Naturwissenschaften 84, 499–502 (1997)

Cannibalism of subordinates' eggs in the monogynous queenless ant *Dinoponera quadriceps*

Thibaud Monnin, Christian Peeters[1]

CNRS URA 667, Laboratoire d'Ethologie Expérimentale et Comparée, Université Paris Nord, 93430 Villetaneuse, France

Received: 15 May 1997 / Accepted: 20 August 1997

About 100 species of ants in the subfamily Ponerinae have lost the morphologically specialized queen caste, and workers can mate and lay fertilized eggs. All workers have identical phenotypes, but only a proportion of them reproduce sexually (these are termed gamergates [1]). Either monogyny (one gamergate) or polygyny (several gamergates per colony) occurs depending on the species [2]. Reproductive division of labor in most queenless ants is regulated by aggressive interactions leading to the formation of hierarchies [3], similarly as in various social wasps lacking dimorphic queens and workers [4]. Subordinate workers generally have undeveloped ovaries, except that in many monogynous species a few high-ranking subordinates can often lay eggs. Unlike wasps where many females mate in each colony, in many monogynous queenless ants only the top-ranking worker ("alpha") can mate, and thus other egg-layers are restricted to producing males. Alpha is often able to destroy subordinates' eggs, which is distinct from the consumption of nonviable "trophic" eggs common in other ants. In *Diacamma* sp., the single gamergate eats most of the haploid eggs occasionally laid by virgin workers [5]. Similarly in some functionally monogynous Polistine wasps, alpha selectively destroys the eggs which are laid by subordinates during the initial stage of colony foundation ("queen policing") [6].

Dinoponera quadriceps is a queenless ant with small colonies (82±29 workers, range 39–141, $n=17$) collected in Bahia state (Brazil). Colonies are monogynous; dominance interactions lead to a linear hierarchy, and only the alpha worker copulates [7]. Oviposition and oophagy were observed directly in 15 colonies kept in the laboratory. We report here that a few high-ranking subordinates infrequently lay unfertilized eggs, but that these are destroyed by alpha. Oophagy seems to be regulated by an identified chemical signal present on the cuticle of alpha workers and on their eggs.

A total of 129 ovipositions were recorded during 885 h, and this low egg-laying rate is characteristic of the Ponerinae [2]. Alpha workers (who can produce eggs even before they mate) laid 111 eggs (Table 1). In nine colonies alpha monopolized oviposition, while in the six remaining colonies subordinate workers (having the beta, gamma, and delta ranks in the hierarchy) laid 18 eggs (14% of total). Dissection of all workers ($n=914$) confirmed that alphas (virgin or gamergate) have much better developed ovaries than the subordinate egg layers (Fig. 1). In colonies where beta workers were not observed to oviposit their ovaries were undeveloped.

The fate of 100 newly laid eggs was monitored for about 30 min to determine the natural pattern of oophagy. New eggs have a pure white color which darkens after a few hours; dark eggs were never eaten. New eggs are generally carried by the mothers for several minutes before being deposited in the egg pile. All but one egg laid by alpha survived, while in contrast most of those laid by subordinates were destroyed (13/18, i.e., 72.2%; Table 1). Alpha was responsible for 12 of 14 observed cases of oophagy (85.7%); virgin alphas behaved similarly to gamergates. Oophagy occurred 22±7 min ($n=9$) after oviposition, but exceptionally an egg was eaten after 65 min. Ten eggs were stolen directly from the mandibles of mothers. Five eggs escaped destruction because alpha did not come across them (in one case she was busy ovipositing). There is good evidence that alpha recognizes new eggs which are not her own and cannibalizes them ("queen policing"). When subordinates carried eggs newly laid by alpha, they were not destroyed if alpha encountered them again. Furthermore, in one case alpha removed and ate a new egg recently added to the egg pile 37 min after it had been laid by a subordinate.

Using solid-phase microextraction (SPME) we previously showed that alpha differs from other colony members by the quantity and relative proportion of a long-chain cuticular hydrocarbon (9-C_{31}: 1 or 9-hentriacontene) [8]. To investigate whether there is more 9-C_{31} on alphas' eggs than on subordinates', we measured 13 newly laid eggs with SPME, which is a non-destructive technique (no solvent is used). Ovipositing workers were removed from their colonies while an egg was already half outside of the gaster. Due to excitement mothers let their egg fall to the ground, and thus

[1] *Present address:* CNRS URA 258, Laboratoire d'Ecologie, Université Pierre-et-Marie Curie, 7 quai St Bernard, 75252 Paris cedex 05, France
e-mail: cpeeters@snv.jussieu.fr

Table 1. Oviposition and oophagy observed during 885 h in 15 colonies of *D. quadriceps*. The beta worker laid eggs in six colonies, gamma laid in three colonies, and delta in two colonies

Status of egg layer	Eggs laid	Eggs removed for chemical analysis or introduction to foreign nest	Eggs left in the nest to check for oophagy within 30 min of oviposition		
			Not destroyed	Destroyed by alpha	Destroyed by subordinates
Gamergate	49	9	39	0	1
Virgin alpha	62	20	42	0	0
Beta	11		4	7	0
Gamma	4		1	3	0
Delta	3		0	2	1
Total	129	29	86	12	2

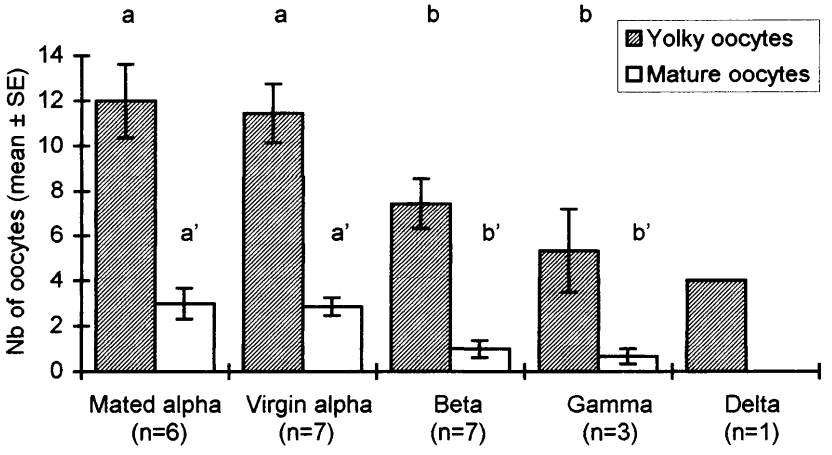

Fig. 1. Pattern of ovarian activity based on the dissection of 914 workers from 15 colonies. Subordinate workers had developed ovaries in six of these colonies, while in others only alpha had developed ovaries. The mean number of both mature oocytes (as large as an egg) and opaque yolky oocytes differed significantly between alpha (virgin or mated) and subordinate egg layers (Mann-Whitney *U* test, $P<0.05$; identical letters refer to values which are not different). All other workers had undeveloped ovaries

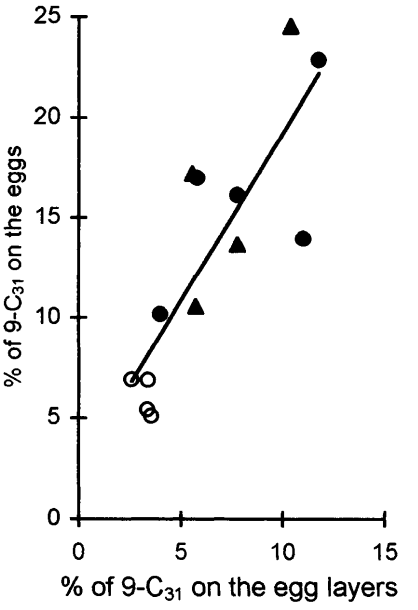

Fig. 2. Relative amount of 9-hentriacontene present both on the cuticle of mothers and on their eggs. ○, Replacement alpha (equivalent to beta); ▲, virgin alpha; ●, gamergate. The percentages of 9-C_{31} extracted from workers and their eggs are correlated by a linear regression ($y=1.68x+2.41$, $r^2=0.687$, Pearson product-moment correlation: $P<0.05$)

there was no direct contact between mandibles and egg. The latter was immediately taken with forceps and sampled with a Supelco 7-μm polydimethylsiloxane fiber designed to extract high-weight hydrocarbons. The glass fiber was rubbed on each side of an egg for 1.5 min, thus totaling 3 min of rubbing (eggs are large: 3.3±0.14 mm long and 0.8 mm wide, $n=34$). Afterwards the fiber was inserted in the injector port of a gas chromatograph for desorbtion (column temperature increased from 70 °C to 300 °C at 5 °C/min, then isothermic at 300 °C for 20 min).

The cuticle of egg-layers was also measured on the day of the oviposition or sometimes 1 day later, following the method described previously [8]. Since oviposition by beta is rare, we removed alpha (virgin or gamergate) in two colonies and used the first eggs laid by the replacement alpha. Beta almost always accedes to the top rank, and both the percentage and amount of 9-C_{31} increase after 2 weeks [9]. Thus eggs initially laid by the replacement alpha are considered to be equivalent to eggs laid by beta. Fourteen major peaks exceeding an arbitrary threshold were used to calculate the relative proportion of 9-C_{31} [8]. As seen in Fig. 2, there was a positive correlation between percentage of 9-C_{31} present on ovipositing workers and that on their eggs. Alpha yielded more 9-C_{31} than the replacement alpha (7.7±2.7 and 3.2±0.4%, $n=9$ and 4 respectively, Mann-Whitney *U* test: $P<0.01$; larger samples described in [9]), and alpha-laid eggs yielded more 9-C_{31} than eggs laid by replacement alphas (16.2±4.9 and 6.1±0.9%, $n=9$ and 4 respectively, Mann-Whitney *U* test: $P<0.01$). Once an egg appears at the tip of the abdomen, 3.6±0.4 min ($n=25$) elapse before it is fully laid, and 9-C_{31} may passively diffuse from the cuticle of the mother to her egg. It is not clear why the proportion of 9-C_{31} should be higher on the eggs than on the cuticle (Fig. 2), but this may be related to surface properties of the chorion.

Sixteen eggs laid by alphas from five colonies, and thus presumably yielding a high percentage of 9-C_{31}, were immediately removed and transferred into foreign colonies ($n=4$) to test the hypothesis that alpha recognizes her eggs on the basis of a high percentage of 9-C_{31}. Indeed, she is the only worker of the colony with this cuticular profile, and thus eggs rich in 9-C_{31} are always her eggs under natural conditions. Only 4 of these 16 introduced eggs were destroyed by the resident alphas. This contrasts with our

previous observations that 13 of 18 eggs laid by subordinates had been destroyed by nestmate alphas. Thus, transferred alpha eggs were less likely to be eaten (χ^2 test: $P<0.01$), although oophagy occurred more often (4/16) than in the normal situation (1/82). This may be due to disturbance while introducing the eggs in a tested colony or to the colony odor of the introduced eggs which are perceived as alien by a resident alpha. Indeed, workers of *Ectatomma tuberculatum* discriminate between larvae and cocoons from same or different colonies [10], and queens of the polygynous *Leptothorax acervorum* preferentially eat nonnestmates' eggs rather than nestmates' [11].

In some Polistine wasps lacking morphologically specialized queens, oophagy could also be regulated by an olfactory signal present on the eggs. In *Polistes dominulus*, alpha destroys new foreign eggs which are experimentally introduced, while introduced 1-day-old eggs are rarely destroyed, and 30-h-old eggs not at all [12]. In *P. dominulus* alpha has also a specific cuticular hydrocarbon profile which is related to ovarian activity [13], and we hypothesize that this odor diffuses to the eggs during oviposition and inhibits oophagy, as in *D. quadriceps*. This is supported by the observation that at the beginning of the egg-laying period in *P. dominulus*, alpha has slightly developed ovaries [14], and sometimes destroyed her own eggs [12]. Since female wasps lacking fully developed ovaries do not have the characteristic hydrocarbon profile of dominant reproductives [13], their eggs may not carry the signal inhibiting oophagy. This also appears to be the case in *D. quadriceps*, where subordinate egg-layers yield less 9-C_{31} than alpha because their ovaries are less developed. Therefore alpha can discriminate their eggs and destroy them.

In honey bees, where the castes are highly dimorphic, very few workers oviposit in queenright colonies, and their eggs are destroyed by other workers [15]. These policing workers can discriminate queen-laid eggs from worker-laid eggs because the former are marked with pheromones apparently originating from the Dufour's gland of the queen [16]. In *Polistes fuscatus*, eggs experimentally rubbed with Dufour's extract from subordinates were destroyed by alpha, while eggs rubbed with secretions from alpha were not destroyed by subordinates [17]. However, it remains unclear whether alpha's secretions really inhibit oophagy because they were only tested with subordinates, and these normally do not destroy eggs [6, 12]. Additional factors may inhibit oophagy in *Polistes*, because eggs are laid in individual cells, unlike ants where they are generally gathered in piles. Thus the alpha wasp may memorize the cells where she recently oviposited and destroy new eggs encountered in other cells [12]. Egg marking has also been reported in *Solenopsis invicta*, where ovipositing queens deposit poison gland products onto their eggs with the sting. These chemicals are not involved in the regulation of oophagy, however, but apparently attract workers to the eggs and protect them from fungus and bacteria [18].

In colonies of *D. quadriceps* that are not recently created by fission of a mother colony, all workers are daughters of the gamergate. Since she mates only once [7], workers are full-sisters and are more related on average to subordinates' eggs developing in nephews (0.375) than to the gamergate's unfertilized eggs developing in brothers (0.25). Thus natural selection is unlikely to favor the destruction of subordinates' eggs by sterile workers ("worker policing" [19]). In contrast, most workers in honey bee colonies are half-sisters (the queen mates multiply) and are more related to the queen's haploid eggs than to nestmate workers' eggs. Thus worker policing leads to the destruction of worker-laid eggs [15]. Furthermore, "queen policing" would not be possible in the huge *Apis* colonies, unlike in *Dinoponera* where there are only very few subordinate egg-layers to watch.

The regulation of selective oophagy by an olfactory cue present on the eggs and related to the ovarian activity of mothers may only be possible in species with significant inequalities among egg layers (i.e., alpha and beta in *D. quadriceps*), such as the queenless ant *Pachycondyla* sp. from West Java [20] and some *Polistes* wasps [6]. In contrast, such regulation seems impossible in species where several individuals have fully developed ovaries and lay eggs equally. All the egg layers would yield the putative "dominant" odor and mark their eggs, preventing differential oophagy. There is indeed no oophagy in the polygynous queenless ants *Pachycondyla berthoudi* [21], *Leptogenys schwabi* [22], and *Harpegnathos saltator* (J. Liebig and C. Peeters in preparation), while oophagy is indiscriminate in the queenright ant *Leptothorax acervorum* [11] and the Polistine wasp *Ropalidia rufoplagiata* [23], both of which are polygynous.

Oviposition by a few high-ranking subordinates appears to be a stable characteristic of reproductive systems regulated by a linear dominance hierarchy. Skew models predict that alpha yields some reproduction to subordinates as an inducement to remain in the society, i.e., "staying incentives" [24]. In *D. quadriceps*, although alpha has an efficient mechanism to recognize and destroy eggs which are not her own, it is adaptive for a few subordinates to oviposit since having developed ovaries gives them an advantage over other nestmates when an opportunity to become alpha arises. Furthermore, several eggs did survive and should develop into males which can copulate in foreign colonies (mating opportunities occur throughout the year [7]). From the colony's perspective, subordinate oviposition should not be counterselected because it allows for the quick replacement of an existing reproductive, following either the decline of an old gamergate or the advent of colony fission (which occurs instead of independent foundation in permanently queenless ants).

We are grateful to C. Malosse (INRA Versailles) for help with the chemical analyses.

1. Peeters C, Crewe R (1984) Insemination controls the reproductive division of labour in a ponerine ant. Naturwissenschaften 71:50–51
2. 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

3. Heinze J, Hölldobler B, Peeters C (1994) Conflict and cooperation in ant societies. Naturwissenschaften 81:489–497
4. Röseler P-F (1991) Reproductive competition during colony establishment. In: Ross KG, Matthews RW (eds) The social biology of wasps. Cornell University Press, Ithaca, pp 309–335
5. Nakata K, Tsuji K (1996) The effect of colony size on conflict over male-production between gamergate and dominant workers in the ponerine ant *Diacamma* sp. Ethol Ecol Evol 8:147–156
6. Reeve HK (1991) *Polistes.* In: Ross KG, Matthews RW (eds) The social biology of wasps. Cornell University Press, Ithaca, pp 99–148
7. Monnin T, Peeters C (1998) Monogyny and regulation of worker mating in the queenless ant *Dinoponera quadriceps.* Anim Behav (in press)
8. Monnin T, Malosse C, Peeters C (1998) Solid phase microextraction and cuticular hydrocarbon differences related to reproductive activity in the queenless ant *Dinoponera quadriceps.* J Chem Ecol (in press)
9. Monnin T (1997) Régulation de la reproduction chez la fourmi sans reine *Dinoponera quadriceps*: structures hiérarchiques et communication olfactive. Unpublished Ph. D. thesis, Université Paris Nord, 139 p
10. Fénéron R, Jaisson P (1995) Ontogeny of nestmate brood recognition in a primitive ant, *Ectatomma tuberculatum* Olivier (Ponerinae). Anim Behav 50:9–14
11. Bourke AFG (1994) Indiscriminate egg cannibalism and reproductive skew in a multiple-queen ant. Proc R Soc Lond 255:55–59
12. Gervet J (1964) Le comportement d'oophagie différentielle chez *Polistes gallicus* L (Hymen. Vesp.). Ins Soc 11:343–382
13. Bonavita-Cougourdan A, Théraulaz G, Bagnères A-G, Roux M, Pratte M, Provost E, Clément J-L (1991) Cuticular hydrocarbons, social organization and ovarian development in a polistine wasp: *Polistes dominulus* Christ. Comp Biochem Physiol 100B:667–680
14. Turillazzi S (1980) Seasonal variation in the size and anatomy of *Polistes gallicus* (L.) (Hymenoptera: Vespidae). Monit Zool Ital (NS) 14:63–75
15a. Ratnieks FLW, Visscher PK (1989) Worker policing in the honeybee. Nature 342:796–797
15b. Ratnieks FLW (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. Behav Ecol Sociobiol 32:191–198
16. Ratnieks FLW (1995) Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. J Apicult Res 34:31–37
17. Downing HA (1991) A role of the Dufour's gland in the dominance interactions of the paper wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). J Insect Behav 4:557–565
18. Vander Meer RK, Morel L (1995) Ant queens deposit pheromones and antimicrobial agents on eggs. Naturwissenschaften 82:93–95
19. Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat 132: 217–236
20. Ito F (1993) Functional monogyny and dominance hierarchy in the queenless ponerine ant *Pachycondyla (= Bothroponera)* sp. in west Java, Indonesia. Ethology 95:126–140
21. Sledge MF, Crewe RM, Peeters CP (1996) On the relationship between gamergate number and reproductive output in the queenless ponerine ant *Pachycondyla (= Ophthalmopone) berthoudi.* Naturwissenschaften 83:282–284
22. Davies SJ, Villet MH, Blomefield TM, Crewe RM (1994) Reproduction and division of labour in *Leptogenys schwabi* Forel (Hymenoptera Formicidae), a polygynous, queenless ponerine ant. Ethol Ecol Evol 6:507–517
23. Sinha A, Premnath S, Chandrashekara K, Gadagkar R (1993) *Ropalidia rufoplagiata*: a polistine wasp society probably lacking permanent reproductive division of labour. Ins Soc 40:69–86
24a. Reeve HK, Ratnieks FLW (1993) Queen-queen conflicts in polygynous societies: mutual tolerance and reproductive skew. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 45–85
24b. Keller L, Reeve HK (1994) Partitioning of reproduction in animal societies. Trends Ecol Evol 9:98–102

Naturwissenschaften 84, 502–507 (1997)

Molecular and Morphological Data Suggest a Single Origin of the Polydnaviruses among Braconid Wasps

James B. Whitfield

Department of Entomology, University of Arkansas, Fayetteville, AR 72701, USA; email jwhitfie@comp.uark.edu

Received: 22 October 1996 / Accepted: 28 August 1997

Endoparasitoids exhibit some of the most intricate and intimate interspecific physiological interactions recognized among animals [1]. These typically wasplike insects feed as larvae within the bodies of other insects (often caterpillars), ultimately killing their hosts. A wide variety of endoparasitoid/host interactions are known [2], which in turn are mediated by diverse agents such as venoms, teratocytes, and viruslike entities. These mediating agents, introduced into the host by the female parasitoid during oviposition [3, 4], misdirect or dismantle the host's defense response and development, thereby enabling the larva(e) to develop freely inside [5].

Perhaps the most remarkable agents introduced into the hosts of some endoparasitoid wasps (Braconidae and Ichneumonidae) are viruslike entities known as polydnaviruses. Polydnaviruses are unusual in two respects. First, they are integrated into the wasp's chromosomal DNA and passed on from parent to offspring in mendelian fashion [6–8]. Second, within the cells of the wasp's ovarian

