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## Slave addition increases sexual production of the facultative slave-making ant *Formica subnuda*

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**Abstract** *Formica subnuda* is a facultative slave-making ant, and colonies without slaves are often found. We studied the effect of slave workers on sexual production of *F. subnuda* by experimentally increasing the proportion of slaves. We added c. 4000 worker pupae of the ant *F. podzolica* to 15 *F. subnuda* colonies and kept 15 colonies as controls. The following year we excavated all colonies, counted the proportion of slaves, the total number of workers (colony size) and the number of sexual offspring. The proportion of slaves was significantly higher in the slave-added colonies than in the control colonies. The total production of sexual offspring increased 57% in the treatment colonies in comparison to the controls. When colony size was adjusted to the number of sexual offspring, the treatment colonies produced significantly more sexual offspring than the controls. Slave addition did not alter sex ratios. We suggest that two alternative mechanisms, not mutually exclusive, caused the increase of sexual production in *F. subnuda* colonies: (1) Most of the added pupae were consumed and stored as fat body in workers at the end of the experimental year; the following spring the excess fat was metabolized and fed to the developing sexual larvae, or (2) a proportion of the added pupae hatched to become slaves; the following spring these slaves foraged actively for protein-rich food for the developing sexual offspring.

**Key words** Ants · Slavemaker · Slave-addition · Sexual production

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### Introduction

Slave-making ants raid nests of other ant species, capture the developing offspring, and rear them as slave workers. Slave-making can be either facultative or obligate. Facultative slavemakers are able to forage, nurse their brood and construct their nest like free-living ants, and hence colonies without slaves are common (Wilson 1971). Obligate slavemakers depend on their slaves, and their colonies contain always a high proportion of slaves (Talbot and Kennedy 1940; Marikovsky 1974; Cool-Kwait and Topoff 1984; Savolainen and Deslippe 1996).

*Formica subnuda* is a facultative slave-making ant, and belongs to the *F. sanguinea* group. None of the *F. sanguinea* group members has external morphological modifications for slave-making, but some members have an enlarged Dufour's gland, a special adaptation to slave-making (Hölldobler and Wilson 1990; Wilson and Regnier 1971). *F. subnuda* has a small Dufour's gland, comparable to the size of that of a free-living *Formica* (Savolainen and Deslippe 1996; Wilson and Regnier 1971), and therefore, physical aggression seems to be the only means of gaining pupae from raids on potential slave colonies. Most *F. subnuda* colonies do not contain slaves, but if they do, the proportion of slaves is low (Savolainen and Deslippe 1996).

Here we study the role of slaves in the production of sexual offspring by the facultative slave-making ant *F. subnuda*. We manipulated the proportion of slaves by experimentally adding *F. podzolica* pupae to colonies of *F. subnuda*. We expected *F. subnuda* to accept the pupae, and that our manipulation would result in an increased proportion of slaves the following summer. Because *F. podzolica* slaves are active foragers, we expected that the increase in slaves would enhance the food harvest of the colony, particularly the following spring when sexual offspring are developing in the nest, yielding more sexual offspring in the colonies with slaves added than in the control colonies.

## Methods

We conducted the experiment in Elk Island National Park (53° 37'N, 112° 58'W) in central Alberta, Canada. The slave-making ant *F. subnuda* is relatively common in the park, nesting along forest edges in stumps, under stones and in abandoned earthen mounds. It enslaves *F. podzolica* of the *F. fusca* group which is abundant throughout the park (Deslippe and Savolainen 1994), and there are approximately ten *F. podzolica* colonies for every *F. subnuda* colony (Savolainen and Deslippe 1996).

In May and June 1993 we selected 30 moderate-sized colonies of *F. subnuda* which contained developing sexual offspring, and which were in earthen mounds, to facilitate excavation the following year. Because the colonies were located over a large area, we assigned them equally as treatments or controls in different sites. Before the experiment colony size could be assessed only superficially, because disturbance may cause *F. subnuda* to relocate its colony. In ants colony size correlates positively with sexual production (Hölldobler and Wilson 1990), and hence, if there was any noticeable difference between two colonies to be allotted as a treatment and control colony, the smaller one was assigned as a treatment colony to make our test conservative.

In August 1993 we added pupae of *F. podzolica* workers to the 15 treatment colonies. We took all pupae of the experiment from one large *F. podzolica* mound (c. 3.6 × 2.0 m in area). We counted 15 sets of about 1000 pupae and poured them on top of the *F. subnuda* nests, on warm and dry days. We repeated this during 4 consecutive weeks, and hence all treatment colonies received 3400–4200 *F. podzolica* pupae. *F. subnuda* workers always carried all pupae into their nest immediately. Later in September slaves were seen foraging around the nest.

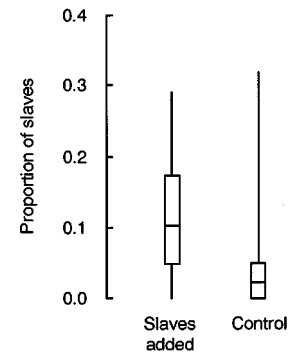
In July 1994 we excavated all 30 colonies. We dug up each nest into a bucket, mixed the nest material thoroughly and took a subsample of it and counted approximately 500 individuals noting the numbers of slavemakers and their slaves. We then counted the number of individuals in the remaining nest material for colony size, and collected all hatched sexuals and pupae of sexuals, and determined their sex. Because worker offspring are produced later than sexual offspring, we did not measure worker production of the colonies.

## Results

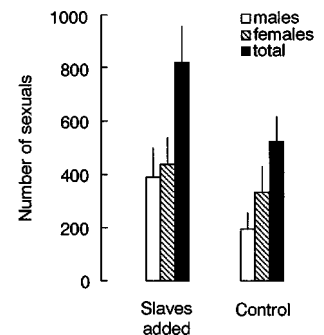
The proportion of slaves was significantly higher in the treatment colonies than in the controls (Fig. 1). Extrapolation of total slave numbers by the proportion of slaves and colony size gave a median estimate of 333 (range 0–1037) and 71 (range 0–640) slaves in the treatment and control colonies, respectively. The treatment colonies had on average 3700 ± 395 (mean ± SE) individuals and the control colonies 3522 ± 407 (mean ± SE) individuals; colony size did not differ significantly between the groups ( $t = 0.29$ ,  $df = 29$ ,  $P = 0.77$ ).

All colonies produced sexual offspring. Sexual production increased 57% in the treatment colonies (a total of 12357 sexual offspring produced) in comparison to the control colonies (7869 sexuals produced). When the number of sexual offspring was adjusted for colony size, slave-added colonies had significantly more sexuals than the controls (ANCOVA  $F = 4.37$ ,  $df = 1, 27$ ,  $P = 0.046$ ; one-tailed test, as the only expected change after adding food or slaves is an increase of sexuals) (Fig. 2). The total investment of treatment colonies was higher than that of the control colonies

**Fig. 1** Proportion of slaves (median, quartiles, minimum and maximum) of the slave-added and control colonies (Kruskal-Wallis,  $P = 0.015$ ,  $df = 1$ ,  $n = 15$  for both groups)



**Fig. 2** Total numbers + SE of males (white bars) females (shaded bars) and males and females combined (black bars) of the colonies with slaves added and control colonies



(ANCOVA  $F = 4.06$ ,  $df = 1, 27$ ,  $P = 0.054$ ; one-tailed test) when 20.01 ± 0.27 mg (mean ± SE) and 17.69 ± 0.42 mg was used for females and males, respectively. The mean fresh weight of females ( $t = 0.16$ ,  $df = 19$ ,  $P = 0.86$ ) and males ( $t = 0.73$ ,  $df = 18$ ,  $P = 0.46$ ) did not differ between the treatment and control colonies. The mean sex ratios (proportion of females from the total of females and males) for treatment and control colonies were 0.49 ± 0.09 (mean ± SE) and 0.54 ± 0.08, respectively.

## Discussion

There are two alternative explanations, not mutually exclusive, for the increase of sexual production in the slave-added colonies.

1. The added pupae were consumed by the workers and stored as fat body at the end of the experimental year. The following spring the excess fat body was metabolized and fed to the developing sexual larvae.

2. A proportion of the added pupae was allowed to hatch to become slaves. The following spring these slaves foraged actively for protein-rich food for the developing sexual offspring.

In the following we discuss these two factors as potential mechanisms for the increased production of sexual offspring in the treatment colonies.

In ants a link between food resources and reproductive parameters is well documented. In general, small colonies produce fewer sexual offspring than do

large ones. With increasing worker number colonies are expected to harvest more food, and hence to produce more sexual offspring (Wilson 1971). In mature colonies the number of sexual offspring depends on food resources: in habitats with low food supply, owing to either poor habitat quality or competition for food, colonies produce less sexual offspring than in habitats with high food supply (Boomsma et al. 1982; Savolainen 1990; Deslippe and Savolainen 1994). Also sex allocation is sensitive to food resources; when food is abundant more diploid eggs become female sexuals than in situations where food is limiting (Boomsma et al. 1982; Brian 1979; Deslippe and Savolainen 1995).

It has been shown experimentally that supplementation of protein-rich food increases production of sexual offspring within one reproductive season (Backus and Herbers 1992; Deslippe and Savolainen 1994). Furthermore, if the food supplementation is superabundant, proportionately more female offspring are produced in comparison to the control colonies without extra food (Deslippe and Savolainen 1995). Thus, ants are capable of assessing their food resources and will direct extra resources to the developing offspring within one reproductive season. The situation becomes complicated, if the extra food is given at the end of one reproductive season, but the production of sexual offspring is studied the following season.

Wood ants of the European *F. rufa* group are known to store carbohydrates, protein and fat in their body during the autumn, and metabolize them in the spring after the winter dormancy (Kirchner 1964). Young workers, in particular, have large reserves of fat or lipids in their body between September and April. As the young workers mainly stay within the nest nursing the developing offspring, the main function of their fat reserves is, according to Kirchner (1964), to feed the developing sexual larvae.

It is known that both facultative and obligate slave-making ants eat large amounts of pupae (Cool-Kwait and Topoff 1984; Czechowski 1994). For example, Czechowski (1994) added 20 000 pupae of the typical slave species, *F. fusca*, to one *F. sanguinea* colony (size unknown), which resulted in only 20% of slaves the following spring. Our experiment shows that a large proportion of the added pupae were presumably consumed, as the median estimate of slaves for the experimental colonies was only about 300, although all experimental colonies received nearly 4000 *F. podzolica* pupae the previous August. Consequently, it is possible that *F. subnuda* workers stored part of the added pupae as fat reserves.

However, the reproductive cycle of *F. subnuda* in Elk Island National Park differs from that of wood ants. Because wood ants lay their sexual eggs in early spring (Collingwood 1979), their fat body may be the sole food resource for their sexual larvae for quite some time. The egg masses of *F. subnuda*, however, appear later

than those of the wood ants, in the middle of May (R. Savolainen, unpublished work). Nevertheless, *F. subnuda* workers are already active in April, and therefore the workers inside the nest may have metabolized a large proportion of their fat reserves by the time the sexual eggs are laid. Thus, the sexual offspring would depend on the foragers for food. In mid-May insect prey is plentiful in Elk Island (Deslippe and Savolainen 1994), and foraging ants bring actively various kinds of prey to their nest (R. Savolainen and R.J. Deslippe, unpublished work).

Because the treatment colonies had a significantly higher proportion of slaves, their foraging activity could explain the increased sexual production in the treatment colonies. This is supported by our findings on foraging *F. subnuda* and their *F. podzolica* slaves: in early May a few slaves (2–14% of the total colony workforce) brought to the nest more prey items per unit time than did their enslavers (R. Savolainen and R.J. Deslippe, unpublished). Similar results were obtained for tasks related to nest construction in *F. sanguinea*, a slave-making ant almost identical to *F. subnuda* with respect to morphology, ecology and behavior (Creighton 1950; Savolainen and Deslippe 1996). In mixed trials *F. sanguinea* excavated sand clearly less than its *F. fusca* slaves, and an increase of *F. fusca* slaves from one to eight individuals speeded up the excavation 5 times, while a similar increase of slavemakers did not notably affect the excavation rate (Sakagami and Hayashida 1962).

Our experiment is not able to distinguish between the two plausible mechanisms in explaining the increase of sexual production in the treatment colonies. It is possible that both mechanisms have acted in concert. But it seems that the slaves of the *F. fusca* group are particularly suitable slave workers and foragers, and seem to function as an extra caste within the slave-maker colony, as suggested by Wilson (1955).

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## References

- Backus VL, Herbers JM (1992) Sexual allocation ratios in forest ants: food limitation does not explain observed patterns. *Behav Ecol Sociobiol* 30:425–429
- Boomsma JJ, Van der Lee GA, Van der Have TM (1982) On the production ecology of *Lasius niger* (Hymenoptera: Formicidae) in successive coastal dunes. *J Anim Ecol* 51:975–991
- Brian MV (1979) Habitat differences in sexual production by two co-existent ants. *J Anim Ecol* 48:943–953
- Collingwood C (1979) The Formicidae (Hymenoptera) of Fennoscandia and Denmark. *Fauna Entomol Scandinavica* 8:1–174

- Cool-Kwait E, Topoff H (1984) Raid organization and behavioral development in the slave-making ant *Polyergus lucidus* Mayr. *Insectes Soc* 31:361–374
- Creighton WS (1950) The ants of North America. *Bull Mus Comp Zool* 104:1–585
- Czechowski W (1994) Emancipation of slaves in *Formica sanguinea* Latr. colonies (Hymenoptera, Formicidae). *Ann Zool* 45:15–26
- Deslippe RJ, Savolainen R (1994) Role of food supply in structuring a population of *Formica* ants. *J Anim Ecol* 63:756–764
- Deslippe RJ, Savolainen R (1995) Sex investment in a social insect: the proximate role of food. *Ecology* 76:375–382
- Hölldobler B, Wilson EO (1990) *The ants*. Springer, Berlin Heidelberg New York
- Kirchner W (1964) Jahreszyklische Untersuchungen zur Reservestoffspeicherung und Überlebensfähigkeit adulter Waldameisenarbeiterinnen (Gen. *Formica*, Hym. Formicidae). *Zool Jahrb, Abt Allg Zool Physiol Tiere* 71:1–72
- Marikovskiy PI (1974) The biology of the ant *Rossomyrmex proformicarum* K.W. Arnoldi (1928). *Insectes Soc* 21:301–308
- Sakagami SF, Hayashida K (1962) Work efficiency in heterospecific ant groups composed of hosts and their labour parasites. *Anim Behav* 10:96–104
- Savolainen R (1990) Colony success of the submissive ant *Formica fusca* within territories of the dominant *Formica polyctena*. *Ecol Entomol* 15:79–85
- Savolainen R, Deslippe RJ (1996) Facultative and obligate slavery in formicine ants: frequency of slavery, and proportion and size of slaves. *Biol J Linn Soc* (in press)
- Talbot M, Kennedy CH (1940) The slave-making ant *Formica sanguinea subintegra* Emery, its raids, nuptial flights and nest structure. *Ann Entomol Soc Am* 33:560–577
- Wilson EO (1955) Division of labor in a nest of the slave-making ant *Formica wheeleri* Creighton. *Psyche* 62:130–133
- Wilson EO (1971) *The insect societies*. Harvard University Press, Cambridge
- Wilson EO, Regnier FE (1971) The evolution of the alarm-defense system in the Formicine ants. *Am Nat* 105:279–289

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