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Host nest usurpation and colony foundation in the European amazon ant, *Polyergus rufescens* Latr. (Hymenoptera: Formicidae)

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Summary

The socially parasitic mode of founding new colonies by queens of the European amazon ant *Polyergus rufescens* was analysed in the laboratory. Newly-mated females of this obligatory slave-maker were individually introduced into queenright and queenless artificially established colonies of both *Formica cunicularia* (the slave present in the natal dulotic nest) and *F. rufibarbis* (another potential *Serviformica* host). Particular attention was devoted to the behavioural patterns displayed by these young queens during the usurpation phases. Our observations, supported also by video-taping, show that the slave-making female, before laying her eggs, must penetrate the host colony, kill the resident queen, become accepted by the adult workers and appropriate the host brood. The parasite was almost always adopted in the colonies of *F. cunicularia*, whereas in the presence of *F. rufibarbis* it was generally killed in a short time. The failure in the attempt of usurping the colonies of *F. rufibarbis* is discussed in relation to the host specificity typical of this slave-maker. Finally, egg-laying by *Polyergus* successful usurpers, the subsequent eclosion of the brood, and its complete social integration in the newly-established mixed colonies were also recorded.

Introduction

Though the mode of colony founding varies greatly in ants, two main strategies are known: independent foundation, in which young queens after the insemination start a new colony without the aid of workers; and dependent foundation, in which newly-mated queens need the help of a worker force from their natal nest or from another colony (Hölldobler and Wilson, 1977, 1990).

Parasitic ants normally exhibit a dependent mode of colony formation. In particular, queens of inquilines (permanent parasites which generally have lost their worker caste) gain acceptance into complete host colonies, coexisting with the resident queen/s and producing only sexual offspring, which is reared by the host workers (Buschinger and Klump, 1988).

By contrast, the elimination and replacement of host queen/s during colony foundation is the rule for temporary parasitic and slave-making (or dulotic) ants.

Young queens of temporary parasites are assisted by the host workers only during the initial stage of colony establishment. In slave-making species, the host workers rear the offspring of the parasite until its worker population is sufficiently large to supplement the slave force by slave raids. During the raid, the dulotic workers attack other nests of the host species and pillage the brood from which the slaves emerge (Buschinger, 1986; Buschinger and Klump, 1988; Hölldobler and Wilson, 1990; Topoff, 1990).

Ants of the genus *Polyergus*, the so-called Amazons, are all obligatory slave-makers living in dependence upon workers of the related genus *Formica* for food, brood rearing, and colony maintenance.

As is the rule for obligatory social parasites, also *Polyergus* queens are not capable of founding new colonies independently. Therefore, before laying her eggs, a newly-mated female generally should locate and penetrate a nest of her host, eliminate the resident queen/s, appropriate the resident brood, and obtain the care of the resident workers for herself and, subsequently, for her brood.

In this connection, the mating and post-mating behaviour of *Polyergus* females includes some adaptations for reaching and penetrating colonies of *Formica*. Concerning the American amazon ants, after the mating flight, dealate females of *P. lucidus* often reached a dulotic nest, wait for a raid and follow the raiding swarm to the target colony (Talbot, 1968; Marlin, 1971; Cool-Kwait and Topoff, 1984). On the contrary, the females of *P. breviceps* copulate directly during a raid of the homocolonial workers (Topoff and Greenberg, 1988). Invasions of host colonies by dealate queens with raiding swarms were also observed in the Japanese *Polyergus samurai* (Hasegawa and Yamaguchi, 1994). Females of the European amazon ant *P. rufescens* adopt a more complex strategy, since they copulate after mating flights, during slave-raids and, in addition, on the ground close to their nest (Mori et al., 1994a).

Whatever is the mating behaviour adopted by *Polyergus* females, following a slave-raid swarm by newly-inseminated queens is considered an advantageous strategy to locate and invade host nests and to establish a new dulotic colony. In fact, the raiding swarm leads the young queens directly to a colony which is demoralized, depleted of brood and probably saturated with the odour of *Polyergus* ants (Talbot, 1968; Topoff, 1990; Topoff and Mendez, 1990).

Among the five species belonging to this genus of slave-makers, host-colony usurpation has been exhaustively described only in *P. breviceps* (Topoff et al., 1988, 1990; Topoff and Mendez, 1990; Topoff and Zimmerli, 1993). Considerably less is known about this process in *P. rufescens*, since the information concerning this parasite is due mostly to the early studies of Emery (1908, 1909, 1911, 1915), Forel (1920) and Zaayer (1967).

In this paper we present the results of a detailed research on colony founding in *P. rufescens*. In particular, our aim was (*i*) to analyse the process by which the newly-mated females of this slave-maker penetrate colonies of *Formica cunicularia* (i.e. the *Serviformica* slave present in their natal dulotic colony), eliminate the resident queen, appropriate the brood and are adopted by *Serviformica* workers; (*ii*) to prove the occurrence of host specificity in colony founding by this parasite, testing the possibility of colony usurpation also in the presence of *Formica rufibarbis* (i.e. another potential slave belonging to the *Serviformica* subgenus as well as *F. cuni-*

cularia, but not present in the mother dulotic nest); and (*iii*) to obtain the foundation of new mixed colonies.

Since it is impossible to make detailed observations of this critical event in the field, we carried out laboratory experiments under controlled conditions. Although this study cannot prove what the slave-maker really does in nature, it enables us to explore the potentialities of this parasite and provide insight into the kind of behavioural patterns which are likely to occur.

Materials and methods

During July 1992, for both the *Serviformica* species (i.e. *F. cunicularia* and *F. rufibarbis*), fragments of pure colonies consisting of queens, workers, brood and nest materials, were collected from polygynous colonies in a selected area of the Apennines near Parma (Northern Italy) at an altitude of about 700 m. Wingless queens of *P. rufescens* were collected during several slave-raids conducted by workers of different colonies (all populated by *F. cunicularia* workers as slaves) in the same area and period of study. Evidence of the mated status of these females was confirmed after their death by examining the spermathecal contents after dissection.

In the laboratory, a total of 28 artificial nests, each consisting of a darkened box linked to a foraging arena (Le Moli and Passetti, 1978), was set up under controlled conditions of temperature (24–26 °C during summer; 10–12 °C during winter) and relative humidity (60–80%). Two days before each tests, the *Serviformica* workers (about 200), brood (about 100 pupae and larvae) and queen (when applicable) were transferred into the artificial nests under a Petri dish (used as a brood chamber in the darkened box) with four entrance/exit holes on the sides. For both species, 7 colonies contained a homocolonial queen (queenright colonies) and 7 were lacking her (queenless colonies). Ants were supplied with water, honey and fragments of insects.

The test began when a single *Polyergus* queen was introduced into the foraging arena. The behaviour of the intruding female and resident ants was observed continuously for 2 h (also by the use of a video-tape). Afterwards, the colony was monitored every 30 min for additional 3 h, followed by observations at the end of 24, 48, and 72 h. After this experimental period, the colonies were observed twice a week up to the autumn of the following year. Successful colony take-over and adoption were recorded after: (*i*) the killing of *Serviformica* queen (when applicable) by the introduced *Polyergus* female; (*ii*) the feeding and grooming of *Polyergus* female by the resident workers; and (*iii*) the resting of *Polyergus* queen on or near the *Serviformica* brood (Topoff et al., 1988).

Results

Interaction with F. cunicularia

For both queenright and queenless colonies of *F. cunicularia*, successful take-over by *Polyergus* queens occurred in 6 (85.7%) trials.

When an alien female was introduced into artificial nests of this host species, in a short time the colony became alarmed: the adult workers ran around the nest, attacked the parasite, and began to abandon the brood chamber with the brood. The parasitic queen fled from encounters with *Serviformica* ants, but finally counterattacked in brief and violent fights. Generally, in such a combat, the workers lost antennae or legs, whereas the slave-making female was never visibly injured. Moreover, the parasite remained motionless on the top of injured or killed workers, or carried them in their mandibles around the nest.

In queenright colonies, when the alien female finally approached the resident queen, she immediately pounced on her, using the sharping mandibles to bite the head, thorax and gaster of the opponent. Moreover, after the seizing, *Polyergus* queen usually curved the gaster towards the *Serviformica* queen. By contrast, we never observed the latter initiating aggressive encounters with the intruder. Many workers approached the fighting queens and began biting indiscriminately each other, the alien female and their own queen. In all successful adoptions, the *Serviformica* queen was killed in few minutes after the attack by the *Polyergus* female or the resident workers (average time for killing from the beginning of the trial was 1 h and 30 min). In 2 cases, after the death of the resident queen, the alien female stood on the top of the dead queen for about 5–10 sec. The attacks performed by the workers against the alien female began to alternate with grooming, and in some hours the *Polyergus* female stood on the host brood surrounded with a court of *Serviformica* workers (average time for adoption was 20 h and 50 min, with a range from 2 h and 30 min to 48 h).

In queenless colonies, the degree of aggression performed by the resident workers against the intruding female seemed to be even higher, but finally in all but one of the trials the *Polyergus* queen was adopted (average time from the beginning of the test was 28 h and 57 min, with a range from 24 h and 15 min to 72 h). However, there is no statistical difference between the adoption times recorded in queenright and queenless colonies (Two-tailed Mann-Whitney *U* test).

During the final stage of take-over, in both queenright and queenless colonies, the usurping queen performed a peculiar behaviour consisting of rapid movements up and down of the gaster, which seemed to attract and appease the workers. Moreover, the *Polyergus* queen stood with her legs fully extended, exposing all surfaces to the grooming *Serviformica* ants (cf. Topoff et al., 1988).

Interaction with F. rufibarbis

When *P. rufescens* queens were introduced into queenright colonies of *F. rufibarbis*, neither take-over nor adoption was recorded (see also Zaayer, 1967), but the alien female was always killed by the *Serviformica* ants by the end of the first 24 h of observation. The aggressive behaviour of the resident workers towards the *Polyergus* female was relentless, so that in all but one of the trials involving queenright colonies, the alien queen was not able to enter the nest and reach the resident queen in the brood chamber. When it succeeded, the *Serviformica* female attacked the intruder, showing gaster flexing and seizing her petiole.

In one (14.3%) queenless colony, the *Polyergus* queen was adopted after 72 h. By contrast, in all other trials of this test, she was fiercely attacked by the resident ants and killed by the end of the first 24 h.

New colony founding

From August 1992, several egg-layings were observed in the artificial nests in which the *Polyergus* female was adopted by the resident *Serviformica* workers. The eggs and subsequently the larvae and the pupae of the slave-maker were cared for by the slaves. In October 1992, the first *P. rufescens* callow workers emerged. They were accepted as homocolonial members by the host and during the winter 1992–1993 we observed a complete social integration between the *Polyergus* and the *Serviformica* workers into the newly-established mixed colonies. The following spring, new egg-layings by the usurping *P. rufescens* queens were observed. Again, the eggs, larvae and cocoons were cared for by the slaves and, after the hatching, the young Amazons were immediately accepted by all the adults present. Nevertheless, during the summer 1993, one of these queens was unexpectedly attacked and killed by the host workers, suggesting the possibility of occurrence of a slaves' emancipation. This behaviour has been previously observed also in the facultative slave-maker *F. sanguinea* (Czechowski, 1990, 1994), though circumstances of the phenomenon and its mechanism were different.

Discussion

Though field observations are not available, these laboratory experiments provide insight into the kind of behaviour which is likely to occur after the penetration of *Serviformica* nest by newly-mated females of *P. rufescens*.

P. rufescens females found new colonies through usurpation, as suggested also by the early observations on the biology of this species (Emery, 1908, 1909, 1911; Forel, 1920; Zaayer, 1967). In both queenright and queenless colonies of *F. cunicularia*, an aggressive take-over with active invasion (Stuart, 1984) occurred. In fact, during this process, not only the resident queen but also many host workers were killed. In particular, after the introduction of alien queen, the colony became alarmed, the resident workers ran around the nest, attacked cooperatively the parasite, and began to abandon the brood chamber in the darkened box. Moreover, the parasitic queen moved quickly and erratically within the host arena and was generally involved in fierce fights with *Serviformica* ants. The usurping queens of *P. rufescens* finally were adopted by a large proportion of resident workers. In this connection, laboratory experiments on *P. breviceps* (Topoff et al., 1988) showed that *Polyergus* females use a pheromone from their enlarged Dufour's gland to reduce aggression by workers before attacking the host queen.

Our results are in agreement with those previously recorded in *P. breviceps*. The only exception is the case of the females introduced into queenless host colonies, since in the American amazon ant the alien female was almost always killed by the resident workers in the absence of the resident queen (Topoff et al., 1988). In this

species, it seems likely that chemical transfer of foreign queen odours plays a key role in colony take-over (Topoff and Zimmerli, 1993). Early experiments on *P. rufescens* showed that fertilized queens were not accepted in orphaned colonies of *F. fusca* if a possibility of flight was present (Zaayer, 1967). In our experiments, the contact of the usurper with the resident queen seems to be not indispensable but only facilitating her acceptance by host workers, as suggested by the shorter time of adoption in queenright colonies. We hypothesize that not only the host queen, but also the workers are the source of the relevant chemicals for the success in colony usurpation and founding. In fact, Yamaoka (1990) demonstrated that *P. samurai* obtains the cuticular hydrocarbon composition from its slave *F. japonica* by continuous contact. This agrees with our observation of *P. rufescens* queen resting on the top of dead workers, during which chemicals of colony odour may be transferred. A gas-chromatographic analysis on surface pheromones and Dufour's gland content could prove this chemical-transfer hypothesis also in *P. rufescens*.

The failure in usurping the *F. rufibarbis* colonies supports the occurrence of host specificity in the European amazon ant, like in the American amazon ants (cf. Hölldobler and Wilson, 1990). Free-living workers of *F. rufibarbis* have been described to be fiercely aggressive towards *P. rufescens* (Habersetzer, 1993; Mori et al., 1994b), and the present results confirm their high level of interspecific aggression. In fact, it seems likely that *F. rufibarbis* workers are more successful than *F. cunicularia* workers in defending their nest and therefore less suitable (Buschinger, 1991). This factor, together with ecological determinants, may favour the choice of *F. cunicularia* as host species by *P. rufescens*. In this connection, in the field we observed scouts of *P. rufescens* being attacked and killed by *F. rufibarbis* workers near their nest, and some raiding swarms rejected (cf. also Forel, 1920; Scortecci, 1972). Moreover, in this slave-maker, host fidelity may be based on a chemically grounded tradition. In fact, if the parasite imprints on the host species present in the dulotic nest (which is also the more available), workers would raid colonies belonging to this species and young queens would try to usurpate colonies of the same species (Goodloe et al., 1987; Schumann and Buschinger, 1994).

Finally, the survival of ants during the artificial over-wintering allow us to obtain permanent mixed colonies, which are the ultimate criterion for colony foundation success of this obligatory slave-maker (Emery, 1911; Bellini, 1982).

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