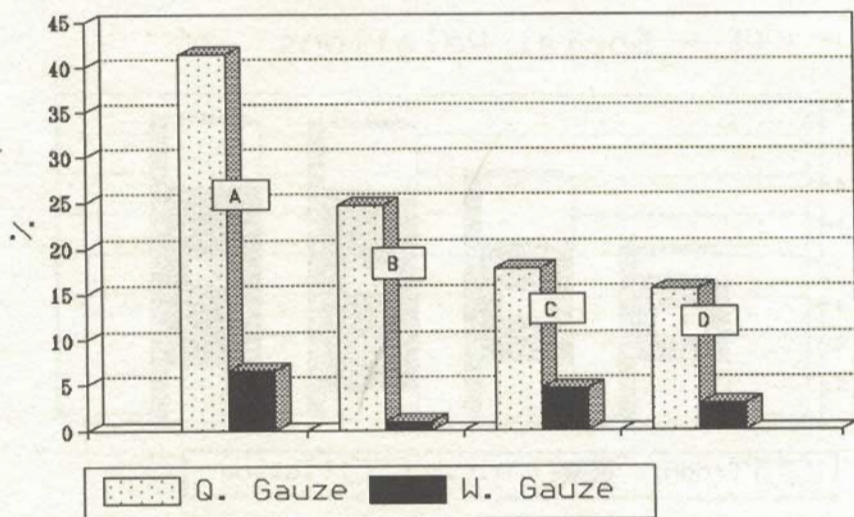
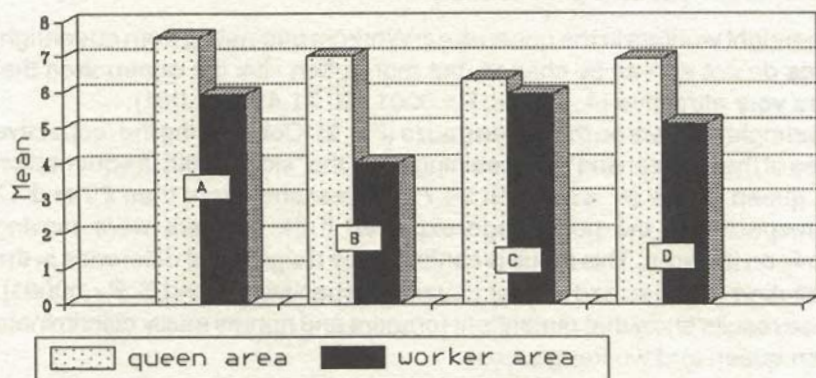
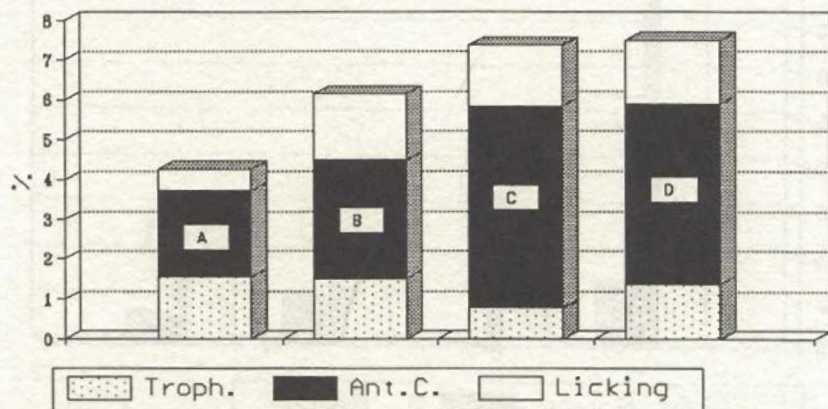


Fig. 2. Frequentation (mean percentage: %) of queen and worker gauzes.
 Experiment 1 A: queenright foragers B: queenright nurses
 Experiment 2 C: queenless foragers D: queenless nurses

I - Repertoire



II - Social Relations



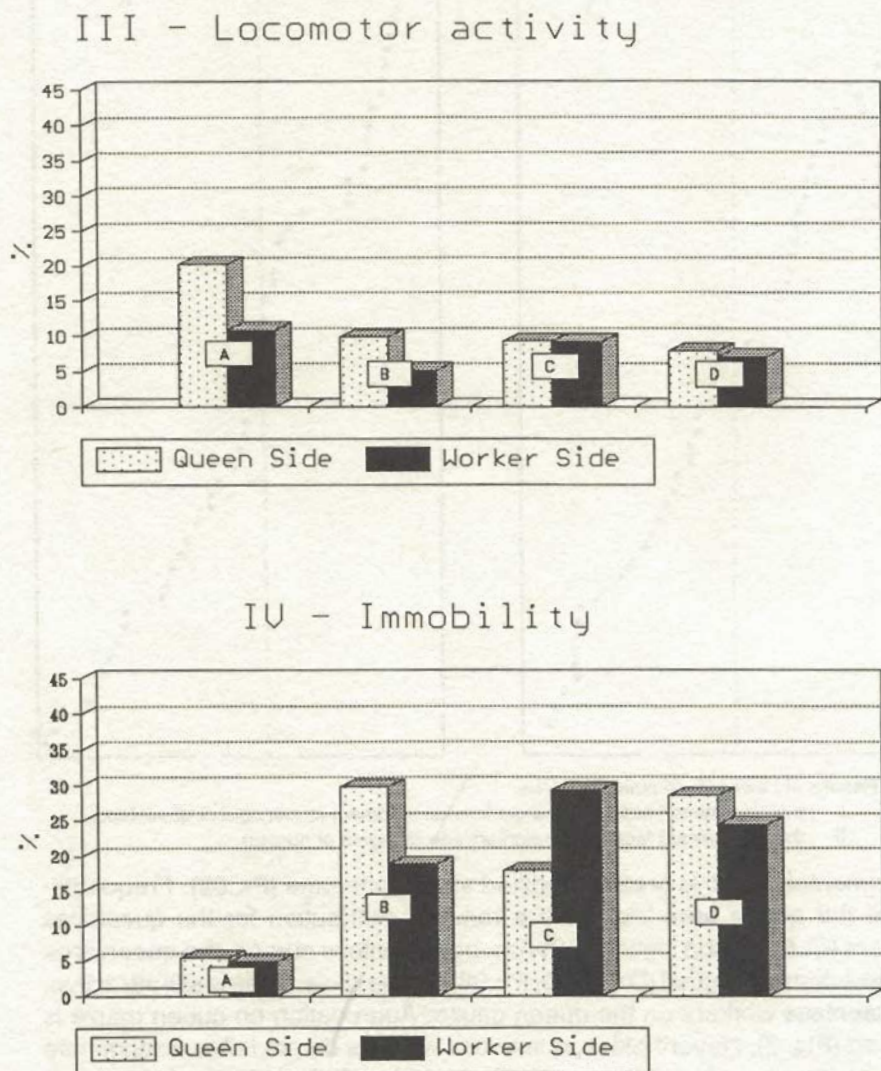


Fig. 3. Behavioral recordings

I- mean number of different behavioral acts

II-, III-, IV- mean percentage of recordings (%), respectively; Social Relations (trophallaxis, antennal contact and licking grouped together); Locomotor Activity; Immobility.

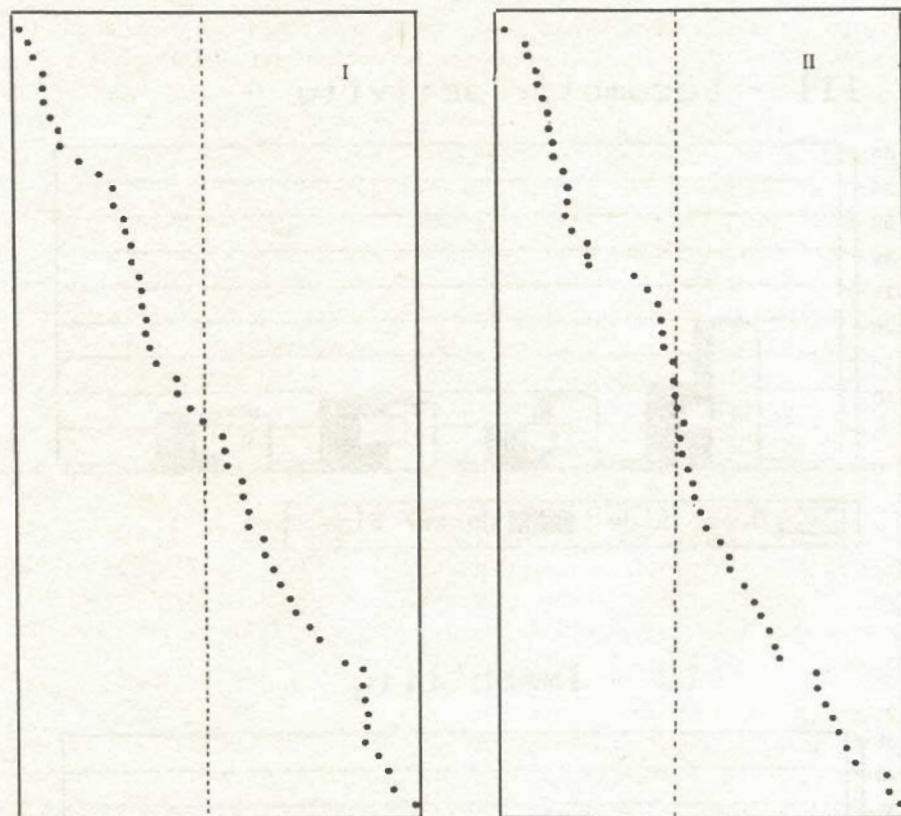


Fig. 4. Results of Linear Discriminant Analysis.

I- : the discriminant factor is the experimental treatment (queenright or queenless).

II- : the discriminant factor is the workers' age (foragers or nurses).

experimental set-up is greatly modified after orphanage ($P < .02$). Frequentation of the queen area looks like a random distribution for the queenless foragers (C: 51%, non significant). Among queenless nurses, the queen area frequentation is reduced (D: 59.8%, $P < .02$) but the queen area is still attractive.

Queenless workers on the queen gauze: Aggregation on queen gauze is reduced (Fig. 2). Nevertheless, queenless workers do not fail to discriminate between queen and worker gazes (C, $P < .0001$); D, $P < .001$).

Repertoire (Fig. 3)

The number of different behavioral acts (Fig. 3-I) is the greatest in queenright foragers (A). Behavioral analysis brought out qualitative and quantitative changes. The results of the various paired observations (queen area and worker area) of different behavioral acts are compared by the

Wilcoxon test. Significant differences are related respectively to groups (A) ($P < .001$) and (B) ($P < .001$). In the experimental set-up, queenright workers have a richer repertoire in the proximity of the queen. The comparison of the behavioral response in group (C) of queenless foragers does not reveal any significant difference between queen and worker areas. In group (D) of queenless nurses, the number of behavioral acts is reduced but still greater in the queen area than in the worker area ($P < .005$). Response profiles to queen attraction of foragers and nurses differ after orphanage.

Social relations (Fig. 3-II): Antennal contacts are grouped together with licking and trophallaxis and named "social relations". During the experiments, queenless workers (C) and (D) have more antennal contacts (Mann-Whitney U, significant) and licking (significant) than queenright workers (A) and (B). Trophallaxis is not significantly changed.

Self-grooming recordings are the highest in group (A).

Worker activity (Figs. 3-III and 3-IV) (Mann-Whitney U): Greatest changes concern the bites of queen gauze (comparisons A/B, $P = .0228$; A/C, $P = .0192$; A/D, $P = .003$) and locomotor activity, moving on queen side (comparisons A/B, B/B, A/C, A/D, $P < .001$), moving on worker side (A/B, $P = .0012$; A/D, $P = .0069$; B/C, $P = .0392$), lack of activity as immobility on queen side (A/B, $P < .001$; A/C, $P < .0019$; A/D, $P < .001$; C/D, $P = .0287$), immobility on worker side (A/B, $P = .008$; A/C, $P < .001$; A/C, $P < .001$; B/D, $P = .0359$).

Data Analysis (Fig. 4)

A linear discriminant analysis (DA) with two groups, using both the Fisher method and the Factorial Multiple Correspondence Analysis (FMCA) were performed. Nine discriminant variables were chosen. In the first DA (Fig. 4-I), discriminant factor is the queenright or queenless experimental treatment. The classification is good and 43 individuals out of 54 (79.63%) are assigned to the adequate group. Exceptions are a10, b6, b8, b10, b11, b12 in the queenright group and c7, c9, c11, d1, d8 in the queenless group. The decision depends principally on the original modalities which are: moving on queen gauze, social relations, immobility on worker side, and moving on queen side. Worker age is the discriminant factor in the second DA (Fig. 4-II). 39 individuals out of 54 (72.22%) are assigned to the right group. Exceptions in the forager group are a4, a15, c1, c2, c3, c5, c6, c11 and b1, b7, b8, b12, d6, d8, and d9 in the nurse group. The decision depends principally on the categories: moving on queen side, moving on worker side, and bites of worker gauze.

To summarize, group (A) is homogenous and easily identified. Individuals, queenright foragers, have a great activity, specially locomotor activity, much efficiency in experimental conditions with few social relations. A second group (D), made of queenless nurses, is relatively homogenous. Selected parameters generally reflect much immobility, few responses to queen attendance

(few bites of queen gauze), but many social relations. A third group is a mixture of queenright nurses (B) and queenless foragers (C). Heterogeneity is striking. Half the individuals are badly filed, either characterized by their age, either characterized by their queenright or queenless experimental treatment. Four individuals, b8, b12, c11, d8 are bad filed in all cases.

DISCUSSION

Under our laboratory experimental conditions, selected nurses for experiments 1 and 2 were aged two months and even up to four months. This could have been because of the specific *Cataglyphis cursor* reproduction cycle, without winterbrood or because of an early and rich production of workers in May and June or a late and a small production in July or August. This could partially explain the heterogeneity of group (B) and the difficulty to distinguish, in DA, this group from group (C).

Our experiments showed that queenlessness modified the worker response to queen attraction. Several hypotheses are considered concerning *Cataglyphis cursor* peculiarity when the queen is missing:

Alterations in colony odor after queenlessness may explain differences in the effectiveness of queen attraction. The following three points are considered of importance.

1°. Queen attraction is less efficient because of a colonial odor difference. Cariou and Passera (1990) observed in *Iridomyrmex humilis* that dead queen's bodies were still attractive for a time and that the loss of efficiency of the attraction was greater when a queen corpse was kept outside the nest than when it was maintained in the nest without licking. This indicates the importance of colonial odor in queen attraction.

2°. Nevertheless, the explanation of odor differences between queenright and queenless colonies should not be sufficient. If colonial odor is linked to queen attraction, what is the queen part? Crosland (1990) demonstrated that natural queenless and queenright colonies of *Rhytidoponera confusa* had similar capacities of kin recognition and that this ability was not diminished when no queen was present. This suggested that the queen does not produce the colony odor and is in agreement with our previous work on *Cataglyphis cursor* (Berton & Lenoir 1986).

3°. In previous experiments (Berton, 1989), we tested queenless *C. cursor* workers after the experimental removal of their queen four months earlier. In a first example of temporary orphanage, when queenless colonies could produce a queen thanks to thelytokous parthenogenesis, possible in spring or early summer, the results showed that attractiveness of the original maternal queen to her removed workers was not weakened. In a second example, when the workers are removed from their nest just after emergence and adopt an

alien queen, the results are similar. Several months after the removal of the queen, maternal queen attraction is efficient for the removed workers. These results suggest modifications in the workers' ovarian physiology. There is a direct relation between queen control upon worker's reproduction and queen attractant effectiveness. The alien adopted or parthenogenetically produced queen is competent to control worker ovarian activity and is therefore very attractive. In case of permanent orphanage, such a response to the queen's attraction is different. After the removal of the queen, queenless workers become reproductive females and thus engage in competition against their queen, whose privileged status of unique queen is henceforth missing. Queen attractant stimulus becomes effective in two-months old workers of *Cataglyphis cursor* (Berton *et al.*, 1991). Worker production is copious but with time limits. Such physiological changes alter individual interactions. Alterations of the worker response to queen attraction were observed both in foragers and nurses. Retana & Cerda (1990), Mayade & Suzzoni (1990), demonstrated in *Cataglyphis cursor*, that inactive workers have non-developed ovaries, interior workers have non-developed and mature ovaries, foragers have totally degenerated ovaries, and behavioral changes are related to age. In our experiments, the orphanage effect should have more deeply modified the response of nurses as compared to foragers. This was not the case. It was difficult to differentiate the two samples (B) and (C) in Data Analysis, and frequentation of queen gauze was not significantly different.

Orphaning effect on queen attraction effectiveness to queenless workers may be temporary if the original maternal queen is readopted by her removed workers. The workers' behavioral repertoire is progressively modified and recovers the level sustained before the removal of the queen (F. Berton, unpublished).

In fact, after the queen removal, a behavioral drift appears which is the expression of social flexibility. The society structure is little disturbed with regard to basic functions such as the closure of the society (Berton and Lenoir, 1986). These capabilities are time dependent. The hypothesized finality is to recover another queen.

In the field, despite worker thelytokous parthenogenesis ability, no observation of queenless colonies of *Cataglyphis cursor* has been related (Lenoir *et al.* 1988). Some arguments can explain this fact. Colony foundation of this species is a budding process which leads, in the same habitat, to the existence of inter-nest relations such as transports between neighboring nests, adoptions of workers, robbing of cocoons or prey. In the case of queen death, queenless workers could easily abandon their nest and enter a new but closely related colony. During thelytokous production, copious production of female and male winged sexuals can take place, but this is nevertheless an accessory mechanism, because it is too expensive and time-dependent.

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RÉSUMÉ

L'efficacité de l'attraction de la reine sur les ouvrières, pourvoyeuses ou nourrices, est mesurée chez *Cataglyphis cursor*. L'effet de l'orphelinage est étudié quatre mois après la partition de la société, juste avant l'hibernation, lorsque l'activité de reproduction de la société est arrêtée. Les résultats montrent d'abord que la reine d'origine maternelle est aussi attractive pour les pourvoyeuses que pour les nourrices. Ils montrent ensuite que l'orphelinage modifie l'efficacité de l'attraction de la reine aussi bien sur les pourvoyeuses que sur les nourrices. L'activité des pourvoyeuses et des nourrices est grandement réduite lorsque la reine est absente depuis quatre mois. Des différences d'odeurs entre colonies normales et colonies orphelines n'expliquent que partiellement ce phénomène. La reine de *Cataglyphis cursor* n'est pas seule responsable de l'odeur coloniale. Son rôle est plus important comme élément essentiel d'une organisation sociale infiniment complexe. La déviation comportementale, observée chez les sociétés orphelines, est plutôt l'expression d'une flexibilité sociale qui permet à la société de fonctionner normalement.

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