Worker policing by egg eating in the ponerine ant *Pachycondyla inversa*

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We investigated worker policing by egg eating in the ponerine ant *Pachycondyla inversa*, a species with morphologically distinct queens and workers. Colonies were split into one half with the queen and one half without. Workers in queenless colony fragments started laying unfertilized male eggs after three weeks. Worker-laid eggs and queen-laid eggs were introduced into five other queenright colonies with a single queen and three colonies with multiple queens, and their fate was observed for 30 min. Significantly more worker-laid eggs (range of 35–62%, mean of 46%) than queen-laid eggs (range of 5–31%, mean of 15%) were eaten by workers in single-queen colonies, and the same trend was seen in multiple-queen colonies. This seems to be the first well-documented study of ants with a distinct caste polymorphism to show that workers kill worker-laid eggs in preference to queen-laid eggs. Chemical analyses showed that the surfaces of queen-laid and worker-laid eggs have different chemical profiles as a result of different relative proportions of several hydrocarbons. Such differences might provide the information necessary for differential treatment of eggs. One particular alkane, $3,11\text{-dimeC}_{27}$, was significantly more abundant on the surfaces of queen-laid eggs. This substance is also the most abundant compound on the cuticles of egg layers.

Keywords: reproductive conflicts; worker policing; egg discrimination; *Pachycondyla inversa*

1. INTRODUCTION

Most animal societies are non-clonal, and this gives rise to potential and actual conflicts among individuals over reproduction (Clutton-Brock 1991; Ratnieks & Reeve 1992; Bourke & Franks 1995; Johnstone 2000). One mechanism that can reduce conflict is worker policing, in which workers prevent each other from reproducing (Ratnieks 1988; Crozier & Pamilo 1996; Barron *et al.* 2001; Monnin & Ratnieks 2001). Empirical studies of eusocial Hymenoptera have documented worker policing in two distinct reproductive contexts: first, in preventing the overthrow of the breeder female in species without morphologically distinct female castes, such as queenless ponerine ants (Monnin *et al.* 2002); and, second, in reducing male production by workers in species with morphologically distinct queen and worker castes (in most species of eusocial Hymenoptera workers cannot mate but can lay unfertilized eggs, which may develop into males (arrhenotokous parthenogenesis; Crozier & Pamilo 1996)).

In single-queen (monogynous) societies of eusocial Hymenoptera, policing of worker male production by workers is selectively favoured on relatedness grounds when queens are mated to more than two males (polyandry; Ratnieks 1988), because workers are then on average more closely related to the queen's sons ($r = 0.25$) than to other workers' sons ($r < 0.25$). Similarly, it is favoured when colonies are headed by multiple related queens (polygyny) (Crozier & Pamilo 1996). When queens mate singly (monandry), however, worker policing is selected in monogynous societies only when it enhances colony productivity (Ratnieks 1988) or helps to produce a female-biased sex-allocation ratio in the colony (Foster & Ratnieks 2001).

Two mechanisms of policing of male production by workers have been observed: egg eating and aggression against workers with activated ovaries. Worker policing by egg eating has been directly observed in several species of honeybee, *Apis* (Ratnieks & Visscher 1989; Barron *et al.* 2001; Oldroyd *et al.* 2001) and in vespine wasps (Foster & Ratnieks 2001; Foster *et al.* 2002). There is also evidence for worker policing by aggression in both honeybees (Visscher & Dukas 1995) and wasps (Landolt *et al.* 1977). Although ants are the largest group of eusocial insects and worker policing by aggression has been reported in several species (Gobin *et al.* 1999; Liebig *et al.* 1999; Monnin *et al.* 2002; Iwanishi *et al.* 2003; Hartmann *et al.* 2003), there is, at present, little information on the occurrence of worker policing by egg eating. In *Diacamma* sp. from Japan, in which a morphological queen caste is absent and instead a mated worker (gamergate) reproduces, Kikuta & Tsuji (1999) observed workers eating the unfertilized eggs laid by other workers. However, most worker-laid eggs (60%) were destroyed by the gamergate, and only 29% were eaten by other workers.

The aim of our study was to investigate worker policing of male production by other workers in colonies of *Pachycondyla inversa*, a ponerine ant in which colonies are headed by one or several morphologically distinct queens. We used a methodology previously employed in studies of honeybees (Ratnieks & Visscher 1989) and vespine wasps (Foster & Ratnieks 2001; Foster *et al.* 2002), in which non-nest-mate worker-laid eggs, obtained from queenless colonies, and non-nest-mate queen-laid eggs, obtained from queenright colonies, were transferred into queenright discriminator colonies and observed to determine their fate. The main difference between our study and previous

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work on bees and wasps is that, because ants do not rear eggs in cells, we transferred eggs one by one into the alien nest and observed each egg continuously rather than placing numerous eggs simultaneously into adjacent cells and checking them at intervals.

Our results show that significantly more worker-laid than queen-laid eggs were eaten in both single-queen and multiple-queen discriminator colonies, which strongly suggests that worker policing by egg eating occurs. In addition, chemical analyses (by gas chromatography (GC) and mass spectrometry (MS)) revealed consistent differences between the relative proportions of surface hydrocarbons of queen-laid and worker-laid eggs. Egg discrimination might be based on these chemical differences.

2. MATERIAL AND METHODS

(a) Study species, collection and rearing of colonies, and source of eggs

Pachycondyla inversa (cf. Lucas *et al.* 2002) (Formicidae: Ponerinae) colonies were collected from knotholes in tree trunks and branches and from cocoa pods in a plantation near Ilhéus, Bahia, Brazil. Twelve monogynous (single-queen colonies with 16–120 workers; mean ± s.d. = 50 ± 35 workers) and three polygynous (two with two queens and one with three queens, 20–43 workers; mean ± s.d. = 29 ± 12 workers) colonies were brought to Regensburg, Germany. Queens in polygynous colonies are typically not related (Heinze *et al.* 2001; Kolmer *et al.* 2002) and queen mating frequency is unclear. There is some evidence that queens typically mate only once (Trunzer *et al.* 1998; Heinze *et al.* 2001), but a genetic study could not rule out multiple mating (Kolmer *et al.* 2002).

Laboratory colonies were divided into queenright (+) and queenless (−) halves of equal population, which served both as source colonies for queen-laid and worker-laid eggs and as discriminator colonies in egg-acceptance bioassays. Colonies were kept under near-natural conditions (27 °C and 60% humidity, 12 L : 12 D photoperiod) in plastic boxes (19 cm × 19 cm × 9 cm) with a regularly moistened plaster floor. A chamber in the plaster (6 cm × 6 cm × 1.5 cm) served as a nest cavity. Food (diluted honey and cockroaches, *Nauphoeta cinerea*) was provided three times per week. Egg laying by workers in queenless colonies started three weeks after the colonies were split. Worker-laid eggs from queenless colonies, which were not used in the bioassays, were viable and gave rise to male offspring. Workers of *P. inversa* lack a functional spermatheca and so cannot mate or lay fertilized female-destined eggs.

Pachycondyla inversa, like most of the tropical ant species, produces sexuals throughout the year (J. H. C. Delabie, personal communication; P. D'Ettorre, personal observation). In our policing bioassays, queen-laid eggs were probably a mixture of haploid male and diploid female eggs, thus we could not discriminate the possible effect of egg sex. Nevertheless, to our knowledge, no study has yet shown that queen-laid male eggs are treated differently from queen-laid female eggs. Indeed, in several ant species, it has been shown that workers perform sex-allocation biasing by killing male larvae and not eggs (cf. Chapuisat & Keller (1999) and references therein). Moreover, in the honeybee, a control policing experiment showed that fertilized and unfertilized queen-laid eggs were treated in the same way by workers (Oldroyd & Ratnieks 2000).

(b) Policing bioassays

Worker policing by egg removal was investigated by transferring queen-laid or worker-laid eggs one at a time into three types of discriminator colony: (i) monogynous queenright colonies (*n* = 5; table 1*a*); (ii) polygynous queenright colonies (*n* = 3; table 1*b*); and (iii) queenless colonies (*n* = 5; table 1*c*). A test egg was carefully removed from its source colony using clean forceps 0–48 h after oviposition and individually placed within 1 cm of the nest of a discriminator colony. The behaviour of the workers towards the egg was observed for 30 min. Eggs could either be eaten or be transferred near to or onto the egg pile. An egg that was transferred to the egg pile was considered to be accepted. The time until an egg was either eaten or placed onto the egg pile was recorded. If the fate of an egg was undecided after 30 min, observations were continued for an additional 30 min or until the egg was eaten or placed onto the egg pile. Because colonies are small and few eggs are laid in *P. inversa* (Trunzer *et al.* 1998; Kolmer & Heinze 2000), a maximum of five eggs per day were introduced into a discriminator colony, with an interval of at least 30 min between eggs. The numbers of eggs introduced into each discriminator colony varied according to the availability of eggs (table 1*a–c*). Data from the monogynous colony C+ are shown in table 1*a* but were not used in the overall statistical analysis to avoid pseudoreplication. This is because this colony received eggs from colonies F+ and F−, and eggs from these two colonies were also introduced into discriminator colonies A+ and B+.

(c) Statistical analysis

Data had to be analysed in several 2 × 2 contingency tables (table 1), corresponding to the various combinations of discriminator and source colonies. We therefore had a contingency 'cube' of data, summarizing the relationships among the three qualitative variables: experimental condition (different discriminator colonies), egg maternity (worker-laid or queen-laid) and result (egg accepted or eaten). We followed a three-step methodology (Everitt 1977). In step 1 we tested the mutual independence of the three variables. If independence was rejected, we proceeded to step 2 to test for independence between experimental condition (discriminator colonies) and the two other variables. If this independence was not rejected, we could pool the data of the different experimental conditions. Step 3 was a new 2 × 2 contingency table based on the pooled data.

(d) Chemical analysis

Surface chemicals of queen-laid and worker-laid eggs were extracted by individually washing eggs in 30 μl of pentane for 1 min. We injected 2 μl of this extract into an Agilent Technologies 6890N gas chromatograph with a flame ionization detector, equipped with a capillary column (Rtx-5, 30 m × 0.25 mm × 0.50 μm; Restek, Bellefonte, PA, USA). The injector was a *split–splitless* type, the carrying gas was helium at 1 ml min⁻¹ and the temperature rose from 70 °C to 180 °C at 20 °C min⁻¹, then from 180 °C to 280 °C at 4 °C min⁻¹ and then was held at 280 °C for 15 min. Compounds were identified on the basis of their mass spectra, which were produced by electron ionization MS using a Hewlett Packard (Palo Alto, CA, USA) 5890A gas chromatograph coupled to an HP 5917A mass selective detector (70 eV electron impact ionization).

For each chemical profile, the proportions of each identified peak were calculated. We used a multivariate statistical analysis (STATISTICA v. 5.1 for Windows, StatSoft, Tulsa, OK, USA) to estimate the similarity of the chemical profiles of worker-laid and

Table 1. (*a*) Proportions of non-nest-mate queen-laid and worker-laid eggs eaten by workers in monogynous queenright discriminator colonies; + and − refer to queenright and queenless colonies. (*b*) Proportions of non-nest-mate queen-laid and worker-laid eggs eaten by workers in polygynous queenright discriminator colonies; ++ two-queen colony; +++ three-queen colony. (*c*) Proportions of queen-laid and worker-laid eggs eaten by workers in queenless discriminator colonies (nest-mates and non-nest-mates).

(Time for acceptance or rejection was not recorded for colony B+. Q, queen; W, worker.)

discriminator colony	egg source colony	egg type	number of eggs introduced	accepted number (%); median time (min)	eaten number (%); median time (min)	Q-laid versus W-laid
(*a*)						
A+	E−	W-laid	16	10 (63%); 29	6 (37%); 14.5	
	F+	Q-laid	20	19 (95%); 12	1 (5%); 10	
B+	F−	W-laid	20	13 (65%)	7 (35%)	
	G+	Q-laid	19	17 (89%)	2 (11%)	
D+	C−	W-laid	16	6 (38%); 5	10 (62%); 4.5	
	C+	Q-laid	16	11 (69%); 6	5 (31%); 5	
X+	Z−	W-laid	16	8 (50%); 5	8 (50%); 4.5	
	Y+	Q-laid	20	17 (85%); 6	3 (15%); 5	
total		W-laid	68	37 (54%); 19.5	31 (46%); 8	$\chi^2 = 14.98$
		Q-laid	75	64 (85%); 10	11 (15%); 9	$p = 0.0001$
C+	F−	W-laid	8	4 (50%); 11.5	4 (50%); 9	$\chi^2 = 3.0$
	F+	Q-laid	8	8 (100%); 7	0 (0%)	$p = 0.08$
(*b*)						
J++	B−	W-laid	11	1 (9%); 30	10 (91%); 9	
	B+	Q-laid	12	10 (83%); 11.5	2 (17%); 13.5	
K++	I−	W-laid	16	1 (6%); 28	15 (94%); 7	
	G+	Q-laid	16	12 (75%); 14	4 (25%); 14.5	
L+++	C−	W-laid	8	0 (0%)	8 (100%); 12	
	A+	Q-laid	8	6 (75%); 24	2 (25%); 14	
total		W-laid	35	2 (6%); 29	33 (94%); 9	$\chi^2 = 34.87$
		Q-laid	36	28 (78%); 14.5	8 (22%); 14.5	$p < 0.0001$
(*c*)						
H−	H+	Q-laid	22	22 (100%); 5	0 (0%)	
C−	C+	Q-laid	18	16 (89%); 5.5	2 (11%); 5	
E−	A+	Q-laid	20	19 (95%); 2	1 (5%); 30	
total		Q-laid	60	57 (95%); 4	3 (5%); 7	
F−	B−	W-laid	9	9 (100%); 4	0 (0%)	
A−	E−	W-laid	11	7 (64%); 2	4 (36%); 4	
total		W-laid	20	16 (80%); 3.5	4 (20%); 4	$\chi^2 = 2.56$
						$p = 0.10$

queen-laid eggs. Peaks were first analysed with principal components analysis (PCA) to reduce the number of variables subsequently used in a discriminant analysis (DA). The standardized discriminant function coefficients and the factor loadings (greater than 0.7) were used to assess the importance of individual compounds. DA was used to determine whether predefined groups (i.e. worker-laid and queen-laid eggs) could be discriminated on the basis of their chemical profiles and to assess the degree of similarity between groups. The correct classification of individual eggs to the respective groups was verified.

3. RESULTS

(a) *Policing assays*

(i) *Monogynous (single-queen) discriminator colonies*

Workers in discriminator colonies inspected and antennated both queen-laid and worker-laid eggs within 2 min of egg introduction. Almost all eggs (98%) were either eaten or accepted within 30 min. In all five discriminator colonies workers ate a greater proportion of worker-laid than queen-laid eggs (table 1*a*; colony C+ was not used in the analysis, see § 2). For the other four colonies, the three-step statistical analysis showed that mutual independence of the three variables was strongly rejected (step 1: $\chi^2 = 24.57$, $p = 0.006$, d.f. = 10). By contrast, the independence of the experimental conditions and the other two variables could not be rejected (step 2: $\chi^2 = 9.18$, $p = 0.42$, d.f. = 9) and the overall analysis showed a strong effect of egg origin (worker-laid or queen-laid) on acceptance (step 3: $\chi^2 = 14.98$, Yates correction, $p < 0.0001$; table 1*a*).

In total, 46% (range of 35–62%) of worker-laid and 15% (range of 5–31%) of queen-laid eggs were eaten. Additionally, worker-laid eggs took longer to become accepted than queen-laid eggs (median time and 25% and 75% quartiles for acceptance; worker-laid eggs: 19.5, 5.5

and 30 min; queen-laid eggs: 10, 6 and 15 min; Wilcoxon matched pairs test on the medians: $Z = 2.47$, $p = 0.013$). However, for eggs that were eaten, maternal origin was not significant in determining when eating began (median time and 25% and 75% quartiles for eating; worker-laid eggs: 8, 3.5 and 14.5 min; queen-laid eggs: 9, 4.5 and 13.5 min; $Z = 0.50$, $p = 0.61$).

(ii) Polygynous (multiple-queen) discriminator colonies

In the three discriminator colonies, almost all (94%, range of 91–100%) worker-laid eggs but only 22% (range of 17–25%) of queen-laid eggs were eaten (table 1b). As in the monogynous discriminator colonies, the three variables were not independent (step 1: $\chi^2 = 38.33$, $p < 0.0001$, d.f. = 7). However, the experimental conditions were independent of the other two variables (step 2: $\chi^2 = 1.08$, $p = 0.98$, d.f. = 6) and the overall analysis showed that worker-laid eggs were less frequently accepted than queen-laid eggs (step 3: $\chi^2 = 34.87$, Yates correction, $p < 0.0001$; table 1b). Those queen-laid eggs that were eaten by workers appeared to be eaten later after the introduction than worker-laid eggs, but the difference was not statistically significant (median time and 25% and 75% quartiles for eating; queen-laid eggs: 14.5, 11 and 15.5 min; worker-laid eggs: 9, 6 and 12 min; $Z = 0.7$, $p = 0.48$). Time until acceptance appeared to be larger for worker-laid eggs than for queen-laid eggs, but the difference was not significant, presumably because of the small sample size (only two worker-laid eggs were accepted) (median time and 25% and 75% quartiles for acceptance; queen-laid eggs: 14.5, 11 and 18.5 min; worker-laid eggs: 29, 28 and 30 min; $Z = 1.34$, $p = 0.18$).

(iii) Queenless discriminator colonies

When eggs were put into queenless discriminator colonies, 95% (range of 89–100%) of the queen-laid and 80% (range of 64–100%) of the worker-laid eggs were accepted. The difference in the acceptance proportions of the two types of egg was not significant ($\chi^2 = 2.56$, Yates correction, $p = 0.10$; table 1c). In addition, there was no significant difference between the median acceptance times of queen-laid and worker-laid eggs (table 1c; median time and 25% and 75% quartiles for acceptance; worker-laid eggs: 3.5, 1.5 and 6 min; queen-laid eggs: 4, 2.5 and 5 min; Mann–Whitney U-test: $U = 345.5$, $p = 0.30$).

(iv) Comparison among different types of discriminator colonies

The proportion of queen-laid eggs eaten by workers did not differ between monogynous and polygynous queenright discriminator colonies (figure 1a; M+ versus P+: $\chi^2 = 0.52$, Yates correction, $p = 0.47$). The proportion of worker-laid eggs eaten was significantly higher in polygynous than in monogynous colonies (figure 1b; M+ versus P+: $\chi^2 = 21.27$, Yates correction, $p < 0.0001$). In queenless colonies, policing of worker-laid eggs was less common than in monogynous and polygynous colonies (figure 1b; M+ versus Q−: $\chi^2 = 3.22$, Yates correction, $p < 0.07$; P+ versus Q−: $\chi^2 = 28.62$, Yates correction, $p = 0.0001$). The same trend was shown by queen-laid eggs (figure 1a; M+ versus Q−: $\chi^2 = 2.39$, Yates correction, $p = 0.12$; P+ versus Q−: $\chi^2 = 4.99$, Yates correction, $p = 0.02$).
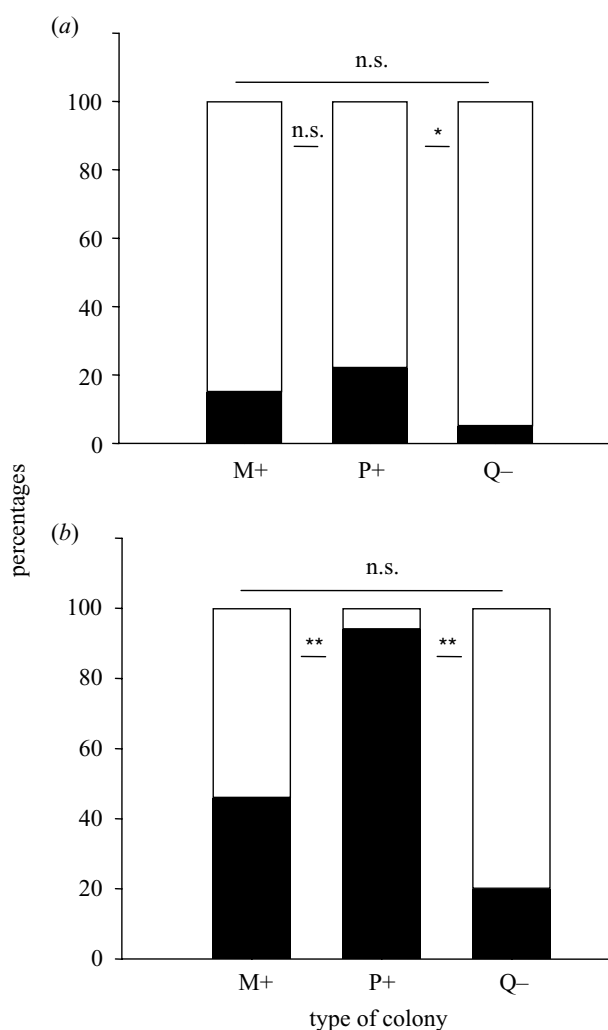
Figure 1. Proportions of (a) queen-laid eggs and (b) worker-laid eggs, accepted (open bars) and eaten (filled bars) in each of the three kinds of discriminator colony (queenright monogynous, M+; queenright polygynous, P+; and queenless, Q−; $^*p < 0.05$ and $^{**}p < 0.01$; χ^2-test).

(b) Chemical analyses of eggs

About 30 compounds or mixtures of compounds, 24 of which were identified as hydrocarbons, were observed in pentane washes of eggs. These were linear alkanes, alkenes and methyl-branched alkanes (with chain lengths of between C_{25} and C_{29}). Figure 2 shows chromatograms of extracts from the surfaces of a typical queen-laid egg (figure 2a) and a typical worker-laid egg (figure 2b). Queen-laid eggs and worker-laid eggs differed in the relative abundance, but not presence, of the detected chemicals.

We compared the chemical profiles of worker-laid eggs ($n = 16$, from three colonies) and queen-laid eggs ($n = 15$, from four colonies). PCA on the 30 compounds produced eight principal components with eigenvalues greater than 1, which explained 88.8% of the total variance. DA performed on 11 variables (factor loading greater than 0.7) significantly differentiated the chemical profiles of worker-laid and queen-laid eggs (Wilks' $\lambda = 0.000\,0022$, $F_{66,80} = 12.64$, $p < 0.000\,01$). All samples were 100% correctly assigned to their group (four groups of queen-laid eggs from four different colonies and three groups of worker-laid eggs from three different colonies; figure 3). On the basis of their proportions of surface compounds,
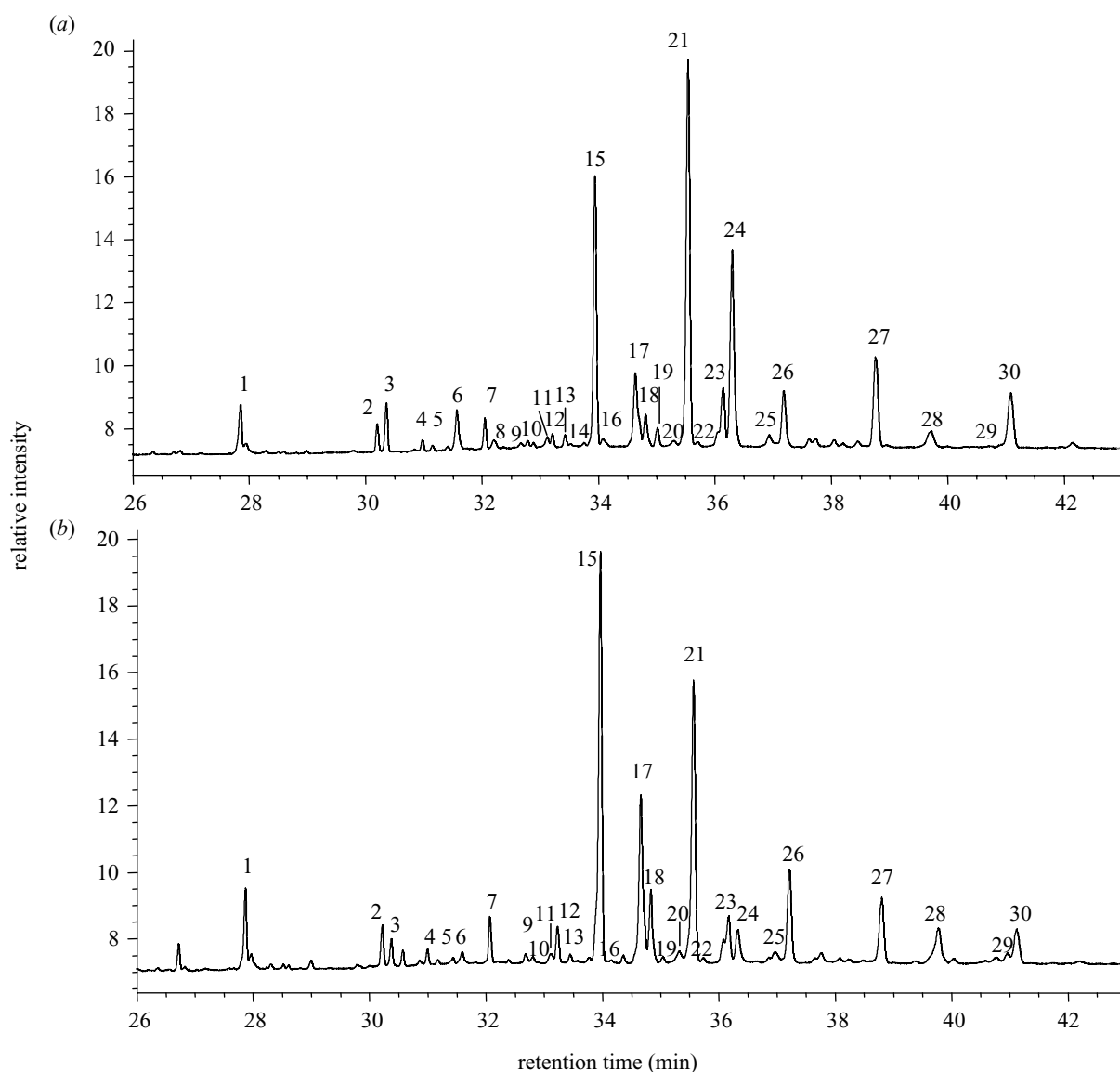
Figure 2. Gas chromatograms of surface extracts from (*a*) a representative queen-laid egg and (*b*) a representative worker-laid egg. 1, unknown; 2, C_{25}; 3, unknown; 4, 11-meC$_{25}$; 5, unknown; 6, 3-meC$_{25}$; 7, C_{26}; 8, 3,9-dimeC$_{25}$; 9, 10 + 12-meC$_{26}$; 10, 6-meC$_{26}$; 11, unknown; 12, 4-meC$_{26}$; 13, 2-meC$_{26}$; 14, x-meC$_{27}$:1; 15, C_{27}; 16, 2,11-dimeC$_{26}$; 17, 9 + 11 + 13-meC$_{27}$; 18, 7-meC$_{27}$; 19, 5-meC$_{27}$; 20, 9,y-dimeC$_{27}$; 21, 3-meC$_{27}$; 22, 5,y-dimeC$_{27}$; 23, C_{28}; 24, 3,11-dimeC$_{27}$; 25, 10 + 12-meC$_{28}$; 26, unknown; 27, C_{29}; 28, 11 + 13 + 15-meC$_{29}$; 29, unknown; 30, 3-meC$_{29}$.

queen-laid eggs were clearly separated from worker-laid eggs by function 1, which accounted for 53% of the total variance. It appears that queen-laid eggs from different queens have very similar odours, because the profiles of queen-laid eggs from three out of the four single-queen colonies largely overlapped (figure 3). Eggs from the same colony clustered together, suggesting that there are also colony-specific profiles. Nevertheless, when comparing the queenright and the queenless parts of the same colony, the profiles of queen-laid and worker-laid eggs diverge (Q–D and W–D; figure 3).

Comparison of the relative proportions of the 11 compounds sorted by the PCA (factor loading of greater than 0.7) revealed several differences between the two kinds of egg. Peak numbers 2 and 12 were more abundant on the surfaces of worker-laid eggs, whereas peak numbers 19, 25, 27 and 30 were more abundant on queen-laid eggs. Particularly striking was the difference in the amount of

peak 24, 3,11-dimeC$_{27}$, which was more abundant on queen-laid eggs (figure 4).

4. DISCUSSION

Our results clearly show that *P. inversa* workers discriminate against worker-laid eggs compared with queen-laid eggs in both monogynous and polygynous colonies. This is, to our knowledge, the first clear case of worker policing by egg eating in ants with a morphologically distinct queen caste. Large proportions of worker-laid eggs were eaten in both monogynous (46%) and polygynous (94%) discriminator colonies. Significantly fewer queen-laid eggs were eaten in both monogynous (15%) and polygynous (22%) discriminator colonies. By contrast, in queenless discriminator colonies most eggs were accepted, and there was no significant difference in the proportions of worker-laid (20%) and queen-laid (5%) eggs eaten.
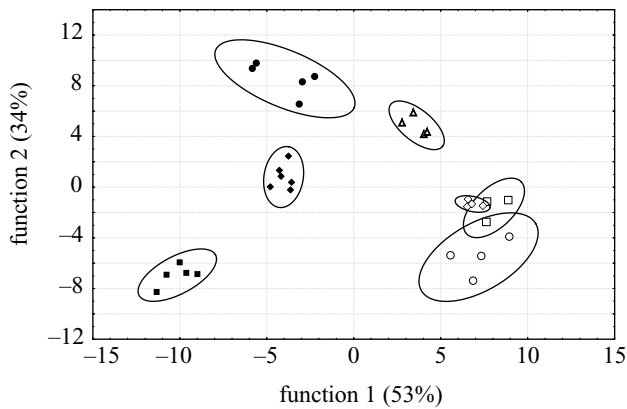
Figure 3. DA of surface-hydrocarbon proportions of queen-laid eggs from colonies A, C, D and E ($n = 15$; Q–A, open triangles; Q–C, open squares; Q–D, open circles; Q–E, open diamonds) and worker-laid eggs from colonies D, F and I ($n = 16$; W–D, filled squares; W–F, filled circles; W–I, filled diamonds). The percentages of the variance explained by each of the two main functions are given in parentheses.
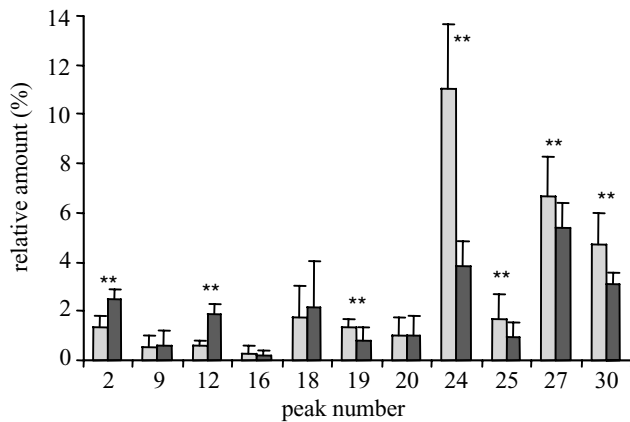

Figure 4. Proportions of the 11 surface compounds sorted by the PCA (factor loadings greater than 0.7) for queen-laid eggs (light-grey bars) and worker-laid eggs (dark-grey bars). Sample sizes are as in figure 3. Peak numbers are as in figure 2 (** $p < 0.01$, Mann–Whitney U-test).

Lack of worker policing is expected in queenless colonies, as these normally rear a last brood of workers' sons before the workers die. In the honeybee, *Apis mellifera*, worker policing is switched off after the death of the queen to allow a final production of many males (Miller & Ratnieks 2001).

The time needed for an egg to be accepted in the colony or eaten revealed a second level of differential treatment. In monogynous discriminator colonies, worker-laid eggs that were accepted took significantly longer (19.5 min) to be placed onto the egg pile than accepted queen-laid eggs (10 min). The same trend also occurred in polygynous discriminator colonies. This is similar to the situation in the ant *Myrmecia gulosa*, in which worker-laid eggs were handled for longer before being placed onto the egg pile than queen-laid eggs (Dietemann 2002). However, no eggs were eaten in *M. gulosa* (Dietemann 2002).

Given that all the eggs used in our study came from alien colonies, our data suggest that, in *P. inversa*, a general mechanism exists that allows workers to discriminate between worker-laid and queen-laid eggs. Chemical analyses of egg surface extracts revealed clear quantitative differences between worker-laid and queen-laid eggs in the relative proportions of hydrocarbons, and in an overall DA of hydrocarbons, the two types of egg were clearly separated by the first principal component. In particular, 3,11-dimeC$_{27}$ was significantly more abundant on queen-laid eggs. This chemical has also been shown to be the most abundant hydrocarbon on the cuticles of reproductive queens and egg-laying workers from queenless colonies (Heinze *et al.* 2002). Similarly, a long-chain branched hydrocarbon was shown to be characteristic of both the reproductive worker's cuticle and its eggs in the queenless ant *Dinoponera quadriceps* (Monnin & Peeters 1997).

In *P. inversa*, the relative quantities of cuticular 3,11-dimeC$_{27}$ are correlated with the degree of ovary activation: it is more abundant on the cuticles of laying workers than on those of non-laying workers and also on the cuticles of laying queens than on those of young queens that have not yet mated and have laid at most a few eggs (Heinze *et al.* 2002; D'Ettorre *et al.* 2004). Laying queens have significantly higher relative and absolute amounts of 3,11-dimeC$_{27}$ on their cuticles than laying workers. In addition to being the most abundant cuticular chemical, 3,11-dimeC$_{27}$ also elicits a strong response in worker antennae during electroantennography, making it a promising candidate for a fertility signal (D'Ettorre *et al.* 2004).

Does 3,11-dimeC$_{27}$ play a role in egg recognition by signalling to the workers that the egg was laid by the queen? Because the chemical is present on eggs laid by both queens and workers, albeit in differing proportions, this hypothesis requires that policing workers can assess its relative abundance on the egg surface. In addition, the ability to produce very high levels of this chemical would need to be restricted to laying queens. Otherwise, workers could lay eggs that mimic queen-laid eggs, as has been observed in 'anarchistic' honeybees (Oldroyd & Ratnieks 2000). Further research is needed to clarify the role of 3,11-dimeC$_{27}$ and of other possible key substances. We are currently investigating the role of surface chemicals in egg recognition to clarify the process that allows the discrimination between worker-laid and queen-laid eggs. Our preliminary results show that worker-laid eggs that have been in close contact with queen-laid eggs are policed at a lower rate than control worker-laid eggs (P. D'Ettorre and A. Tofilski, personal observation). This is probably a result of a process of cue scrambling that would allow laying workers to shuffle some eggs into the egg pile of the colony.

Although the kin structure of *P. inversa* colonies is not completely known, queens appear to be unrelated (Heinze *et al.* 2001) and it is likely that most queens are singly mated (see § 2). This suggests that worker policing is favoured by factors other than the greater average relatedness of workers to the queen's sons than to males produced by other workers. Worker reproduction can result in costly competition among workers, which lowers overall colony efficiency (Cole 1986; Woyciechowki & Lomnicki 1987; Gobin *et al.* 2003), and this can select for worker policing even in colonies with a single queen mated to a single male (Ratnieks 1988). Worker policing of worker-laid eggs occurs even in the Cape honeybee, *A. mellifera capensis*. In this subspecies, queens mate with many males, as in other *A. mellifera*, but unmated workers can produce genetically

identical female offspring by thelytokous parthenogenesis. Therefore, there are no relatedness benefits to policing worker-laid eggs (Pirk *et al.* 2003). Similarly, worker policing by aggression has recently been observed in an ant with thelytokous parthenogenesis (Hartmann *et al.* 2003). Here, and possibly also in *P. inversa*, policing appears to help maintain high colony efficiency. In addition, policing of worker-laid eggs can be selected as a means for workers to achieve a female-biased sex-allocation ratio (Foster & Ratnieks 2001).

Genetic studies of male parentage have revealed that worker production of males is typically rare in ants, suggesting that policing mechanisms occur more commonly than expected (Walin *et al.* 1998; Villesen & Boomsma 2003). More studies are needed to determine whether worker policing by egg eating is more abundant in ants. These studies should involve the transfer of worker-laid eggs into queenright colonies, as carried out in this study, and ideally also determine whether eggs laid by workers in natural queenright colonies are discriminated against.

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