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Evolution

Reproductive cooperation between queens and their mated workers: The complex life history of an ant with a valuable nest

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ABSTRACT **The life history of *Harpegnathos saltator* is exceptional among ants because both queens and workers reproduce sexually. Recently mated queens start new colonies alone, but later some of the offspring workers also become inseminated and take over the egg-laying role. This alternation seems associated with the existence of very complex underground nests, which are designed to survive floods. Longevity of ponerine queens is low (a consequence of limited caste dimorphism in this "primitive" subfamily), and upon the death of an *H. saltator* foundress, the nest represents a substantial investment. The queen's progeny should thus be strongly selected to retain the valuable nests. Unlike the flying queens, the workers copulate with males from their own colonies, and, thus, their offspring are expected to be highly related to the foundress. Colony fission appears not to occur because a daughter fragment would lack an adequate nest for protection. Thus, the annual production of queens in colonies with reproductive workers remains essential for the establishment of new colonies. This contrasts with various other ponerine species in which the queens no longer exist.**

The fundamental feature of insect societies is the inability of a majority of their members to reproduce sexually. In ants particularly, reproductives and helpers are morphologically specialized for the performance of their contrasting roles (queens and workers, respectively). Nonetheless, individual ant workers commonly compete with the queen(s) and each other over various aspects of reproduction. However, they generally have only a limited number of options to increase their fitness: (*i*) production of males, (*ii*) control of queen differentiation, and (*iii*) control of sex allocation (1–6). Intracolonial conflict takes on a different dimension in various species of the morphologically "primitive" subfamily Ponerinae, where workers are able to mate and thus produce diploid offspring (7). This is possible because, in contrast to ants in other subfamilies, workers in some of the Ponerinae have retained a functional sperm receptacle. Thus, one or several "gamergates" [this functional term emphasizes that inseminated reproductives do not necessarily belong to the queen caste (8)] occur in the colonies of some ponerine species, and queens no longer exist (9). Queenless colonies reproduce exclusively by fission (an existing colony divides and produces two autonomous units) since, unlike queens, gamergates lack the necessary opportunities to establish new colonies independently (7). In ponerine species, founding queens need to hunt outside their nest to feed the first generation of workers (9, 10). In contrast, gamergates exhibit a limited behavioral repertoire, and they never forage outside.

Harpegnathos saltator is one of the few ponerine ants known in which queens occur together with gamergates. It affords a unique opportunity to contrast the adaptive benefits of two morphologically distinct types of reproductives. We suggest that the life history of *H. saltator* is based on an exceptional form of cooperation between queens and their worker offspring—although gamergates are able to continue reproduction in an established colony, queens are necessary to start new colonies.

Table 1. Worker populations and reproductive status in 15 colonies of *H. saltator*

Colony code	Colony size	Workers dissected	Mated workers	Gamergates
RE-1	24	19	12	3
RE-2	74	69	25[*]	14
RE-3	31 + Q[†]	23	2	0
RE-4	72 + Q[†]	72	0	0
RE-5	67	55	7	3[‡]
RE-6	32 + Q[†]	26	11	5
RF-1	78 + Q[†]	74	40	0
RF-2	33 + Q[†]	26	12	0
RF-5	36 + Q[†]	33	23	5
RF-6	33 + Q[†]	22	5	0
RH-1	182	170	56	9[‡]
RL-15	66 + Q[†]	66	36	0
RB-3	>300	73	25[*]	12[‡]
RB-4	302	88	5	2
RB-5	525	49	9	5[‡]

All living individuals in 12 colonies were dissected, while in 3 large colonies (RB series, from Bangalore) only a sample of the workers was dissected. Collection dates are given in Table 2. The occurrence of maturing yolky oocytes in the ovaries was used to determine which of the mated workers were gamergates.

[*] Four young workers mated in the laboratory.

[†] Q indicates the occurrence of a mated fertile queen.

[‡] Many dark yellow bodies present in most gamergates.

MATERIAL AND METHODS

We excavated 44 colonies of *H. saltator* in Karnataka State, southern India (11), during four trips in the period 1991–1994. The physical structure of nests, with all inhabited chambers close together and near the soil surface, made it possible to collect all adults and brood. The average colony size in Mudigere and Jog Falls was 51 ± 23 workers (n = 34). Reproductive activity was determined by dissecting the ovaries and sperm receptacles of 865 workers belonging to 15 colonies (Table 1), as well as those of 30 dealate queens. We recorded the presence of yellow bodies, which are the remains of nurse cells deposited at the base of the ovarioles whenever an egg is laid.

RESULTS

Dealate queens were found in 28 of the 44 colonies that were excavated. They were dissected in 22 colonies, and there was always only one inseminated queen with active ovaries and many dark yellow bodies, except in colony RM-2, where two queens were inseminated and had dark yellow bodies.

Table 2. Production of sexuals in 11 colonies collected at the end of May 1992 (RE series) and the beginning of June 1993 (RF and RH series)

Colony code	Alate queens	Males
RE-1	8	7
RE-2	22	33
RE-3*	24	15
RE-4*	9	0
RE-5	19	72
RE-6*	7	32
RF-1*	12	15
RF-2*	1	10
RF-5*	2	1
RF-6*	0	0
RH-1	16	57
Total	79	159

Counts include adults and pupae (483 cocoons were opened and sexed). June data are incomplete because the departure of sexuals had already started (following the first pre-monsoon showers). There were no sexuals in 10 colonies collected during October 1991 (RB series). *Founding queen still present.

A dealate queen was the sole egg-layer in 6 colonies (RE-3, RE-4, RF-1, RF-2, RF-6, RL-15) of the 15 colonies in which the ovarian activity of workers was determined, while gamergates reproduced as well in two other queenright colonies (Table 1). However, these particular gamergates lacked dark yellow bodies, suggesting that they had only recently started to oviposit. In the seven remaining colonies, gamergates reproduced exclusively. They had many dark yellow bodies in four colonies (Table 1), indicating that they had been laying eggs for some time already. Three to 14 gamergates were found per colony. Their fecundity (0.5–1 egg per day) is about half that of the queens (C.P., J. Liebig, and B.H., unpublished data).

In total, inseminated workers were found in all but one (RE-4) of the 15 colonies that were dissected (Table 1). The proportion of mated workers varied considerably among colonies (from 9% in RE-3 to 70% in RF-5). However, only a few of these mated workers laid eggs—i.e., were gamergates—and none oviposited in five queenright colonies.

Field data indicate that sexuals are active only during a few weeks of the year in May and June (Table 2). Even the small colonies produced both winged queens and males. We never succeeded in observing mating activity in the field, despite being present during the appropriate season. Five dealate and inseminated queens were collected on the ground in June 1994 and later laid eggs and produced workers in the laboratory (C.P., J. Liebig, and B.H., unpublished data), which confirms that newly mated queens shed their wings and establish new colonies without the help of workers.

Our field and laboratory results reveal that there are different stages in the ontogeny of colonies in *H. saltator* (Fig. 1). Colonies are founded by queens, but the latter are eventually replaced by gamergates. Dissection data suggest that young workers mate during the first period of sexual activity following a colony's initiation, even when the founding queen is still actively laying eggs. In the laboratory, young workers copulated successfully with males produced in their own colonies, and we expect this to occur in nature also. While the queen is present, however, workers usually do not have active ovaries. The occurrence of both the founding queen and gamergates in two colonies confirms that colonies with gamergates were originally queenright, and it suggests that once the queen's fecundity decreases (presumably with age), workers are no longer inhibited. Such declining queens soon die.

DISCUSSION

The key element underlying the occurrence of both queens and gamergates in *H. saltator* is the exceptionally complex, waterproof nests, which ensure survival in a flood-prone habitat (11). The nest of an average-sized colony consists of a vaulted, impermeable earthen shell, which encloses a few superimposed chambers. This shell is separated from the surrounding soil by an empty space, which connects to another cavity deeper in the

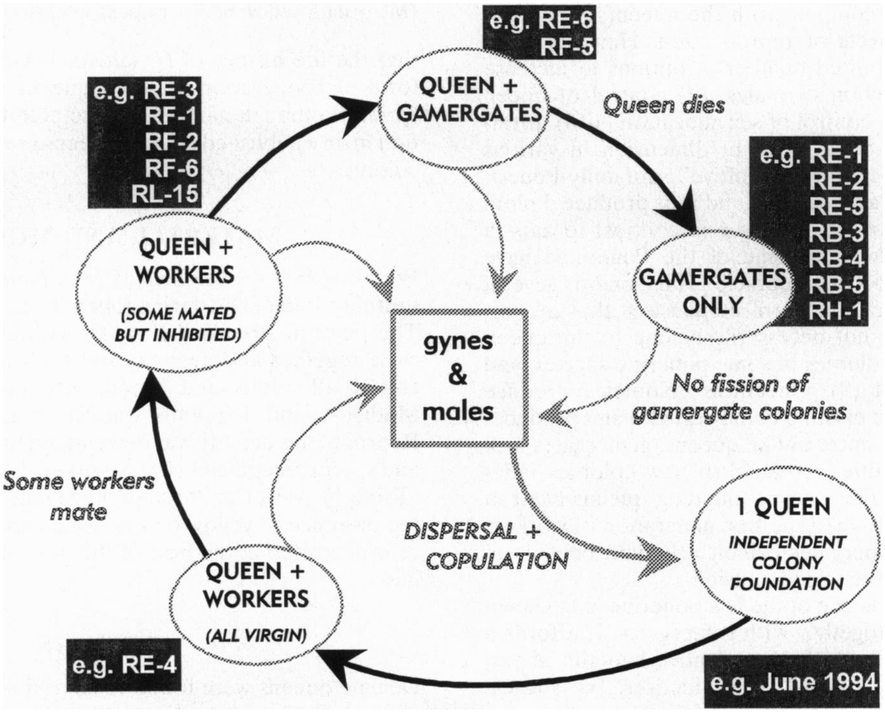

FIG. 1. Life cycle of *H. saltator* reconstructed from field data and dissections of ovaries and sperm receptacles. The different transitional stages are illustrated by colonies (see Table 1) in which the reproductive status of all members was known. Sexuals are only active for a short period of the year. Males mate with workers from their own colonies, or they fly out and presumably search for queens.

soil; the latter is usually filled with prey remains but may also serve to evacuate run-off water (11). Thus, *H. saltator* creates a highly valuable and persistent microhabitat, in contrast to most other Ponerinae, which have simple nests and readily move from one nest site to another (12). This has several consequences, which may explain (*i*) why gamergates replace the founding queen and (*ii*) why gamergate colonies continue to produce new queens every year.

Founding queens in ants sometimes live for many years (13). Although we have no direct observations on queen longevity in *H. saltator*, our demographic data suggest that they die relatively young (2–3 years?). Short life-spans seem associated with the limited queen–worker dimorphism typical of the Ponerinae and are not unique to *H. saltator.* By the time the fecundity of a founding queen decreases, her nest represents a substantial investment in labor, and her lineage would be selected to retain it. The foundress is thus succeeded by several gamergates whose offspring are likely to be highly related to her. Indeed, not only do workers inbreed within the colony, but the males, as well as the workers, are produced by the founding queen (virgin workers with active ovaries were never found). In this situation, selection against letting foreign genes enter the colony is expected. Similarly, many termites exhibit a regular alternation between inbreeding and outbreeding (14), which corresponds also to periods of colony growth and dispersal.

In queenless ponerine ants, fission is the obligate means of colony reproduction (7, 9). Few details are known about the mechanisms of fission, but it probably results from fragmentation of a colony during above ground emigration. Nest relocation is frequent in many ants (13). In *H. saltator*, the existence of a specialized nest appears essential for colony survival: since a newly emigrated colony would lack an adequate nest for protection, such emigration is unlikely. Furthermore, the elaborate nests represent valuable "estates" which should not be readily abandoned. Accordingly, we expect that colony fission does not occur in this species. Gamergate colonies can perpetuate themselves (cohorts of young workers mate annually), but they are confined to the original nests. Thus, a regular production of queens remains essential for the initiation of new colonies.

The coexistence of queens and gamergates has been documented in only a few ponerine ants. In *Rhytidoponera confusa*, they are never found together (15) because workers appear to mate and reproduce in orphaned colonies only. The gamergate colonies can multiply through fission, and they seldom produce new queens. In *Platythyrea arnoldi* (ref. 16; C.P., unpublished data) and *Pachycondyla tridentata* (17), both morphological categories of reproductives coexist within the same colonies, but their respective roles in the life history are incompletely understood. The advantages of aerial dispersal and the colonization of new habitats have generally been invoked as the adaptive basis for the persistence of winged queens. In the ant *Technomyrmex albipes* (subfamily Dolichoderinae), winged queens disperse and establish new colonies but are eventually replaced by wingless intercastes—i.e., phenotypic intermediates between queens and workers (18). Intercastes are produced in large numbers, and almost all are inseminated and reproduce in the huge colonies (several millions of adults) of this species. Such secondary polygyny in *T. albipes* seems adaptive in terms of the exploitation of local habitats through colony fission.

Colony survivorship exceeds queen life-span in a number of ants (13), which is possible because newly mated queens can be adopted into established colonies—e.g., various species with multiple queens of *Formica* and *Camponotus* (subfamily Formicinae) (19, 20). This strategy, however, would not be economical in those ponerine species in which workers are competent to reproduce sexually. In *H. saltator*, natural selection should favor gamergates to replace the foundress, but the production of the specialized queen caste remains adaptive to disperse aerially and found new colonies elsewhere. As in most ants, the founding stage represents a bottleneck, and many incipient *H. saltator* colonies will probably fail.

In several ponerine ants, the queens have been permanently replaced by gamergates since colonies with the latter are able to multiply (7). Such fission of gamergate colonies cannot occur in *H. saltator*, due to the exceptional nature of the nests. Queens and gamergates exhibit distinct reproductive capabilities, and they perform interdependent roles in this species.

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1. Bourke, A. F. (1988) *Q. Rev. Biol.* **63,** 291–311.
2. Seger, J. (1991) in *Behavioural Ecology*, eds. Krebs, J. R. & Davies, N. B. (Blackwell, London), pp. 338–373.
3. Ratnieks, F. K. & Reeve, H. K. (1992) *J. Theor. Biol.* **158,** 33–65.
4. Boomsma, J. J. (1993) in *Queen Number and Sociality in Insects*, ed. Keller, L. (Oxford Univ. Press, Oxford), pp. 86–109.
5. Nonacs, P. (1993) in *Queen Number and Sociality in Insects*, ed. Keller, L. (Oxford Univ. Press, Oxford), pp. 110–131.
6. Heinze, J., Hölldobler, B. & Peeters, C. (1994) *Naturwissenschaften* **81,** 489–497.
7. Peeters, C. (1991) *Biol. J. Linn. Soc.* **44,** 141–152.
8. Peeters, C. & Crewe, R. M. (1985) *Behav. Ecol. Sociobiol.* **18,** 29–37.
9. Peeters, C. (1993) in *Queen Number and Sociality in Insects*, ed. Keller, L. (Oxford Univ. Press, Oxford), pp. 234–261.
10. Haskins, C. P. (1970) in *Development and Evolution of Behavior*, eds. Aronson, L., Tobach, E., Lehrman, D. & Rosenblatt, J. (Freeman, San Francisco), pp. 355–388.
11. Peeters, C., Hölldobler, B., Moffett, M. & Musthak Ali, T. M. (1994) *Insectes Soc.* **41,** 211–218.
12. Peeters, C. (1995) in *Social Competition and Cooperation in Insects and Arachnids*, eds. Choe, J. & Crespi, B. (Princeton Univ. Press, Princeton), Vol. 2, in press.
13. Hölldobler, B. & Wilson, E. O. (1990) *The Ants* (Harvard Univ. Press, Cambridge, MA).
14. Myles, T. G. & Nutting, W. L. (1988) *Q. Rev. Biol.* **63,** 1–23.
15. Ward, P. S. (1983) *Behav. Ecol. Sociobiol.* **12,** 285–299.
16. Villet, M. (1993) *S. Afr. J. Zool.* **28,** 56–57.
17. Sommer, K. & Hölldobler, B. (1992) *Naturwissenschaften* **79,** 470–472.
18. Yamauchi, K., Furukawa, T., Kinomura, K., Takamine, H. & Tsuji, K. (1991) *Behav. Ecol. Sociobiol.* **29,** 313–319.
19. Rosengren, R., Sundström, L. & Fortelius, W. (1993) in *Queen Number and Sociality in Insects*, ed. Keller, L. (Oxford Univ. Press, Oxford), pp. 308–333.
20. Hölldobler, B. (1962) *Z. Angew. Entomol.* **49,** 337–352.