Lack of Plasticity in the Behavior of Queens of the Ant *Leptothorax rugatulus* Emery (*Formicidae: Hymenoptera*)

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INTRODUCTION

Eusociality has evolved independently several times (Hölldobler and Wilson, 1990; Choe and Crespi,1997). Most cases are known from insects, and over 10,000 species of ants are exclusively eusocial, with the exception of some social parasites (Hölldobler and Wilson,1990). In ants, eusociality involves in most cases a pronounced morphological and behavioral differentiation between queens, the female reproductive caste, and the nonreproductive, female workers. In an established colony, queens concentrate solely on egg- laying, while workers perform all other necessary tasks, most importantly brood care and foraging. However, the majority of ant queens perform worker tasks in the early stages of colony establishment when they raise their first brood (Hölldobler and Wilson, 1990). Thus, they exhibit a full behavioral repertory early in their life cycle, but later they specialize in egg- laying and abandon most other behavioral tasks.

In many ancestral ant species workers can take over the queen role and adopt a respective behavioral profile (e.g., Liebig et al., 1998), and in some species queens have been evolutionary lost altogether (Peeters, 1993). Among more derived ant species, similar transitions are not the rule. However, in a considerable number of species some workers in queenles colonies start egg-laying (by arrhenotokous parthenogenesis), accompanied by changes in their overall behavior (Bourke, 1988; Choe,1988). Thus, functional caste changes from sterile worker role to reproductive queen role are widespread in ants. This behavioral plasticity in workers is probably maintained by opportunistic fitness gains when queens are lost (Bourke, 1988; Liebig et al.,1998).

In contrast, almost nothing is known about whether established queens are plastic enough in their behavior to take over worker tasks when needed. Queens are shielded by workers from most sources of mortality (e.g., predators, starvation). Consequently a relative queen surplus may arise after adverse conditions, which would be survived by most queens but few workers. This is particularly true for polygynous ant species, where often a large number ofqueens share one colony. While a relative queen surplus leads to queen excecution in extreme cases (Keller et al.,1989), excess queens could increase their fitness (and probably avoid excecution) by

performing worker tasks. Some reports about unfertilized queens that remain in the colony without reproducing and engage in worker tasks such as brood care (Liebig, personal communication; Ortius, 1997) indicate that the behavioral repertory of queens is not invariably reduced to egg-laying with age. In this study, we addressed the question whether mature, reproductively active queens expand their behavioral repertoire to worker tasks when necessary.

MATERIALS AND METHODS

The Formicoxenine ant *Leptothorax rugatulus* (Emery) is distributed widely in North America and it is characterized by alternative reproductive tactics (Rüppell et al., 2001): Readoption and independent colony foundation usually coexist in any population. One population was discovered that contained some colonies with exceptionally high numbers of queens per worker (Table I). Colonies were found in crevices of rocky outcrops on an unprotected mountain top in the Chiricahua Mountains (Cochise County, Arizona) ata 2540-m altitude. Among locations in which we found *Leptothorax rugatulus*, this habitat was most exposed to environmental conditions. Thus, the queen surplus could have arisen through worker mortality due to adverse weather conditions. Regardless of the ultimate reason for the relative worker shortage in these colonies, they constitute a natural situation in which mature queens would be expected to display an extended behavioral repertoire if their behavior was plastic enough. The queen-to-worker ratio in some of these colonies was clearly adverse to maximum colony productivity (Oster and Wilson, 1978), unless queens partly take over worker duties, such as brood care, nest maintenance, and foraging. Therefore, we investigated, by behavioral observations in the laboratory, whether queens

Table I. Collection Data on our Focal Population (Chiricahua I) Compared to Three Other Populations of *L. rugatulus*

	Chiricahua I	Manzano	Magdalena	Chiricahua II
Colonies with queen-to-worker ratios				
>1:1	5.1%	0%	1.8%	0%
>1:2	3.1%	3.3%	0%	0%
>1:5	11.2%	6.7%	0%	0%
>1:10	23.5%	16.7%	3.5%	0%
≤1:10	57.1%	73.3%	94.7%	100%
No. of colonies collected[a]	98	60	57	67
Average No. of queens per colony ± SD	5.6 ± 7.0	6.1 ± 6.9	3.2 ± 5.7	1.3 ± 0.8
Average No. of workers per colony ± SD	52.3 ± 45.6	88.8 ± 113.9	111.4 ± 109.5	62.8 ± 35.0

[a] Queenless colonies were excluded from the analysis.

in these particular colonies performed more brood caring, nest maintenance, or foraging or differed otherwise in their behavioral repertoire from queens in colonies with a more well-balanced queen/worker ratio.

Overall, 282 colonies with at least one queen were collected in July and August from four populations (Table I). Colonies were brought to the laboratory alive, and their worker and queen numbers determined immediately. To amplify the necessity for queens to engage in worker tasks, we removed the few existing workers from the six colonies with the naturally highest queen/worker ratios 1 month prior to our behavioral investigations. Another six colonies with similar queen numbers but at least six times more workers than queens (Table II) were chosen as controls and kept under identical

Table II. Composition of the Observation Colonies upon Collection (Before Removal of the Workers from the Treatment Group)

Group	No. of workers	No. of queens
Treatment		
Colony 1	0	16
Colony 2	4	21
Colony 3	7	12
Colony 4	9	16
Colony 5	12	13
Colony 6	62	35
Control		
Colony 1	63	7
Colony 2	69	10
Colony 3	145	12
Colony 4	128	10
Colony 5	129	11
Colony 6	202	34

standard laboratory conditions (Buschinger,1974). A behavioralcatalog was determined by 20 h of ad libitum sampling in several colonies (Martin and Bateson, 1988) 2 weeks prior to the experimental observations. This catalog was complemented from the literature to be congruent with published behavioral catalogs of Leptothorax ants (Wilson and Fagen, 1974; Herbers and Cunningham, 1983; Cole, 1986). Initially, the duration of occurrence of 21 behaviors (inactivity, movement in the nest, antennate queen, antennateworker, antennate brood, grooming queen, grooming worker, grooming brood, self-grooming, food requesting, trophallaxis, avoiding queen, avoiding worker, brood transport, transport of nest material, oviposition, defecation, cannibalism of brood, fighting, entrance guarding, and foraging) was recorded by direct observation. Behaviors that were difficult to distinguish or functionally similar were combined for statistical analyses, resulting in 12 behavioral classes (Fig. 1). From each colony, six queens were selected at random and the behavior of each was observed for approximately 30 (range, 27–43) min. This observation schedule was repeated once by direct observation and once from video recordings, resulting in an average total observation time of 566 min per colony. As queens were not individually marked, repeated observations of identical queens could not be excluded.
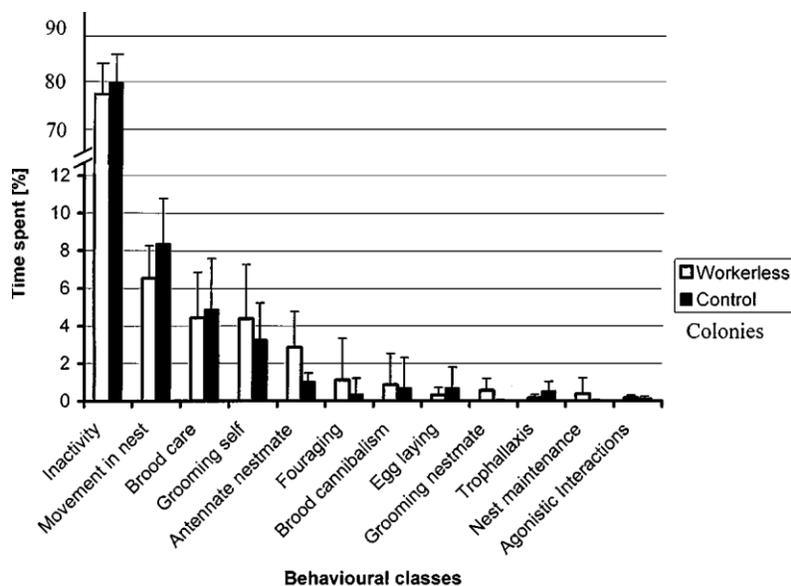
Fig. 1. Behavioral repertoires of queens kept without workers and with workers (control). Behavioral acts are summarized in behavioural classes which are self-explanatory and/or defined in the literature (Hölldolber and Wilson, 1990).

Consequently, colonies and not individual queens were analyzed as independent data points. However, results did not change significantly when the analyses were performed on individual queen observations.

RESULTS
The behavioral repertoire of queens in colonies with workers and of queens without workers was similar (Fig. 1). No significant difference after Bonferroni correction was found with respect to any single behavioral class (evaluated by t-tests on angular-transformed values: t ranged between 0.32 and 3.29, df =10, P ranged between 0.008 and 0.752 without Bonferroni correction). No increased foraging activity, nest maintenance, or brood care was observed in workerless queen groups. Furthermore, increased competition among queens for decreasing colony resources was not apparent because agonistic behavior among queens was similar in colonies with and without workers. The most pronounced difference (marginally significant, with a P value of 0.096 after Bonferroni correction) was that queens in workerless groups groomed each other, whereas queens in colonies with workers did not groom any nestmates.

For multivariate evaluation, three principal components were derived from the 12 behavioral classes and checked for differences between groups. The first principal component correlated most strongly with self-grooming (r = 0.87) and inactivity (r = —0.85), the second with brood care (r = 0.85) and trophallaxis (r = —0.71), and the third with movement (r = 0.82) and egg laying (r = 0.79). No significant differences in scores of any of those principal components existed between groups.

Thus, queens did not change their behavior and performed worker tasks. In contrast, a single *Leptothorax rugatulus* founding queen (observed over 3 weeks for a total of 344 min), which successfully raised her initial brood in the laboratory, spent significantly more time outside the colony [11.5%; t(11) = 3.69, P = 0.004] and cared more for her brood, although the latter difference was not significant [10.0%; t(11) = 1.76, P = 0.112].

The fact that queens failed to fulfil worker tasks in the workerless colonies was apparent also from additional observations. In three of the six experimental workerless colonies, the brood started to mold over the course of 6 months in the laboratory, which never happened in "regular" colonies. Queen mortality in the workerless colonies was also elevated (21.2% per year, compared to an overall laboratory average of 13.8% per year). The increased mortality could be caused by indirect factors, such as disease and starvation, because no increased aggression in the workerless queen groups was observed.

DISCUSSION

Our results suggest that established, reproducing *Leptothorax rugatulus* queens, and perhaps ant queens in general, lack the behavioral plasticity to expand their behavioral repertoire and fulfill worker tasks, even in situations that would clearly favor such adjustments. This lack of flexibility contrasts with the plasticity of worker behavior and with functional caste changes in eusocial wasps, where both castes display considerable behavioral plasticity (e.g., Mead and Gabouriaut,1993).

It has been repeatedly reported that uninseminated queens start egg laying or maintain their nonreproductive state as a plastic response to their social environment (e.g., Passera and Aron, 1993). Queens in functionally monogynous species assume a reproductive role only as the dominant queen (e.g., Ortius and Heinze, 1999). Furthermore, when ant queens cooperate during colony foundation, a division of labor among founding queens commonly arises (Rissing et al.,1989; Trunzer et al.,1998). Thus, queen behavior in ants shows considerable plasticity in many circumstances. Flexible behavioral strategies are especially expected in changing environments (Foster, 1999), such as the versatile social environment of insect colonies.

The occurrence of the first workers in a colony probably results in major hormonal or physiological changes in the queens that are irreversible, although this has not yet been investigated in any ant species. However, it is known that the presence of workers radically alters the behavior of co- founding queens from cooperation to lethal fighting (e.g., Bernasconi and Keller, 1996; for review see Hölldobler and Wilson, 1990). A reduction of the central nervous tissue has been reported in worker ants that become active reproductives (Gronenberg and Liebig, 1999) and it will be interesting to investigate whether similar changes might account for the decreased behavioral repertory of ant queens after colony establishment.

Whatever its proximate mechanism, the low plasticity in queen behavior ultimately limits the evolutionary prospects of reverse social evolution (Gadagkar,1997) and may help explain why all ants, except social parasites, are eusocial. The circumstances in which it would be selectively advantageous for ant queens to assume worker roles may be rare and thus the overall selection pressure to maintain behavioral flexibility should be weak. On the other hand, queens might be caught in a prisoner's dilemma: a single queen assuming worker behavior may not benefit the colony's productivity enough to outweigh her loss in direct fitness, even though cooperation by all queens would enhance the colony productivity significantly. The plasticity in worker behavior, particularly in species with a low queen/worker dimorphism (Peeters, 1993), suggests that reverse social evolution would occur more likely via solitary, omnipotent workers than queens.

Two further, general conclusions emerge from this study. First, studies on behavioral mechanisms and constraints are important for understanding social evolution and they should be emphasized in future research programs. Second, intraspecific phenotypic plasticity of behavior not only is determined by genotype (Foster, 1999) but also maybe constrained by the physiological state of individuals.

REFERENCES
Bernasconi, G., and Keller, L. (1996). Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). *Proc. R. Soc. Lond. B* **263:** 509–513.
Bourke, A. F. G. (1988). Worker reproduction in the higher eusocial Hymenoptera. *Q. Rev. Biol*. **63:**291–311.
Buschinger, A. (1974). Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameisen *Harpagoxenus sublaevis* (Nyl.).*Insectes Soc*. **21:** 381 –406.
Choe, J. C. (1988). Worker reproduction and social evolution in ants (Hymenoptera: Formicidae). In Trager, J. C. (ed.), *Advances in Myrmecology*, E. J. Brill, Leiden, pp. 163–187.
Choe, J. C., and Crespi, B. J. (1997). *Social Behavior in Insects and Arachnids*, Cambridge University Press, Cambridge.
Cole, B. J. (1986). The social behaviour of *Leptothorax allardycei* (Hymenoptera, Formicidae): Time budgets and the evolution of worker reproduction. *Behav. Ecol. Sociobiol*.**18:**165– 173.
Foster, S. A. (1999). The geography of behaviour: an evolutionary perspective. *Trends Ecol. Evol*.**14:**190–195.
Gadagkar, R. (1997). Social evolution—Has nature ever rewound the tape? *Curr. Sci*. **72:** 950–956.
Gronenberg, W., and Liebig, J. (1999). Smaller brains and optic lobes in reproductive workers of the ant *Harpegnathos. Naturwissenschaften* **86:** 343–345.
Herbers, J. M., and Cunningham, M. (1983). Social organization of *Leptothorax longispinosus* Mayr. *Anim. Behav*. **31:** 759–771.
Hölldobler, B., and Wilson, E. O. (1990). *The Ants*, Belknap Press, Harvard, Cambridge, MA. Keller, L., Pallera, L., and Suzzoni, J.-P. (1989). Queen excecution in the argentine ant, *Iridomyrmex humilis. Physiol. Entomol*.**14:**157–163.
Liebig, J., Hölldobler, B., and Peeters, C. (1998). Are ant workers capable of colony foundation? *Naturwissenschaften* **85:**133–135.
Martin, P., and Bateson, P. (1988). *Measuring Behaviour—An Introductory Guide*, Cambridge University Press, Cambridge.
Mead, F., and Gabouriaut, D. (1993). Post-eclosion sensitivityto social context in *Polistes dominulus* Christ females (Hymenoptera, Vespidae). *Insectes Soc*. **40:**11–20.

Ortius, D. (1997). *Einfluss von reproduktiver Dominanz auf die soziogenetische Kolonie- und Populationsstrukturvon* Leptothorax *sp. A*, Ph.D. dissertation, Tectum Verlag, Marburg.

Oster, G. F., and Wilson, E. O. (1978). *Caste and Ecology in SocialInsects*, Princeton University Press, Princeton, NJ.

Passera, L., and Aron, S. (1993). Factors controlling dealation and egg laying in virgin queens of the argentine ant *Linepithema humile* (Mayr) (=*Iridomyrmex humilis*). *Psyche* **100:**51 –63.

Peeters, C. (1993). Monogyny and polygyny in ponerine ants with or without queens. In Keller, L. (ed.), *Queen Number and Sociality in Insects*, Oxford University Press, Oxford, pp. 234–261.

Rissing, S. W., Pollack, G. B., Higgins, M. R., Hagen, R. H., and Smith, D. R. (1989). Foraging specialization without relatedness or dominance among co-founding ant queens. *Nature* **338:** 420–422.

Rüppell, O., Heinze, J., and Hölldobler B. (2001). Alternative reproductive tactics in the queen-size-dimorphic ant *Leptothorax rugatulus* (Emery) and their consequences for genetic population structure. *Behav. Ecol. Sociobiol*. **50:**189–197.

Trunzer, B., Heinze, J., and Hölldobler, B. (1998). Cooperative colony founding and experimental primary polygyny in the ponerine ant *Pachycondyla villosa. Insectes Soc*. **45:**267–276.

Wilson, E. O., and Fagen, R. M. (1974). On the estimation of total behavioral repertories in ants. *J. N.Y. Entomol. Soc*. **82:**106–112.