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Reproductive conflict among ant workers in *Diacamma* sp. from Japan: dominance and oviposition in the absence of the gamergate

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Summary

Queens do not exist in *Diacamma* sp. from Japan, and a single worker ("gamergate") mates and monopolizes reproduction in each colony. We isolated small groups of workers without the gamergate, and confirmed that after 7–15 days many workers were able to oviposit (Tab. 1). These egg-laying individuals engage in stereotyped attacks towards each other. In six groups of individually marked workers (Tabs. 3 to 7), the pattern of aggressive interactions always indicated that one worker was dominant. She usually initiated a large number of attacks, but was herself never attacked. This dominant worker ("alpha") also ate the eggs just laid by others. There was no linear dominance hierarchy, although a second highest-ranking worker could be recognized (she was only attacked by alpha). When these workers were dissected 4–6 weeks after being orphaned, only the alpha worker had active ovaries; other individuals that had been observed to oviposit earlier exhibited resorbed ovaries. Dissection of another 12 orphaned groups, kept together for different periods of time (Tab. 2), confirmed that one dominant worker is able to suppress the ovarian activity of all others in her group. We discuss how these aggressive interactions also function to regulate the production of males in other contexts, even when the gamergate is present. This aggression is separate, however, from another competitive interaction, mutilation of the gemmae, that functions as a control of mating activity in this species.

Introduction

Reproductive division of labor is a fundamental characteristic of insect sociality. Female sterility is regulated by a variety of mechanisms, ranging from competitive dominance orders to pheromonal inhibition. In primitively eusocial hymenopterans (i.e. bees and wasps that lack morphologically distinct queens and workers), a large

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proportion of female adults have equivalent reproductive potentials, and personal fitness is then determined by one's position in the dominance hierarchy. In highly eusocial species (defined by the occurrence of morphological differences between queens and workers), caste membership specifies the available reproductive options. Queens thus monopolize diploid reproduction, and workers are mostly sterile. Dominance orders can lead to differential reproduction in various polygynous ants, such as leptothoracines (Heinze and Smith, 1990; Bourke, 1991; see review Heinze, 1993) or *Odontomachus chelifer* (Medeiros et al., 1992). Dominance orders may also exist among virgin ant workers, where they affect the production of males (Cole, 1981; Franks and Scovell, 1983; Bourke, 1988; Oliveira and Hölldobler, 1990).

A distinct situation prevails in a small number of ponerine ants in which queens no longer exist. All the workers are morphologically identical and have a spermatheca, but only a minority of them mate and reproduce. While in some species several gamergates (= mated laying workers) occur in each colony, in others there is strict monogyny (see review Peeters, 1993). In *Pachycondyla sublaevis*, stereotyped dominance interactions occur between the gamergate and a few unmated workers, and the latter have undeveloped ovaries (Ito and Higashi, 1991). If the gamergate is killed, one of the subordinate workers becomes dominant and will probably mate when foreign males visit the nests. In *Diacamma australe* and *Diacamma* sp. from Japan (previously referred to as *D. rugosum*), mating and oviposition are also regulated by competitive interactions among workers, the most conspicuous of which is the mutilation of a pair of dorsal thoracic appendages termed "gemmae". These appendages are highly modified structures unique to the genus *Diacamma*, and they are filled with exocrine glands in *D. australe* (Billen and Peeters, 1991; Peeters and Billen, 1991). Unlike other ponerines without queens, the gamergate in both species of *Diacamma* differs morphologically from all other workers because she alone retains the gemmae (Fukumoto et al., 1989; Peeters and Higashi, 1989). All workers eclose with the tiny gemmae, but these are soon bitten off by the single gamergate. When there is no gamergate (following her natural death or colony fission), one of the callow workers that subsequently emerge retains the gemmae and proceeds to mutilate all other callows. This individual is then sexually attractive, and mates when the opportunity arises. The gamergate monopolizes egg production in the colonies; the other workers (that are all virgin) have undeveloped ovaries (except 1–2 individuals). When the gamergate is experimentally removed, a large number of these workers soon lay eggs (Fukumoto et al., 1989; Peeters and Higashi, 1989).

This study on the Japanese species of *Diacamma* focused on the interactions among mutilated workers following experimental removal of the gamergate. Since these workers lack gemmae, they can never mate and are restricted to the production of males. Fukumoto et al. (1989) divided one colony into 6 groups, and observed that, after 12 days, eggs were laid in the 5 groups lacking the gamergate. We have extended these investigations and found that one worker in each group soon becomes aggressively dominant and inhibits others from laying eggs.

Methods

Field collections

Only one species of *Diacamma* occurs in Japan. It should be referred to as *Diacamma* sp. (Morisita et al., 1989) and not *D. rugosum*. Our colonies were collected in Nakijin Castle and Chinen, Okinawa island, southern Japan. In both localities colonies occur in disturbed wooded habitats. Ten colonies were excavated between April and September 1990. Care was taken to collect every adult and all the brood.

Culture methods

Colonies were maintained in the laboratory and studied soon after collection. They were housed in plastic boxes lined with white absorbent paper and with a raised roof of clear perspex. The ants soon tore up the paper (which was kept moist) and constructed chambers with distinct entrances. Fresh insect food (cockroaches and mealworms) was provided.

Experimental treatment

Eight colonies were divided into 4–7 (depending on initial colony size) orphaned groups of workers (this treatment is hereafter termed "sociotomy"). Exclusion of the gamergate is an easy procedure in this species of *Diacamma* since she can be visually recognized (gemmae are present). Workers were randomly distributed among the groups. All cocoons were removed in order to study interactions involving mutilated workers only. No eggs were initially present in the experimental groups. At varying intervals of time, all newly laid eggs were removed, counted, and destroyed (Tab. 1). After different predetermined time periods, all ants belonging to one group were killed simultaneously and their reproductive status determined by dissection (Tab. 2).

Observation methods

In 6 of the above orphaned groups, all individuals were painted with unique color markings. Due to the cuticular sculpturing and pilosity, color marks persisted for several weeks. These 6 groups were observed daily for 1–2 weeks. Entire nest arenas were routinely placed under a dissecting microscope for detailed study of interactions between ants. In two groups, the dominant worker was removed after a few days. All individually marked workers were dissected after completion of the observations.

Descriptions of behaviors

In each observation round we recorded every occurrence of oviposition, oophagy, and aggressive interactions. Egg-laying is a conspicuous act during which the gaster is

Table 1. Time course of oviposition in 43 orphaned groups created from 8 colonies "1-A1" means group 1 from colony A1. Eggs were counted and destroyed after various intervals of time. Group size ranged from 15 to 30 workers. Blanks (–) indicate no information; workers were dissected in some groups (see Table 2)

Group code	1–3 days	4–6 days	7–9 days	10–20 days
1-A1	3	1	4	–
2-A1	0	0	0	25
3-A1	0	0	0	22
4-A1	1	1	7	72
5-A1	0	0	2	28
1-A2	0	0	–	38
2-A2	2	7	–	–
3-A2	1	7	–	27
4-A2	0	1	–	16
5-A2	0	1	–	18
1-A4	0	–	1	42
2-A4	1	–	5	–
3-A4	0	–	0	20
4-A4	0	–	4	19
5-A4	0	–	3	30
6-A4	0	–	4	4
7-A4	5	–	3	23
1-B2	0	0	0	22
2-B2	0	0	0	29
3-B2	0	0	0	22
4-B2	0	0	0	31
5-B2	7	6	11	43
1-B3	3	3	6	–
2-B3	0	0	0	17
3-B3	0	0	0	20
4-B3	0	0	0	18
5-B3	0	0	0	33
6-B3	7	6	13	40
1-E1	0	0	2	16
2-E1	0	0	0	31
3-E1	0	0	3	31
4-E1	0	0	5	31
5-E1	0	0	2	47
6-E1	0	0	0	49
1-F1	0	0	3	43
2-F1	0	0	5	45
3-F1	5	5	5	39
4-F1	0	0	2	36
5-F1	0	0	5	32
1-F2	0	0	1	38
2-F2	0	0	0	24
3-F2	0	0	0	56
4-F2	0	0	0	88

Table 2. Dissection results from 17 orphaned groups of workers without cocoons, isolated for different periods of time. Eggs were laid in each group. All workers alive in each group were dissected

Group code	Weeks together (n)	Workers dissected (n)	Active ovaries (n)	Resorbed ovaries (n)
1-A1	1	16	1–2[1]	0
6-F3	2	14	4	1
7-F3[2]	2	26	4	4
4-F1	3	16	5–6	3
1-E2	4	13	2	5
3-F1[2]	4	8	1	5
5-F1[2]	4	15	1	7
3-F2[2]	4	32	16	1
2-F2[2]	5	23	1	10
4-F2[2]	6	24	1	6
5-A1	6	15	1	6
1-B2	7	12	1	2
2-B2	7	10	1	1
4-B2	7	16	1	5
5-B2	7	11	1	5
1-C1	11	8	1	4
0-C2	11	16	1	4

[1] In others, oocytes had begun to develop.
[2] These workers were individually marked and their interactions were observed before dissection (see Tables 3 to 7).

flexed, the sting is extruded, and the genital valves open and close. This sometimes lasts for 5–15 min before an egg appears.

Different levels of aggression were noted. (a) *Antennal boxing* – vigorous and prolonged beating of the antennae above the head of another worker. Challenged ants either ignored this or responded timidly by crouching down with antennae drawn back. (b) *Bite and jerk* – forward lunge with open mandibles, followed by biting of limbs or antennae of the challenged ant, that are sometimes jerked to-and-fro. Challenged ants usually responded passively, but in a few instances "bite and jerk" escalated into mutual fighting, with stinging attempts and dragging. (c) In a few instances, *avoidance* was observed – one ant with antennae stretched forward approached another and the latter crouched submissively before physical contact occurred. For each experimental group, a matrix of dominance relationships was constructed on the basis of the above aggressive acts (Tabs. 3 to 7). A maximum of two interactions per day were recorded for each pair of workers. This was done to avoid biassing the cumulative totals for each ant, since the same act between two specific individuals was sometimes repeated over and over again during a short period of time. In this way, a *qualitative* picture of the pattern of aggression was obtained, that emphasized the direction rather than the frequency of aggression. Colony snapshots were also made to document the activities of individual ants, e.g. foraging or carrying eggs.

Table 3. Aggressive intractions among orphaned workers and correlates of dominance in group 3-F2. Observations started 3 weeks after sociotomy and continued for 6 days (6 hours). The alpha worker (BB), and RR, were dissected 2 days before the others

		Subordinate																Total times dominating
		BB	RG	WR	RR	GR	WG	WX	RB	WB	YY	RW	YG	XB	XW	GW	XG	
	BB	–	5		5	6	1							2				19
	RG		–	5	4	4	4	2				3		1				23
	WR			–	9	11	3	2	1	1		3	1	3	1			35
	RR				–	5	6	4	3	3		1		1	1			24
	GR			1	7	–	1	2	1					2				14
	WG						–	3				1		6				10
Dominant	WX				1	1		–						4			1	7
	RB							4	–	2					1			7
	WB							1		–					1		2	4
	YY								1		–		1		2			4
	RW						1					–		3				4
	YG					2		2					–	1				5
	others																	0
Total times dominated		0	5	6	26	29	16	20	6	6	0	8	2	23	6	0	3	156
Oviposition records		5	3	0	0	3	3	2	2	2	1	1	0	1	4	1	0	N = 28
Oophagy records		7	5*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N = 12
Ovaries		b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	
Mature oocytes		7	4	1	2	1	1	2	3	3	4	5	3	2	2	4	2	

* These were stolen after the alpha worker had been removed; only one was eaten.
a: undeveloped; b: active; c: resorbed.

Table 4. Aggressive interactions among workers and correlates of dominance in orphaned group 2-F2. Observations started 2 weeks after sociotomy and continued for 3 weeks. The alpha worker (RY) was removed on the 7th day of observations; the number of interactions before (5h45 of observations) and after (4h00) her removal are indicated separately. The alpha worker was observed to steal 11 eggs, 7 of which were eaten; after the alpha worker was removed, YY stole 3 eggs

		Subordinate												Total times dominating
		RY**	YY	GY	RX	YR	XG	XR	RG	GR	WX	GG	GX	
	RY	–	10	1	3	2			4	1	5	2		28
	YY		–	2; 6	2; 8	1; 7	0; 1	1; 2	0; 1		3; 1		0; 1	9; 27
	GY			–		3; 5					4; 1	1; 0		8; 6
Dominant	RX				–		2; 2	2; 1	0; 1		4; 0			8; 4
	YR				1; 1	–	2; 3	1; 1	1; 0			2; 1		7; 6
	XG									2; 0	1; 0	2; 0		5; 0
	XR				1; 0			–			4; 1			5; 1
	others													0; 0
Total times dominated		0	10; 0	3; 6	7; 9	6; 12	4; 6	4; 4	5; 2	3; 0	21; 3	7; 1	0; 1	70; 44
Oviposition records		1	2; 3	2; 0	0; 0	0; 2	0; 1	0; 0	2; 1	3; 0	2; 0	2; 0	1; 0	N = 15; 7
Ovaries			b	c	c	c	c	c	c	c	?	?	c	
Mature oocytes			0	0	0	0	0	0	0	0			0	

a: undeveloped; b: active; c: resorbed.
** transferred to 4-F2.

Table 5. Aggressive interactions among orphaned workers and correlates of dominance in group 4-F2. Observations began 3 weeks after sociotomy and continued for 11 days (4 hours of observation). The alpha worker (GG) was observed to eat 4 eggs. RY was formerly the alpha worker in 2-F2, and was transferred to this group when observations began. A few other workers that were never attacked exhibited resorbed ovaries

		Subordinate												Total times dominating
		GG	RY	XW	GR	BX	WY	BY	RR	YW	YB	RX	YBY	
Dominant	GG	–	8	8		9	6	3	3	4			2	43
	RY		–								1			1
	XW			–					1			1		2
	BX			1		–			1				1	3
	WY					3	–							3
	RR				1				–		1			2
	others													0
Total times victim		0	8	9	1	12	6	3	5	4	2	1	3	54
Oviposition records		4	2	2	1	0	0	1	0	0	0	0	0	N = 10
Ovaries		b	c	?	?	c	c	c	c	c	?	c	c	
Mature ocytes		3	0			2	0	0	0	0		0	3	

b: active; c: resorbed.

Table 6. Aggressive interactions among orphaned workers and correlates of dominance in group 5-F1. Observations started two weeks after sociotomy and continued for 10 days (6 hours). The alpha worker (RR) was responsible for all 10 records of oophagy

		Subordinate									Total times dominating
		RR	XY	YY	GG	GR	YR	YX	XG	GY	
Dominant	RR	–	4	3	3	3	1	1	1	1	17
	XY		–					3			3
	YY			–				1		1	2
	GG				–	1					1
	GR					–		1			1
	others										0
Total times dominated		0	4	3	3	4	1	6	1	2	24
Oviposition records		11	3	2	3	1	4	0	0	0	N = 24
Ovarian activity		b	c	c	?	c	c	c	c	c	
Mature oocytes		6	3	3		2	0	3	0	1	

a: undeveloped; b: active; c: resorbed.

Table 7. Aggressive interactions among orphaned workers and correlates of dominance in group 7-F3. Observations started 8 days after sociotomy (first egg was then laid) and continued for 6 days (2h30). The alpha worker (RW) was observed to steal 2 eggs, only 1 of which was eaten

		Subordinate												Total times dominating
		RW	GX	RX	RG	GR	GY	BX	WW	WR	BB	WY	RR	
Dominant	RW	–	7			1		1		5				14
	GX		–				7	2		6	1	3		19
	RX			–			3	1	3	1				8
	RG			1	–	3				2				6
	GR					–				2	3			5
	others													0
Total times dominated		0	7	1	0	4	10	4	3	16	4	3	0	52
Oviposition records		2	2	0	0	1	0	0	0	0	0	0	0	N = 5
Ovaries		b	b	c	b	b	a	a	a	c	c	a	c#	
Mature oocytes		5	5	2	5	3	–	–	–	0	2	–	3	

distinct yellow bodies present in this ant.
a: undeveloped; b: active; c: resorbed.

Ovarian dissections

In *Diacamma* sp. from Japan, unmated workers living with a gamergate characteristically exhibit completely undeveloped ovaries (Fukumoto et al., 1989). In groups of orphaned workers, however, significant numbers have enlarged ovaries with yolky oocytes, and in this study it was necessary to determine who was able to oviposit. Various characteristics are normally considered to assess reproductive activity (e.g. number and size of yolky oocytes, including "mature" ones which are as large as an egg and have a chorion; length of ovarioles relative to the eggs; presence of yellow bodies). In this study these characters did not always vary concordantly. Although yellow bodies ("corpora lutea") are a useful indicator of previous oviposition, and are conspicuous in the gamergates of *Diacamma*, they were seldom detected in the ovaries of workers that had begun to lay eggs following removal of the gamergate. This is possibly because the yellow bodies only become distinguishable several days after oviposition. Another difficulty was the occurrence of previously ovipositing workers that had resorbed ovaries as a consequence of subordinate status. Accordingly, we decided to classify ovaries into the following categories:

(a) *non-functional* – oviposition cannot occur. Included are ovaries in which immature oocytes are present.

(b) *active* – capable of oviposition. Individuals in this category exhibit considerable variation in fecundity, as evidenced by differences in ovariole length and number of mature oocytes. As mentioned earlier, yellow bodies may not yet be visible if the onset of oviposition was recent.

(c) *resorbed* – oogenesis has stopped. Developing oocytes and nurse cells are not found; ovarioles are shorter; large oocytes may be found, but the yolk appearance is abnormal (i.e. mottled). The differences between (b) and (c) were very clear when egg production had stopped several weeks earlier, but there was uncertainty when oogenesis had ceased more recently.

Results

Oviposition by orphaned workers

In 10/43 groups of workers isolated from the gamergate, eggs were laid within 1–3 days (Tab. 1). This suggests that one or two virgin workers were ovipositing in the presence of the gamergate in 6/8 original colonies.

The onset of oviposition by previously inactive workers varied between groups. In 16 groups, the first eggs were laid after 7–9 days, while in all remaining groups they were found after 10–20 days. The number of eggs found quickly increased (Tab. 1), suggesting that several workers in each group had begun to oviposit.

Ovarian activity in orphaned workers

A total of 275 workers were dissected (Tab. 2); these originated from 17 experimental groups that had been isolated from the gamergate for increasing periods of time. In one group that had been isolated for only one week, one worker with very well-developed ovaries (and faint yellow bodies) was found. Since eggs were laid immediately following sociotomy, it seems that this worker already oviposited while the gamergate was present.

Two to three weeks after sociotomy, several workers with active ovaries were found in the various groups. In contrast, four or more weeks after sociotomy, only one worker (irrespective of group size) had active ovaries in all but two groups (Tab. 2). The ovaries of several other workers were then clearly resorbed, reflecting that they had previously laid eggs.

Aggressive interactions among orphaned workers

One or two weeks after sociotomy, frequent and conspicuous aggression was observed between some of the members of all marked groups. The proportion of workers that initiated or were the victims of attacks ranged from 38 % to 60 % in each group; such ants never foraged outside the nest. "Bite and jerk" (see Methods) occurred most frequently and provided unambiguous information about dominance status. Examination of the matrix of dominance relationships for each group (Tab. 3 to 7) always revealed the same pattern of aggression. One worker attacked others more or less frequently (this ranged from 80 % of all attacks in 4-F2 to 12 % in 3-F2), although she herself was never attacked. Most of her aggressive acts were directed towards only a few of the workers involved in the dominance orders. Another reliable characteristic of this dominant individual (hereafter called *alpha*) was her frequent destruction of the eggs just laid by others. In contrast, oophagy by subordinate individuals was never observed.

With the exception of groups 4-F2 and 5-F1, a second-highest ranked worker (called *beta*) could be recognized, on the basis that she was only attacked by the alpha worker. The beta worker was aggressive towards other subordinates, but only in 7-F3 did she initiate most attacks. When the alpha worker was removed from groups 2-F2 and 3-F2, she was replaced by the beta worker. In 2-F2, the number of interactions (antennal boxing mostly) initiated by the beta worker increased from 13 % to 61 % (Tab. 4). In both groups, the beta worker started to steal freshly laid eggs within a few hours, although she only began to steal and eat them after a few days.

The alpha worker was transferred from 2-F2 (where she initiated 40 % of all attacks) to another group (4-F2) originating from the same colony. She was then attacked exclusively by the resident alpha worker, and she exhibited little aggression towards other workers. When dissected (12 days after the transfer), her ovaries were resorbed.

Besides the two highest-ranking workers, the relationships among other aggressive workers were unclear. The absence of a linear dominance hierarchy was confirmed by using various statistical tests (Appleby 1983). While some workers initiated attacks and were themselves victims, others never initiated attacks. In 7-F3, ant WR was frequently aggressed by both alpha and beta workers (she was the victim in 31 % of the total number of attacks), although she was never aggressive. The direction of attacks between pairs of ants was always the same, with few exceptions.

Correlates of dominance

The alpha worker laid eggs more often than the other workers (except in group 2-F2, Tab. 4). With the conspicuous exception of the alpha worker, all workers capable of ovipositing (as determined by observations or dissections) were attacked. Although many workers were observed to oviposit, this was not a complete record since dissections revealed that additional workers had mature oocytes in their ovaries. Out of 56 workers who were victims of attacks, 43 had active ovaries (76%); some of the others may have been resorbed. Out of 35 workers who initiated attacks, 31 had active ovaries (89%). Therefore, there is a distinct association between dominance order and ovarian activity (sometimes this is obscured by the time lag between observations and dissection).

Many subordinate individuals that had previously oviposited, exhibited resorbed ovaries at the time of dissection. Thus, the establishment of a dominance order is associated with temporal changes in the ovarian activity of participants; only the alpha worker continues to produce eggs. In group 7-F3, workers were dissected two weeks after sociotomy. Four workers (including alpha and beta) had active ovaries and were involved in attacks on others (Tab. 7). Another four workers had resorbed ovaries with mature oocytes still present. In contrast, only the alpha worker had active ovaries in another group dissected after four weeks; in 5-F1, five workers that had been observed earlier to lay eggs, exhibited resorbed ovaries (Tab. 6). In another group dissected after four weeks (3-F2), 16 workers had active ovaries (including alpha) and 12 of these had oviposited earlier (Tab. 3). There were no workers with resorbed ovaries, presumably because the hierarchy was not yet established. Finally, in groups that were dissected six weeks after sociotomy (2-F2 and 4-F2), ovarian resorption in subordinate workers (12 of which had oviposited earlier) was more marked; no mature oocytes were found (Tabs. 4 and 5). Only the alpha worker had active ovaries.

The characteristics of ovarian resorption could be determined accurately since some of the dissected workers had been observed to lay eggs a few days or weeks earlier. The extent of ovarian resorption varied not only with the length of the isolation periods, but also between individuals in each groups. Four weeks after sociotomy, various workers had ovaries with one or more large and chorionated oocytes, although oogenesis had obviously stopped (no developing oocytes). The presence of a chorion appears to impede oosorption, since mature oocytes may remain (it is not known whether they are sometimes laid; in 3-F1, ant GR had ovaries classified as "resorbed", but she had oviposited 2 ½ days earlier). Seven weeks after sociotomy, ovarian resorption was more advanced, because no more yolky oocytes remained. The bases of ovarioles were sometimes swollen with undifferentiated oocyte material. In some marked workers, ovarioles were so resorbed that they could not be distinguished from those of workers that presumably never oviposited. The physiology of oosorption is reviewed by Bell and Bohm (1975).

Oophagy

The usual sequence of events leading to oophagy began with the alpha worker antennating rapidly towards a subordinate worker in the egg-laying position. The alpha worker often stayed close to the latter, sometimes antennating the subordinate (however, beta workers were occasionally attacked). Once an egg had been laid, it was either stolen by the alpha worker while its end was still in the mother's gaster, or picked up by the mother and then stolen from her mandibles. Eggs were punctured and the contents were sucked until only the chorion remained. The eggs laid by the alpha worker were never stolen or destroyed by other workers.

In not all cases did the alpha worker succeed in abducting an egg quickly. The alpha worker was unable to steal eggs while she was herself ovipositing, or if a mother managed to defend her egg by running away or by using her gaster to block the alpha worker's attempts. In this species the eggs are always held together in distinct packets. In some cases a mother was able to incorporate her newly laid egg into an existing packet; it was then never stolen. The alpha workers in some groups destroyed eggs more efficiently than those in others; some stopped eating the eggs they stole.

An idea of the characteristics of egg cannibalism can be obtained from the details of a 2-hour observation session on group 5-F1:

First day of observations (no eggs yet present); RR is the alpha worker; XY is the beta worker; the other three workers (GG, GR, YR) were all involved in dominance interactions (see Table 6)

0935h	XY lays an egg; stolen by RR as it comes out of gaster, and eaten
0940h	GG lays an egg; stolen by RR as it comes out of gaster, and eaten
1017h	YR and RR both in oviposition posture, next to each other; no interactions; after respective eggs are laid, they are taken between the mandibles; RR antennates YR and shows interest in her egg, but she obviously cannot steal it
1026h	egg of YR is taken by GR and held between mandibles
1030h	GG and XY are again in oviposition posture
1040h	RR drops her egg on the floor; picked up by GR
1045h	GG lays an egg; stolen by RR, licked and moved between mandibles, but not eaten; egg is then dropped, and picked up by GR
1047h	XY lays an egg, and holds it between her mandibles; RR tries to steal it, but XY manages to defend her egg
1100h	XY walks to GR (who is still carrying the other eggs) and adds her egg
1115h	XY is now carrying the packet of 4 eggs

Six hours later, 7 eggs are in the packet. Next day at 1600h, only 10 eggs are present, which suggests that many eggs were being destroyed (given that oviposition is so frequent). Four days later, RR pays no interest to the eggs that she frequently lays (they are left on the floor); she is now ruthlessly efficient during oophagy.

Variability between groups

Different levels of aggression were evident in the various experimental groups (although observations started later in some groups). In 4-F2, a hierarchy already seemed established three weeks after sociotomy. Attacks continued, with the alpha worker being responsible for 80% of them (Tab. 5). In contrast, in 3-F2 (also observed three weeks after sociotomy), the alpha worker initiated a minority of the attacks, while subordinates continued to be very aggressive (Tab. 3). Thus, we are seeing different stages along the establishment of a stable dominance order. If a rank order exists in the presence of the gamergate, then some variability may be due to the composition of experimental groups.

Discussion

Our results indicate that dominance interactions (including physical attacks and egg cannibalism) among recently orphaned workers of *Diacamma* sp. result in the regulation of their reproductive activity. Although several workers were initially observed to oviposit in each group, their ovaries were no longer active when dissected a few weeks later. Indeed, the only individual who then had active ovaries was always the one identified as being dominant on the basis of her behavior. It is not known how particular workers are selected for attacks; there is no variability in size between *Diacamma* workers. Either there is personal recognition of ants encountered previously, or there is perception of a rank-specific odor (e.g. chemical cues associated with aggressiveness or oogenesis). In *Polistes gallicus*, dominant status is associated with elevated juvenile hormone titer in the hemolymph, together with ovarian activity (Röseler et al., 1984). However, ovariectomized dominant foundresses behave like dominant foundresses with ovaries (Röseler and Röseler, 1989), indicating that dominance does not depend on high ecdysteroid levels. We may assume that similar physiological mechanisms operate in ants. Being the victim of frequent attacks may lead to changes in juvenile hormone, which in turn affect ovarian activity.

Egg cannibalism is an important component of the dominance order in *Diacamma* sp. In our small experimental groups, most eggs laid by subordinates were eaten. This would be harder to achieve in natural nests. It is possible that chemical cues originating from the tip of the gaster help the alpha worker detect ovipositing nestmates. Eggs do not seem to be eaten once they are several minutes old or after they have been added to a packet. This is unlike the situation in *Pachycondyla obscuricornis* (Oliveira and Hölldobler, 1991), where some workers shuffled their newly laid eggs within the egg pile; 8/42 eggs destroyed were stolen from the egg pile.

Results in Table 1 are further evidence that in *Diacamma* sp. most workers have undeveloped ovaries in the presence of the gamergate (in 33/43 orphaned groups, eggs were only laid after 7–15 days). Nonetheless, there were 10 orphaned groups in which oviposition occurred immediately after sociotomy. This confirms the finding by Fukumoto et al. (1989) that one or two mutilated workers have active ovaries despite the gamergate's presence (they found mutilated laying workers in 9/15 colonies). Taki (1991) has found that in colonies with a gamergate, a proportion of the mutilated workers are involved in aggressive dominance interactions. The highest-ranking worker lays eggs, but these are stolen and eaten by the gamergate. Thus, the occurrence of dominance orders in small groups of orphaned workers (this study) may be a reflection of what happens in much larger groups with a gamergate (average colony size is 118 ± 88 workers; Abe and Uezu, 1977). The physical attacks among recently orphaned workers seem to be more frequent and intense; however, once an alpha worker has established her supremacy, the level of aggression decreases.

In group 3-F1, eggs were laid immediately after sociotomy. Thus, one worker had been ovipositing in the presence of the gamergate and she presumably became the alpha worker. Nevertheless, three other workers began to lay eggs later, indicating that they had been inhibited by the gamergate, but not by the alpha worker. When dissected, these three workers had resorbed ovaries, probably as a consequence of the alpha worker's subsequent aggression.

All cocoons had been removed from our orphaned worker groups, because the emergence of young workers with gemmae would have disrupted the experiments. When colonies become orphaned under natural conditions, diploid cocoons would be present. One of the new workers would keep her gemmae and behave very aggressively. In addition to mutilating all subsequently emerging workers, this unmutilated virgin attacks other workers in a manner identical to that observed among orphaned workers (Peeters, unpublished; also in *D. australe*, Peeters and Higashi, 1989). She soon begins to oviposit and eat those eggs laid by mutilated workers. The attacks between the unmutilated virgin and her nestmates would end after she had mated and become the new gamergate. Indeed, in both *D. australe* and the Japanese species, aggressive interactions between the gamergate and other workers are not observed (except for the amputation of gemmae in new workers) (Peeters and Higashi, 1989; Fukumoto et al., 1989). This strongly suggests that the gamergate signals her presence pheromonally.

In both *Diacamma* sp. and *D. australe*, the incidence of mating and oviposition are regulated by separate mechanisms. Indeed, workers lacking gemmae cannot mate, and thus an alpha worker with active ovaries will remain virgin. This is unlike other queenless ponerines, such as *Pachycondyla sublaevis* and *Streblognathus aethiopicus*, in which those workers having active ovaries appear sexually attractive (Ito and Higashi, 1991; Ware et al., 1990). In *P. apicalis*, in which the queen caste occurs, dominance interactions involve workers only (Oliveira and Hölldobler, 1990). Dominant workers usually had better developed ovaries and laid more eggs than subordinate ones. However, workers never mate in *P. apicalis*, and intracolonial aggression occurs over the production of males (as in *Diacamma* sp.).

Colonies of *Diacamma* sp. are strictly monogynous. Furthermore, there is a strong suggestion that the gamergates are mated by one male only. The male gaster remains attached to that of the gamergate for over 12 hours, even while the male is being dismembered by workers (Fukumoto et al., 1989; Peeters, unpublished). Therefore, the genetic relatedness among nestmates may be close to the maximum possible in outbred hymenopteran societies (depending on the frequency of gamergate replacement). Nevertheless, intensive conflict over personal fitness occurs among orphaned workers. As Bourke (1988) noted for *Harpagoxenus sublaevis*, such a high observed degree of selfishness between highly related workers is only expected when workers can produce males. These results on *Diacamma* sp. add to our understanding of the mechanisms regulating division of labor in queenless ponerines. They should also contribute to a better appreciation of the importance of intracolonial conflict over reproduction in phylogenetically primitive ants.

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References

Abe, T. and K. Uezu, 1977. Biology of *Diacamma rugosum* (Le Guillou) in the Ryukyu Islands with special reference to foraging behaviour. Proc. VIII Congr. IUSSI, Wageningen, pp. 142–143.

Appleby, M. C., 1983. The probability of linearity in hierarchies. *Anim. Behav. 31*:600–608.

Bell, W. J. and M. K. Bohm, 1975. Oosorption in insects. *Biol. Rev. 50*:373–396.

Billen, J. and C. Peeters, 1991. Fine structure of the gemma gland in the ant *Diacamma australe* (Hymenoptera, Formicidae). *Belg. J. Zool. 121*:201–210.

Bourke, A. F., 1988. Dominance orders, worker reproduction, and queen-worker conflict in the slave-making ant *Harpagoxenus sublaevis. Behav. Ecol. Sociobiol. 33*:323–333.

Bourke, A. F., 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum. Anim. Behav. 42*:295–310.

Cole, B. J., 1981. Dominance hierarchies in *Leptothorax* ants. *Science 212*:83–84.

Franks, N. R. and E. Scovell, 1983. Dominance and reproductive success among slave-making worker ants. *Nature 304*:724–725.

Fukumoto, Y., T. Abe and A. Taki, 1989. A novel form of colony organization in the "queenless" ant *Diacamma rugosum. Physiol. Ecol. Japan 26*:55–61.

Heinze, J., 1993. Queen-queen interactions in polygynous ants. In: *Queen number and sociality in insects* (L. Keller, ed.), Oxford University Press (in press).

Heinze, J. and T. A. Smith, 1990. Dominance and fertility in a functionally monogynous ant. *Behav. Ecol. Sociobiol. 27*:1–10.

Ito, F. and S. Higashi, 1991. A linear dominance hierarchy regulating reproduction and polyethism of the queenless ant *Pachycondyla sublaevis. Naturwissenschaften 78*:80–82.

Medeiros, F. N., L. E. Lopes, P. R. Moutinho, P. S. Oliveira and B. Hölldobler, 1992. Functional polygyny, agonistic interactions and reproductive dominance in the Neotropical ant *Odontomachus chelifer* (Hymenoptera, Formicidae, Ponerinae). *Ethology 91*:134–146.

Morisita, M., M. Kubota, K. Onoyama, K. Ogata, M. Terayama, M. Kondoh and H. T. Imai, 1989. A guide for the identification of japanese ants (I). *The Myrmecological Society of Japan* 42 pp. (in japanese).

Oliveira, P. S. and B. Hölldobler, 1990. Dominance orders in the ponerine ant *Pachycondyla apicalis* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol. 27*:385–393.

Oliveira, P. S. and B. Hölldobler, 1991. Agonistic interactions and reproductive dominance orders in *Pachycondyla obscuricornis* (Hymenoptera: Formicidae). *Psyche 98*:215–225.

Peeters, C., 1993. Monogyny and polygyny in ponerine ants with or without queens. In: *Queen number and sociality in insects* (L. Keller, ed.), Oxford University Press (in press).

Peeters, C. and S. Higashi, 1989. Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe. Naturwissenschaften 76*:177–180.

Peeters, C. and J. Billen, 1991. A novel exocrine gland inside the thoracic appendages ("gemmae") of the queenless ant *Diacamma australe. Experientia 47*:229–231.

Röseler, P.-F. and I. Röseler, 1989. Dominance of ovariectomized foundresses of the paper wasp, *Polistes gallicus. Ins. Soc. 36*:219–234.

Röseler, P.-F., I. Röseler, A. Strambi and R. Augier, 1984. Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp, *Polistes gallicus. Behav. Ecol. Sociobiol. 15*:133–142.

Taki, A., 1991. Dominance orders and reproductive division of labour in the ponerine ant, *Diacamma* sp. 22nd International Ethological Conference, Kyoto p. 37 (Poster abstract)
Ware, A. B., S. G. Compton and H. G. Robertson, 1990. Gamergate reproduction in the ant *Streblognathus aethiopicus* Smith (Hymenoptera: Formicidae: Ponerinae). *Ins. Soc. 37*:189–199.

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