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Worker oviposition and policing behaviour in the myrmicine ant *Aphaenogaster smythiesi japonica* Forel

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In eusocial Hymenoptera, worker reproduction is affected by various factors, especially the genetic structure of the colony. Kinship theory predicts that hymenopteran workers prefer to produce sons rather than brothers when a single, once-mated queen is in a colony, while such worker reproduction is expected to be mutually inhibited by other workers under multiple mating of the queen. To test these predictions we observed the behaviour of the myrmicine ant *Aphaenogaster smythiesi japonica*. Observation of the social structure of 60 queenright colonies indicated monogyny and microsatellite DNA analysis of 14 colonies showed monandry. Under laboratory conditions, workers with functional ovaries laid only trophic eggs in the presence of the queen and produced viable eggs in her absence. In an experiment in which colonies split and reunited, workers that had well-developed ovarioles with viable oocytes were frequently attacked by other workers from the queenright groups. The number of oocytes in a worker's ovarioles was positively correlated with the frequency of being attacked. The results show that a worker's production of males in this species is potentially inhibited by worker policing, contrary to the prediction that worker policing is not predominant in monogynous and monandrous societies.

In eusocial Hymenoptera (ants, wasps and bees), there is a division of labour between reproductive and sterile females, with colonies showing cooperative behaviour to rear their brood and striking group level adaptations. However, conflicts over reproduction among castes are also expected because of the existence of genetic asymmetries (Hamilton 1964; Trivers & Hare 1976). In a colony with a single, once-mated queen, workers should rear the queen's daughters, their sisters, which are more closely related to them (r=0.75) than their own daughters are (r=0.5). On the other hand, their sons are always more closely related to them (r=0.5) than the queen's sons, their brothers, are (r=0.25). It is hypothesized that workers try to lay and rear their male eggs selfishly while the queen inhibits this action to force them to rear her male eggs.

In most ant species, workers have functional ovarioles for producing unfertilized male eggs (Bourke 1988; Hölldobler & Wilson 1990). Nevertheless, some studies have shown that the contribution of workers to male production could be of only minor importance in colonies with queens (Bourke 1991; Pearson et al. 1995; Heinze et al. 1997; Walin et al. 1998). Three hypotheses may explain the absence of worker reproduction when a queen is present. First, queens may inhibit workers from producing male eggs by chemical or physical manipulation (Alexander 1974; Oster & Wilson 1978). This parental manipulation can be regarded as policing behaviour by the queen. Second, if the queen mates with several males, or several related queens are present in a colony, the average relatedness between workers will be reduced to below 0.75. In this case, workers would prefer to rear their brothers (r=0.25) because the average relatedness to their nephews is likely to be reduced. As a result, workers are expected to attack fertile workers, or eat male eggs produced by other workers, and mutually inhibit their production of males (worker policing, Ratnieks 1988; see also Starr 1984; Woyciechowski & Lomnicki 1987). Third, worker policing might also evolve as one of the colony's maintenance behaviours if male production by workers reduces colony efficiency, production of new adults or colony maintenance (Ratnieks 1988; see also Cole 1986). When policing invalidates selfish worker reproduction, self-restriction in which

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workers voluntarily control their oviposition (self-policing) may secondarily evolve (Ratnieks 1988). Policing mechanisms are strongly dependent on the social structure of each ant species.

Policing behaviour is currently the focus of attention as an ultimate factor responsible for regulating the selfish behaviour of workers (Ratnieks 1988; Monnin & Ratnieks 2001) and has been reported in a few ant species: *Harpegnathos saltator* (Liebig et al. 1999), *Diacamma* sp. (Kikuta & Tsuji 1999), *Gnamptogenys menadensis* (Gobin et al. 1999). These species belong to the subfamily Ponerinae, in which there are few morphological differences between queen and worker castes. In about 100 species in this group, a morphologically distinguishable queen caste has been lost secondarily and mated workers (gamergates) are capable of reproducing in place of the queen (Peeters 1991). In such species, the workers frequently and aggressively inhibit mating and ovarian development of other workers to increase their own chances of mating and oviposition (Ito & Higashi 1991; Monnin & Peeters 1999). Therefore, it seems to be difficult to distinguish between behaviour that inhibits other workers' mating and policing behaviour that inhibits their production of males. *Diacamma* sp. is an exception since workers with mutilated gammae cannot mate in this species (Fukumoto et al. 1989). To clarify whether worker policing is a common mechanism in ants, we should examine policing behaviour in nonponerine ants in which it is impossible for workers to mate and produce female eggs.

In the present study, we observed the worker policing behaviour of the myrmicine ant *Aphaenogaster smythiesi japonica*, in which queen and worker castes are morphologically distinguishable and differ in reproductive function. With observations and laboratory experiments, we examined the existence of mutual worker policing behaviour. We also investigated the social and genetic structures of the colonies to elucidate the ultimate factor in policing behaviour.

METHODS

Study Species and Field Collection

Aphaenogaster s. japonica Forel is a common species, distributed from Japan to the Korean Peninsula (Watanabe & Yamane 1992). We collected 60 colonies from a broad-leaved deciduous forest dominated by oaks near Kanazawa University in Kanazawa, central Japan, with the University's permission, from May to July in 1998–2000. To minimize impact on the ant population, we changed local collecting sites slightly each time during the research. Colonies were brought to the laboratory and kept in plastic boxes (17.0 × 25.0 cm and 4.5 cm high) with artificial plaster nests under a constant temperature of 23°C and natural 12:12 h light:dark cycle. They were fed mealworms every 3 days. In 1998, we killed six dealate queens and 922 workers, in six of 60 queenright colonies, by crushing the head, and dissected them immediately after field collection, to observe the ovarian development.

Estimation of Queen Mating Number

We conducted microsatellite analysis for samples collected in 1999–2000, to determine the mating frequency of queens. Queens, workers and males were taken from 14 colonies, killed by freezing and stored in ethanol (99.5%). Assuming that a queen mates with more than two males and that one male fathers up to 70% of workers in a colony, the probability that a rare patriline is not represented among 10 workers sampled randomly is less than 5% and therefore it is unlikely that nonsampling error would occur with several observed colonies. Thus, we obtained DNA samples from a queen and 10–11 workers per colony. For each sample, we extracted DNA by the phenol extraction method (Hasegawa 1995). A DNA fragment including a microsatellite region (locus As 1) was amplified by PCR on a thermal cycler (DNA engine, MJ Research Inc., Waltham, Massachusetts, U.S.A.) with genomic DNA as template. The microsatellite region was detected by the methods described in Hasegawa & Takahashi (2002). Primer sequences were as follows: sense primer labelled with Texas Red, 5′–CGA GGC CTA AGC CAC GAA AT and antisense primer, 5′–CTG GTA TCT CGC CCG TTT TT. PCR was conducted in a total volume of 10 µl containing template DNA, 0.2 µM of each designed primer, 0.2 mM of each dNTP, 1 × Ex Taq buffer and 0.625 unit of Ex Taq (TaKaRa). Amplification was performed as follows: initial 3-min denaturation at 95°C, 32 cycles of 1 min at 93°C, 1 min at 53°C, 2 min at 65°C, and a final 10-min extension at 72°C. PCR products and a size standard (pUC18 control DNA sequencing reactions) were separated by a sequencer apparatus (SQ-5500, Hitachi Co., Tokyo, Japan) on a polyacrylamide gel (6% Long Ranger (TaKaRa), 6.1 M urea and 1.2 × TBE (0.1 M Tris, 30 mM EDTA and 0.1 M boric acid)) in 0.6 × TBE buffer. Band sizes were estimated by computer software Fraglys version 2 (Hitachi Electronics Engineering Co., Tokyo, Japan).

To clarify the existence of worker reproduction, we also obtained DNA samples from males in six of these colonies in which paternal alleles differed from maternal ones on locus As 1. In these colonies, we can detect males with patriline-specific alleles with 95% probability by analysing more than five males per colony, under the assumption that all males were produced by workers. By analysing 30 males per colony, we can detect these males with 95% probability under the assumption that the frequency of worker reproduction was 20% (Walin et al. 1998). We therefore examined the genotypes of 5–30 males per colony and compared them with those of their queens.

Observation of Worker Oviposition

In 1999 and 2000, we observed the oviposition behaviour of workers in nine laboratory colonies composed of 50–350 workers and a single queen. Total observation time in each colony was 24–48 h. During the observation period, we counted the ovipositions and noted the fate of each egg to examine whether worker-derived eggs survived. Since it was rare for several workers to oviposit

simultaneously, all eggs produced during the observation periods could be followed. In five of the nine colonies, we also examined worker reproduction in the absence of the queen. After the queen and all eggs were removed, we observed the colonies for 8 h every 2 days for 1 month. We counted ovipositions by workers and noted the fate of each egg as well as the presence of the queen. We also counted the eggs in the egg piles every 2 days. After these observations, we killed each worker by crushing its head, and dissected it under a binocular microscope. We counted the ovarioles and oocytes and checked the shape and colour of the oocyte, the thickness of the follicle cell layer, and the presence of a yellow body to discriminate between viable and trophic eggs (Passera et al. 1968; Gobin et al. 1998; Gobin & Ito 2000; Tay & Crozier 2000).

Egg Fertility

To examine the ability of workers to produce viable eggs, we observed seven queenless colonies in 1999. In July of that year, all eggs, larvae and pupae were removed from these colonies and, for 5 months, we observed the production of eggs by workers to see whether the eggs developed into males.

Colony Splitting and Reunion

This experiment was performed to examine whether mutual inhibition of oviposition by workers, that is, worker policing, was present. In May 2000, we split five queenright colonies composed of 110–280 workers in half, to make queenright and queenless groups. For this observation, all queenless workers in four of five colonies were individually marked on the thorax and gaster with Paint Marker (Mitsubishi, Osaka, Japan). As a control to examine the effect of marking, all queenright workers in the other colony (colony number 000516(1)) were also marked. After 1 month, we observed each group for 24–30 h and recorded oviposition and aggressive behaviour by workers.

After the above observation, we moved all workers in queenless groups one by one to their own queenright counterparts and thereby reunited the two groups. The behaviour of workers and queens was continuously observed for 12 h in each colony. In particular, we noted the occurrence of aggressive behaviour between individuals and the response of workers in ex-queenright groups to those in ex-queenless groups. Finally, all queens and workers were killed by crushing the head and dissected to observe the development of ovaries.

RESULTS

Colony Genetic Structure

Colony size ranged from eight to 624 workers (median 275 workers, N=60). Only one dealate queen was in each colony, suggesting that this species is monogynous. Of 922 workers, 918 (99.6%) had a pair of ovarioles and only four had three or four ovarioles. The ovaries were well developed with a few oocytes but no yellow body. All queens were inseminated and had 16–32 ovarioles ($\bar{X} \pm$ SD=23.3 $\pm$ 5.3, N=6). Their ovarioles were well developed with many oocytes and large yellow bodies.

We examined the genotypes of 303 individuals, 14 queens, 143 workers and 106 males in 14 colonies (Table 1). The microsatellite locus (As 1) had five alleles with a heterozygosity of 0.69. In 13 colonies, the queens were heterozygous. Furthermore, workers in seven colonies had common patriline-specific alleles and queen-derived alleles (Table 1). In the other seven colonies, genotype arrays were consistent with the assumption of monandry of the queen (Table 1). Since only one microsatellite locus was examined, the probability of nondetection error in which each queen mated with several males having the same alleles was 31%. However, in all 14 colonies the genotype of the queen's presumed mating partner was of only one type, suggesting that most *A. s. japonica* queens mated singly.

To determine male paternity, we compared the genotypes of 106 males with those of queens in six colonies. No male had patriline-specific alleles in any of the colonies (Table 1). The probability of worker reproduction based on the assumption that workers produced all the males was less than 5% in each colony suggesting that most males were produced by queens.

Worker Oviposition

In nine queenright colonies, worker oviposition was seen 839 times during 302 h of observation ($\bar{X} \pm$ SD= 93.2 $\pm$ 71.3 times/colony; Table 2). Of these 839 eggs, 389 (46.4%) were immediately given to larvae as food by the layers or other workers; another 385 (45.9%) were eaten by the layers (199 eggs, 23.7%), other workers (79 eggs, 9.4%), and queens (107 eggs, 12.8%) and the remaining 65 eggs (7.7%) were broken by the layers or other workers and were deposited on the nest floor (Table 3). Thus, most eggs laid by the workers were consumed, and no eggs survived. Of 84 ovipositions by queens (mean/h 0.1–0.6 times, N=9), no queen-derived eggs were eaten and were instead brought by workers to egg piles.

In three of five artificial queenless colonies, new egg piles were found in the nests 12–28 days after orphaning. Subsequently, 447 ovipositions by workers were observed in these colonies during 184 h. Similar to the queenright colonies, 403 eggs (90.2%) were consumed by colony members, including the layers, and 34 (7.6%) were deposited. Only 10 eggs (2.2%) were brought to egg piles by the layers or other workers. The proportion of eggs that survived differed significantly between queenright and queenless colonies (Fisher's exact test: P<0.001). These eggs appeared to be reared by colony members.

All dissected queens (N=9) were inseminated and had 20–24 ovarioles ($\bar{X} \pm$ SD=22.9 $\pm$ 1.8). Their ovarioles were well developed with many cylindrical oocytes containing fine yolk, surrounded by a thick layer of follicle cells and large yellow bodies. All workers had a pair of ovarioles. In queenright and queenless colonies, 598 of 886 (67.5%) and 432 of 636 (67.9%) workers had developed ovarioles with more than one whitish oocyte, respectively. Most of

Table 1. Microsatellite genotypes of queens, workers and males

Colony no.	Queen	Presumed genotype of queen's mating partner	Number of workers		Number of males	
000501(1)*	ad	b	ab	bd	a	d
			5	5	9	21
000511(1)*	bc	d	bd	cd	b	c
			6	4	20	10
000514(1)†	be	a	ab	ae	b	e
			7	3	1	4
990417(1)*	ac	d	ad	cd	a	c
			4	6	19	11
990420(2)†	cd	a	ac	ad	c	d
			9	2	2	3
990421(1)	ac	a	aa	ac		
			5	5		
990427(1)	ae	a	aa	ae		
			3	7		
990505(1)†	ab	d	ad	bd	a	b
			4	6	4	2
990508(1)	ac	c	ac	cc		
			5	5		
990514(2)	ac	a	aa	ac		
			4	6		
990709(1)	ab	a	aa	ab		
			5	5		
990727(1)	aa	a	aa			
			11			
990808(1)	ab	c	ac	bc		
			5	5		
990723(1)	ac	a	aa	ac		
			6	5		

Genotype of the queen's mating partner was estimated by comparing the genotype of the queen with those of workers. The groups classified by the microsatellite analysis were named a, b, c, d and e. The lengths of PCR product in the groups were 194, 200, 203, 206 and 209 bp, respectively.

*In these colonies the probability of worker reproduction was less than 5% under the assumption that the frequency of worker-produced males was 20%.

†In these colonies the probability of worker reproduction was less than 5% under the assumption that workers produced all males.

Table 2. Observation of worker oviposition in nine colonies

Colony no.	Colony size	Observation time (h)	Number of ovipositions by workers	
			Total	$\bar{X}$±SD/h*
990418(1)	50	24	8	0.3±0.6
990507(1)	100	24	29	1.2±1.4
990508(1)	100	30	27	0.9±1.2
990723(1)	150	30	55	1.8±1.7
990726(1)	150	32	73	2.3±1.7
990806(2)	250	48	148	3.1±2.9
990727(1)	270	30	140	4.7±3.1
990803(1)	350	48	218	4.5±3.1
990808(1)	350	36	141	3.9±2.5

*Kruskal–Wallis test: H_8=76.5, P<0.001.

these workers had roundish oocytes with coarse yolk particles, surrounded by a thin layer of follicle cells, and no yellow body. Only five workers in the queenless colonies had cylindrical oocytes containing fine yolk and visible yellow bodies. The proportion of workers with yellow bodies differed significantly between queenright and queenless colonies (queenright colonies: 0/886; queenless colonies: 5/636; Fisher's exact test: P<0.05). These five workers had more oocytes than the other workers of queenright and queenless colonies (Kruskal–Wallis test: H_2=33.5, P<0.001).

Male Production by Orphan Workers

In seven queenless colonies, new egg piles were found 2 weeks after the removal of the queen. In five of seven colonies, new males (N=11) had emerged after 4–5 months. This indicates that *A. s. japonica* workers have the potential to produce males on their own.

Aggressive Behaviour

Aggressive behaviour differed between workers in queenright and queenless groups (Table 4). In five queenright groups, aggressive behaviour was observed only three times, whereas it was seen 592 times in five queenless groups. Most aggression consisted of one-to-one conflict, that is, two workers biting each other's antennae and legs.

Table 3. Fate of eggs laid by workers in nine queenright colonies

Colony no.	Colony size	Number of eggs	Number of eggs consumed				Deposited eggs
			By egg layer (%)	By other workers (%)	By queens (%)	By larvae (%)	
990418(1)	50	8	2 (25.0)	1 (12.5)	0	5 (62.5)	0
990507(1)	100	29	5 (17.2)	1 (3.4)	1 (3.4)	22 (75.9)	0
990508(1)	100	27	1 (3.7)	0	4 (14.8)	22 (81.5)	0
990723(1)	150	55	3 (5.5)	11 (20.0)	12 (21.8)	24 (43.6)	5 (9.1)
990726(1)	150	73	26 (35.6)	8 (11.0)	14 (19.2)	19 (26.0)	6 (8.2)
990806(2)	250	148	54 (36.5)	13 (8.8)	13 (8.8)	58 (39.2)	10 (6.8)
990727(1)	270	140	23 (16.4)	15 (10.7)	3 (2.1)	82 (58.6)	17 (12.1)
990803(1)	350	218	81 (37.2)	25 (11.5)	30 (13.8)	63 (28.9)	19 (8.7)
990808(1)	350	141	4 (2.8)	5 (3.5)	30 (21.3)	94 (66.7)	8 (5.7)

Table 4. Number of aggressive behaviours between workers in colony splitting and reunion experiment

Colony no.	Colony size	Number/h			Number after reunion (%)		
		Before reunion		After reunion	Total	By queenright	By queenless
		Queenright	Queenless				
000430(1)	110	0	1.5±1.5	12.8±5.9	153	151 (98.7)	2 (1.3)
000514(1)	224	0	4.5±3.7	2.8±2.0	33	33 (100)	0
000505(1)	250	0.1±0.3	6.0±3.8	4.8±4.2	58	43 (74.1)	15 (25.9)
000518(1)	280	0	5.9±4.8	8.4±2.6	101	87 (86.1)	14 (13.9)
000516(1)*	228	0	4.3±2.3	3.7±4.5	44	39 (88.6)	5 (11.4)

Mean are shown±SD. Each colony was observed for 24–30 h when the colony was split and 12 h when it was reunited.
*All workers in the queenright groups were individually marked with paint.

After the reunion, aggressive behaviour between workers was observed 389 times, but aggression by dealate queens was never observed. Aggression after the colony reunion was more varied and included: (1) single workers bit and pulled antennae, legs, petioles, gasters and thoraces of single nestmates (biting); (2) single workers dragged single workers out of the nest and bit and pulled the antennae or legs (pulling out); (3) several workers simultaneously bit and immobilized single nestmates (immobilization). In five reunited colonies, workers from queenright groups (queenright workers) attacked workers from queenless groups (queenless workers) in 353 of 389 (90%) cases of aggression. In queenright workers, the frequency of aggressive behaviour was higher after than before the reunion (Table 4). In all colonies involving the control colony (colony number 000516(1)), queenless workers never attacked queenright ones, suggesting that the direction of aggressive behaviour was fixed.

Not all queenless workers were attacked. Only 84 (19.9%) of 422 queenless workers were attacked by queenright workers in four reunited colonies in which queenless workers were individually marked. Workers that were attacked had well-developed ovarioles with many oocytes and some had visible yellow bodies and cylindrical oocytes. These workers had significantly more oocytes than workers that were never attacked (Kruskal–Wallis test: H_2=259.8, P<0.001; Fig. 1). Regardless of the experimental treatments (queenless or queenright conditions), nonattacked workers had less developed ovaries. Furthermore, the number of oocytes in ovarioles of attacked workers was positively correlated with the number of attacks on them by ex-queenright workers (Pearson correlation: r_{82}=0.51, P<0.001) in all four

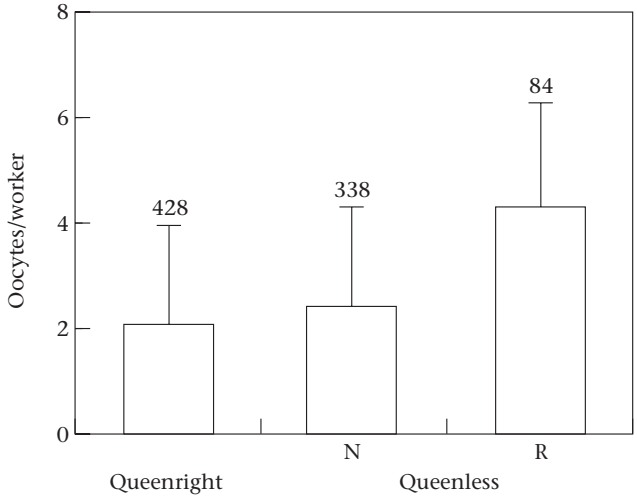

Figure 1. Number of oocytes ($\bar{X}$+SD) in ovarioles of workers in four reunited colonies: queenright workers, queenless workers that were attacked (R), queenless workers that were not attacked (N). Sample sizes are given above the bars.

colonies (colony number 000430(1): r_{21}=0.64, P<0.005; number 000505(1): r_{14}=0.75, P<0.001; number 000514(1): r_8=0.86, P<0.005; number 000518(1): r_{33}= 0.47, P<0.005). These results suggest that workers with viable eggs and yellow bodies were attacked more frequently than those with only trophic eggs.

Worker Oviposition in Colony Splitting and Reunion

Before the reunion, 227 and 266 ovipositions by workers were observed in five queenright and five queenless groups, respectively (mean/h: queenright: 0.6–2.8 times, queenless: 0.5–3.0 times). In queenright groups, all 227 eggs were eaten by colony members. In queenless groups, 13 (4.9%) of 266 eggs were brought to egg piles, although 253 (95.1%) were still consumed. After colony reunion, 191 ovipositions by workers were observed (mean 1.0–6.0 times) and 130 (68.1%) were laid by ex-queenless workers. Of these eggs, 126 (96.9%) were consumed by colony members but four (3.1%) were brought to egg piles. The eggs that survived were produced soon after the colony reunion; the layers were then attacked and their oviposition restricted. The ratio of eggs that survived after reunion was slightly decreased, although it was not significantly different from that before reunion (Fisher's exact test: P=0.59).

DISCUSSION

Our results suggest that behavioural interactions between *A. s. japonica* workers are important for the regulation of a worker's production of males. Three hypotheses may explain the aggression seen after colony reunion: (1) dominance behaviour to monopolize the production of males; (2) hostile behaviour caused by a change in colony odour, and (3) worker policing behaviour. In the queenless groups, aggressive interactions might have been dominance behaviour among workers competing to reproduce. The observations that only a few workers in orphan colonies of this species are aggressive and produce fertile eggs support this interpretation (S. Iwanishi, personal observation). In reunited colonies, however, only ex-queenright, reproductively inactive workers were aggressive, attacking ex-queenless active workers. Yamaoka (1997) reported that, in *Formica* sp. 5, cuticular hydrocarbon profiles of the workers changed after orphaning. Therefore, it is possible that the ex-queenless workers of *A. s. japonica* could not be recognized as colony members by ex-queenright workers after reunion. However, this hypothesis cannot fully account for the aggression, because ex-queenless workers with well-developed ovarioles were attacked much more than ex-queenless sterile workers. The ex-queenright workers selectively attacked the fertile ex-queenless ones, which strongly supports the hypothesis of worker policing. Policing behaviour should be performed by all workers that recognize their queens. We did not observe aggression among ex-queenless workers. Although we do not know why, it is possible that sterile ex-queenless workers had already been regulated by the dominance behaviour of fertile workers in the orphan condition. In addition, ex-queenless workers might not have been able to recognize quickly the presence of a queen in the colonies.

The queen removal and colony splitting experiment shows that the presence of a queen affected workers' reproductive ability in *A. s. japonica*. In queenright colonies, most eggs laid by workers were consumed by colony members and appeared to be trophic eggs that could not develop into males. In contrast, in the absence of queens, some workers started to produce male-destined eggs. Thus the queens appear to restrain worker reproduction by informing workers of their presence, possibly pheromonally. Keller & Nonacs (1993) argued that pheromonal queen control is evolutionarily unstable, and therefore the queen pheromone might be an honest signal of the queen's presence. Furthermore, if worker policing becomes sufficiently common and effective in eliminating workers' male production, self-restriction may evolve (Ratnieks 1988). Thus the putative queen pheromone seems to have two functions: workers receiving the pheromone (1) voluntarily forego their oviposition and (2) police other workers' oviposition. More data are needed to examine the function of the queen pheromone and information mechanism in *A. s. japonica*. In addition, because we examined only one microsatellite locus and the sample size was relatively small, additional research with other microsatellite loci is also needed to estimate the ratio of males produced by workers in detail.

Worker policing of workers' male production is expected to evolve under conditions of reduced relatedness caused by high mating frequency or multiple related queens (Woyciechowski & Lomnicki 1987; Ratnieks 1988; Pamilo 1991). In our study *A. s. japonica* was monogynous and most queens mated singly. Hence it was unlikely that all queens mated multiply in the five observed colonies where worker policing was elicited. Worker policing has also been found in monogynous and monandrous *Diacamma* sp. (Kikuta & Tsuji 1999). In such cases, mutual worker policing may be caused by factors other than genetic structure. The cost of workers' male production should be the most likely factor. Frequent male production by workers may reduce the colony's efficiency (Cole 1986), because the increase in production and rearing of their own eggs by workers reduces the time spent on other colony activities, such as defence, foraging and brood care. Consequently, the inclusive fitness of workers themselves might be reduced. Ratnieks (1988) predicted with a theoretical model that worker policing can evolve if colony-level efficiency is raised by only 4.4%. In the case of *A. s. japonica*, the decreased supply of trophic eggs to colony members seems to be disadvantageous. Empirical testing of these cost hypotheses is needed.

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