A Female Caste Specialized for the Production of Unfertilized Eggs in the Ant *Crematogaster smithi*

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In colonies of the North American ant *Crematogaster smithi* "large workers" occur which are morphologically intermediate between winged queens and small workers and appear to be specialized for the production of unfertilized eggs. In queenless colonies these eggs may develop into males, but most of them are eaten in colonies containing a queen. We discuss the possible significance of this new type of female.

Ant reproduction was once considered to be quite simple and uniform. For example, it is widely thought that a virgin ant queen typically mates with a single male during a nuptial flight, after which she seals herself off in a small cavity in wood or in the soil to start a new colony. In the mature colony she monopolizes egg laying while all other individuals are non-reproductive workers. Recently, however, it has been shown that there are numerous variants of this simple scheme, such as queens' multiple mating, mating within the nest, multiple fertile queens per nest, male production by workers, thelytoky (production of female offspring from unfertilized eggs), and sexual reproduction by mated workers [1–4]. Here we describe a striking addition to the growing range of ant reproductive strategies: in the myrmicine *Crematogaster* (*Orthocrema*) smithi, a distinct female caste exists which appears to be specialized in morphology and behavior for the production of unfertilized eggs. At least in queenless colonies, males are reared from these haploid eggs.

C. smithi, also considered to be a subspecies of C. *minutissima* [5], is currently known only from two mountain ridges, the Huachuca and Chiricahua Mountains in Cochise County of southwestern Arizona in the United States. In summer 1992, 1993, 1997, and 1998 and spring 1996 we excavated a total of 137 colonies of *C. smithi* from their nests in the soil in a juniper-oak–pinyon forest at an elevation of approx. 2000 m. In 94 colonies a single queen, workers, and brood were present ("queenright colonies"), and in 40 colonies with workers and brood no queen was found. These latter colonies were probably incompletely collected. Three additional nests contained only a single founding queen. The number of workers ranged from 4 to 615 (not all colonies were censused; 82 queen- right colonies: median 165). In addition, almost half of all colonies contained between one and ten "large workers" (47% of 137). Of 94 queen- right

colonies (excluding solitary foundresses), 53% contained one or several “large workers.” The median number of “large workers” in 50 censused queenright colonies was two. Queenright colonies containing “large workers” were significantly
larger than queenright colonies without “large workers” (Mann-Whitney U test, n1 = 46, n2 = 36, U = 332, P<0.001), and the numbers of small workers and “large workers” per colony were positively correlated (Spearman’s test of rank correlation; all colonies, n =107, $r_S = 0.510$, P < 0.001; only queenright colonies, n=82, $r_S=0.519$, P<0.001; Fig. 1). “Large workers” were intermediate in size, weight, and morphology between queens and small workers (Fig.2). Weber’s alitrunk length in queens was 1.95 ± 0.08 mm (n = 21), in small workers 0.63 ± 0.04 mm (n = 62), and in “large workers” 1.01 ± 0.05 mm (n =17). Small workers weighed 0.72 ± 0.17 mg (n =150; because of the low precision of the scales the weight of small workers was determined in groups of ten), queens 5.3 ± 1.44 mg (n = 3), and “large workers” 2.2 ± 0.37 mg (n = 8). Body size and weight of “large workers” and small workers did not overlap. In contrast to small workers, which lacked ocelli and whose thoracic sclerites were widely fused, “large workers” showed thoracic sutures, traces of ocelli, and occasionally pigmented patches at the site of wings to a varying degree (for further details on morphology and the composition of a smaller sample of colonies see [6]). “Large workers” could therefore also be referred to as “intercastes” (see Peeters [7]; but see [8]).

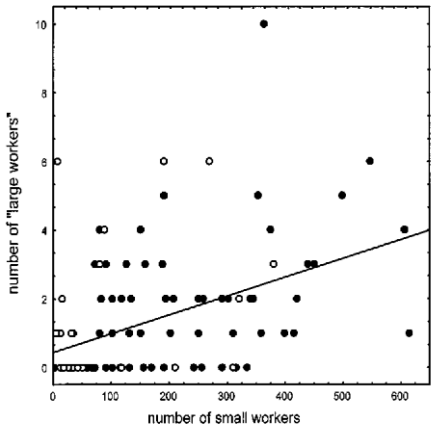

Fig. 1. Number of small workers and “large workers” of the ant *Crematogaster smithi* found in colonies with queen (*black circles*) and without queen (*open circles*). The regression line is based on all colonies

Fig. 2. The three female castes in the ant *Crematogaster smithi*: queen, small worker, and "large worker"

"Large workers" were intermediate between queens and workers also in the anatomy of their ovaries. The ovaries of six dissected queens consisted of a total of 18–24 ovarioles (median 20.5) and a spermatheca, ten dissected small workers invariably had two ovarioles without spermatheca, and the ovaries of ten "large workers" had 6–12 ovarioles (median 10) and lacked a spermatheca.

During more than 100 h of direct and video observation (for details see [6]) both small and "large workers" were observed laying eggs in colonies with and without queen. "Large workers" typically produced more eggs in queenless colonies than in the presence of the queen. In isolation, "large workers" from queenless colonies laid between 6 and 20 eggs in 3 days ($n = 4$; median 13.5). Under similar conditions "large workers" from queenright colonies laid between 0 and 11 eggs ($n = 9$, median 1; Mann- Whitney U test, $U = 3.5$, $P < 0.05$). In groups of 50 small workers each from queenright colonies, kept without queen for 3 days a total of 0–18 eggs were produced ($n = 11$, median 5). Even when corrected for size, "large workers" thus appear to be considerably more efficient egg layers than small workers. Small workers laid on average 0.05 eggs per day and per milligram fresh weight and "large workers" 0.97. While many of the eggs produced by both small and "large workers" were fed to the larvae or eaten by the queen, at least in queenless colonies both types of workers were found capable of producing males. It remains unclear, however, whether worker-laid eggs can develop into males in queenright colonies. Our data do not suggest thelytoky, as has been suggested for another *Crematogaster* species [9]. As for queens, "large workers" were constantly surrounded and groomed by an entourage of small workers in queenless colonies and to a lesser extent also in queenright colonies. No aggressive interactions between nest- mate queen and "large workers" were observed in laboratory colonies. When we experimentally placed a fertile queen into an alien queenless colony containing a "large worker" and one "large worker" each into two alien queenright colonies, the small workers initially reacted with an alarm response typical for *Crematogaster*, with the triangular gaster pointing upwards [10, 11] but were later observed to treat the introduced individual amicably. Within a few of days after introduction, the two unrelated reproductives engaged in fighting, which led to one or both of the two individuals at least temporarily emigrating from the nest and finally resulted in the death of the "large workers" in all three replicates.

A role of "large workers" in colony defense or as repletes for the storage of fat or liquid food as in other species with polymorphic workers [1, 12–14] could be excluded (for details see [6]). It therefore appears that the major function of "large workers" is to produce large amounts of unfertilized eggs. We believe that this is the first known case of a female caste morphologically and behaviorally specialized for this function in the social Hymenoptera.

As long as data are lacking on the maternity of males produced in colonies with both queen and "large workers," we can only speculate on their ecological significance. From our data we conclude, however, that in queenright colonies they convert perishable food carried in by foragers, such as insect prey, into viable eggs, which can be stored over several weeks and may be eaten by the queen or fed to the larvae during periods of prey shortage in the highly seasonal environment of the southwestern United States). Many other species living in xeric habitats rely on the storage of seeds (e.g., *Pogonomyrmex*, *Ephebomyrmex*) or liquids (*Myrmecocystus*) to resist starvation in periods of food shortage [1]. Interestingly, the ovaries were developed in a large percentage of workers of *Novomessor cockerelli* [15], *Ephebomyrmex imberbiculus* (35–68% of all workers in seven colonies; J.H., unpublished), and several *Pogonomyrmex* species (*P. barbatus*: 18 of 50 dissected foragers; *P. maricopa*: 23 of 75 foragers; *P. rugosus*: 12 of 50 foragers, B.H., unpublished) from desert habitats adjacent to our collecting site. Their eggs are also most probably fed to larvae and nestmates.

A highly variable food intake has also been suggested to underlie the evolution of egg eating in other species of social insects [16]. Furthermore, larvae of *C. smithi* and related species do not feed on solid items other than eggs and only infrequently receive liquid food by trophallaxis from small workers [17]. The presence of a caste specialized for the production of food for larvae or the queen might increase colony growth and the output of sexuals, leading to colonies with "large workers" being larger than colonies without. However, only experimental manipulations of the number of "large workers" per colony would be able to clarify the causes of the apparent correlation between colony size and the number of "large workers" in field-collected colonies. Once the queen dies, "large workers" might become highly fertile replacement reproductives, which would strongly increase the output of males in the declining colony. Our observation of aggression between alien queens and "large workers" may support this hypothesis.

Workers of many other *Crematogaster* species exhibit considerable size variation both within and between colonies (e.g., [18]), although a bimodal distribution of worker size has yet to be demonstrated. "Large workers" as found in *C. smithi* apparently occur in at least two other species. Holliday [19] described an "ergatoid female" of C. (*Orthocrema*) *minutissima* from Texas which is very similar in morphology to the "large workers" of *C. smithi* but has the same ovarian anatomy as small workers. Furthermore, we have recently found "large workers" in a shiny black *Crematogaster* [morphologically similar to *Crematogaster* (*Orthocrema*) *curvispinosa*], nesting in young acacia on the Rio Palillo, 25 km east of San Blas, Nayarit, Mexico. In this species normal workers had two ovarioles, and "large workers" had 10–16 ovarioles (n=4, median 11.5).

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1. Hölldobler B, Wilson EO (1990) The ants. Belknap–Harvard University Press, Cambridge
2. Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton
3. Heinze J, Tsuji K (1995) Ant reproductive strategies. Res Popul Ecol 37:135–149
4. Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford
5. Creighton WS (1950) The ants of North America. Bull Mus Comp Zool 104:1–585
6. Heinze J, Cover SP, Hölldobler B (1995) Neither worker, nor queen: an ant caste specialized in the production of unfertilized eggs. Psyche 102:173–185
7. Peeters C (1991) Ergatoid queens and intercastes in ants: two distinct forms which look morphologically intermediate between workers and winged queens. Insectes Soc 38:1–15
8. Heinze J (1998) Intercastes, intermorphs, and ergatoid queens: who is who in ant reproduction? Insectes Soc 45:113–124
9. Soulié J (1970) Des considérations écologiques peuvent-elles apporter une contribution à la connaissance du cycle bio-
10. logique des colonies de *Crematogaster* (Hymenoptera – Formicoidea)? Insectes Soc 7:283–295
11. Buren WF (1958) A review of the species of *Crematogaster*, sensu stricto, in North America (Hymenoptera, Formicidae). I. J NY Entomol Soc 66:119–134
12. Blum MS, Crewe RM, Sudd JH, Garrison AW (1969) 2-Hexenal: isolation and function in a *Crematogaster* (Atopogyne) sp. J Georgia Entomol Soc 4:145–148
13. Rissing SW (1984) Replete caste production and allometry of workers in the honey ant, *Myrmecocystus* mexicanus Wesmael (Hymenoptera: Formicidae). J Kansas Entomol Soc 57:347–350
14. Tsuji K (1990) Nutrient storage in the major workers of Pheidole ryukyuensis (Hymenoptera: Formicidae). Appl Ent Zool 25 :283–287
15. Hasegawa E (1993) Caste specialization in food storage in the dimorphic ant Colobopsis nipponicus (Wheeler). Insectes Soc 40:261–271
16. Hölldobler B, Carlin NF (1989) Colony founding, queen control, and worker re-production in the ant *Aphaenogaster* (=*Novomessor*) *cockerelli* (Hymenoptera: Formicidae). Psyche 96:131–151
17. Crespi BJ (1991) Cannibalism and trophic eggs in subsocial and eusocial insects. In: Elgar MA, Crespi BJ (eds) Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, Oxford, pp 176–213
18. Cassill DL, Tschinkel WR (1996) A duration constant for worker-to-larva trophallaxis in fire ants. Insectes Soc 43:149–166
19. Wilson EO (1953) The origin and evolution of polymorphism in ants. Q Rev Biol 28:136 –156

20. Holliday M (1903) A study of some ergatogynic ants. Zool Jahrb Abt Syst Okol Geogr Tiere 19:293–328