Workers influence royal reproduction

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Understanding which parties regulate reproduction is fundamental to understanding conflict resolution in animal societies. In social insects, workers can influence male production and sex ratio. Surprisingly, few studies have investigated worker influence over which queen(s) reproduce(s) in multiple queen (MQ) colonies (skew), despite skew determining worker-brood relatedness and so worker fitness. We provide evidence for worker influence over skew in a functionally monogynous population of the ant *Leptothorax acervorum*. Observations of MQ colonies leading up to egg laying showed worker aggressive and non-aggressive behaviour towards queens and predicted which queen monopolized reproduction. In contrast, among-queen interactions were rare and did not predict queen reproduction. Furthermore, parentage analysis showed workers favoured their mother when present, ensuring closely related fullsibs (average $r = 0.5$) were reared instead of less related offspring of other resident queens ($r \leq 0.375$). Discrimination among queens using relatedness-based cues, however, seems unlikely as workers also biased their behaviour in colonies without a mother queen. In other polygynous populations of this species, workers are not aggressive towards queens and MQs reproduce, showing the outcome of social conflicts varies within species. In conclusion, this study supports non-reproductive parties having the power and information to influence skew within cooperative breeding groups.

Keywords: multiple queen colony; skew; social evolution; conflict resolution; policing

1. INTRODUCTION

In cooperative breeding animal societies, the close integration of related, but non-clonal, individuals leads to potential conflicts over many aspects of reproduction. Understanding the dynamics and resolution of such conflicts is considered fundamental to understanding how societies evolve and persist [1–7]. An important reproductive conflict, relevant to both cooperative breeding vertebrates and invertebrates, is over the distribution of reproduction among social group members (reproductive skew; [8–11]). In the highly integrated societies of the eusocial Hymenoptera (ants, bees and wasps), a morphologically distinct caste system means colonies consist of queens, which mate and are the primary reproductives, and workers, which are unable to mate and generally do not reproduce. This division of reproductive labour means that reproductive skew among colony members is high overall, and that there is potential for conflict between the two parties because the direct interest of queens may not coincide with the indirect interest of the workers [12–16]. Indeed, intra-colony conflict is often overt in eusocial Hymenoptera and coercive tactics may be used by both queens and workers to get their own way [5,17].

In species with multiple queen (MQ) colonies, a common social organization, there is potential for conflict over reproductive skew among coexisting queens [18,19]. Theoretical treatments of skew attempting to explain the outcome of such conflict nearly always assume that control over skew and group membership rests with those

individuals competing directly over reproduction [8–10,20–25]. In the case of MQ social insect colonies, this assumption about control is supported by queens forming reproductive dominance hierarchies through aggressive interactions in some species [26–31]. However, an understudied possibility is that non-reproductive parties (i.e. workers) also play an influential role in determining skew. For instance, in some ponerine ants, low-ranking colony members use aggression to maintain the reproductive hierarchy [32], or as a mechanism to manage the number of reproductives [33]. Crucially, however, there is to our knowledge no evidence to support worker influence over skew among coexisting queens in social insect species with morphologically distinct castes where workers are incapable of mating and therefore potentially challenging the queen. This is in spite of ample evidence of worker influence in other reproductive conflicts (e.g. sex ratio, policing of worker reproduction, caste development; as reviewed in [5]). Worker influence is expected because skew among queens determines worker relatedness to brood reared, so it should be in the workers' interests to act accordingly. Furthermore, showing worker influence over skew is important because morphologically distinct workers do not have the outside option of leaving the group and breeding alone, a fundamental basis of transactional skew models (reviewed in [25]).

We investigated the role that morphologically distinct queens and workers play in determining skew in MQ colonies of the ant *Leptothorax acervorum*. In a Spanish population, colonies are functionally monogynous as just one queen monopolizes reproduction, even though other coexisting queens are capable of reproducing [34,35]. In these high-skew colonies, queen reproductive dominance might be under 'queen control', with one queen aggressively dominating all other queens so

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forming a dominance hierarchy, or under 'worker control', with workers determining which queen reproduces. We tested the queen and worker control hypotheses by investigating whether a queen's reproductive status was influenced by the amount of aggressive and non-aggressive behaviour she received during the weeks before egg laying began, and importantly which party—queens or workers—was responsible. Furthermore, we investigated whether the genetic relationships within the colony influenced which queen became reproductive.

2. MATERIAL AND METHODS

(a) *Colony collection, maintenance and composition*

Twenty-two *L. acervorum* MQ colonies were collected in Spain from two populations, near Orihuela del Tremedal in the Sierra de Albarracín (OT) and Valdelinares in the Sierra de Gudar (V) in October 2006 [35] and 2007. Colonies were found inside cavities of decaying twigs and were removed 2–6 days after collection, and, as the whole twig was collected, we are confident that all resident queens were collected. Colonies were then censused and provided with an artificial nest in the laboratory (for nest description, see electronic supplementary material, S1). Colonies were kept in a versatile environmental chamber (*Sanyo* MLR-351H) and temperature, light and humidity controlled (conditions described in electronic supplementary material, S2). Colonies experienced the following conditions: autumn (six to eight weeks), winter (six weeks), spring (eight weeks) and summer (six weeks). The composition of the 22 MQ colonies observed (OT = 19; V = 3) was as found in the field, with two to seven queens per colony at the start of winter. After overwintering in the laboratory, colonies had an average of 3.1 queens (range = 2–6) and 77 workers (range = 36–164).

(b) *Behavioural observations*

After overwintering in the laboratory, we observed aggressive and non-aggressive behaviour received by queens (individually marked using Humbrol model paint) in all 22 colonies over the 'spring' period when queen ovary development occurs. Colony behaviour was sampled by taking short videos from the onset of spring until eggs were laid (electronic supplementary material, S3). Behaviour was sampled between 07.00 and 19.00 and recording bouts were made over this period (electronic supplementary material, S4), with each bout for each colony made on separate days and the mean duration per bout being 0.68 h (range = 0.26–1.5 h). Behavioural recording was stopped once egg laying began. However, in three colonies (OT4.09, OT4.15 and OT4.35) recording was stopped early because all but a single queen (the future reproductive) had either been evicted or had left the nest permanently.

Behavioural interactions were analysed by re-watching recordings and behaviour scored using the programme VAR (designed by D. S. Gill & R.J.G. and available from R.J.G.), which helps measure the length of each type of interaction and the individuals involved. All behaviour was sampled using a focal queen approach with the type and duration of all behaviour a queen received or performed recorded. Rates of behaviour were calculated as the duration of behaviour (seconds)/the total length of time a focal queen had been observed (hours). *Per capita* rates were calculated as

the rate of behaviour/number of workers or queens (whichever was applicable) in the colony. During recording, there were occasions where queens strayed outside the nest or out of camera view. This time was deducted from the focal queen observation time, resulting in variation among queens from the same nest in the time they were observed (electronic supplementary material, S5).

Four types of aggressive interactions were scored: (i) a single bite, (ii) prolonged biting, (iii) dragging, and (iv) spreading, each increasing in their degree of aggressiveness (electronic supplementary material, S6). For rate calculations, a standard time of 1 s per bite was used. Non-aggressive 'grooming' behaviour involved individuals cleaning others using their mouthparts, and trophallaxis (an individual feeding another).

(c) *Queen reproductive and mated state, and queen size*

Queen reproductive state was determined after our behavioural observations were completed and once eggs were seen to have been laid. Reproductive queens had an enlarged (physogastric) abdomen and a central position among nest-mates [35], which is known to be a reliable identification method (R. J. Gill & R. L. Hammond 2008/2009, personal observation). Queen thorax widths were measured to provide an estimate of body size [36], and ovaries dissected to determine queen mated state [35].

(d) *Genetic analysis*

After behavioural observations, samples of workers, larvae and all queens still present in 21 of the 22 colonies observed ($n = 58$ queens; nine queens from eight colonies were permanently evicted or left the nest and then escaped during observations) were genotyped at four polymorphic microsatellite loci: LXAGA1, LXAGA2, LXAGT1 [37] and L18 [38]. Worker and larval DNA was extracted using a 10 per cent Chelex solution as previously described [35], and queen DNA was extracted using a Pure Gene extraction kit (Qiagen).

Sibship patterns were investigated by using the program COLONY [39] to group individuals into fullsib families based on queens being singly mated [40]. To identify whether any resident queens were the mothers of colony members, we checked all mated queen genotypes against the COLONY predicted maternal genotype (PMG) for each fullsib family in each colony. Colonies where a resident queen's genotype matched the PMG for the majority fullsib family (the fullsib family containing the majority of workers) were classed as 'maternal' colonies. Colonies with no resident queen matching the PMG of any fullsib family that contained workers were classed as 'non-maternal' colonies. We also calculated regression relatedness [41] using the program Relatedness 5.08 for only diploid (female) individuals.

3. RESULTS

(a) *Mated state of queens*

Ninety-six per cent of successfully dissected queens ($n = 49$ queens) were found to be mated (electronic supplementary material, S5), but the mated state of 20 queens (from 16 colonies) could not be determined because queens were either lost or were too desiccated to dissect. However, based on the proportion of mated queens in this and a previous study [35], a high proportion of these 20 queens were probably mated and all were treated as mated in the behavioural analysis. A single queen in two separate colonies

was found to be unmated, but we included these queens in the behavioural analysis (see below). Separate analyses with these queens excluded did not alter the analysis outcome (electronic supplementary material, S1). Importantly, dissections showed that there was more than one mated queen present in each colony.

(b) Behaviour received by queens

In all colonies, and as expected [35], only a single queen per colony became reproductive, and the first egg was laid an average of 36 days after the onset of spring (range = 17–66 days). During the period up to egg laying each colony recorded an average of nine separate bouts (range = 2–17), and a total of 354.6 h of queen behaviour distributed across all resident queens ($n = 69$, mean per queen = 5.14 h, range = 0.11–12.09 h) was analysed. Variation in focal queen recording time among queens in the same nest was caused by some queens being evicted or leaving the nest during recordings, and some queens periodically straying out of camera view.

(i) Aggression

Behavioural observations showed that aggression received by queens was strikingly biased, with more than 99 per cent carried out by workers towards queens ($W \rightarrow Q$, mean per queen $\pm$ s.e.m.: 292.4 ± 67.4 s h^{-1} ; figures 1 and 2a; electronic supplementary material, video), and less than 1 per cent carried out by queens ($Q \rightarrow Q$, mean per queen: 0.6 ± 0.4 s h^{-1} ; Wilcoxon signed-rank test: $Z = -6.51$, $n = 69$, $p < 0.001$). The numerical superiority of workers did not explain this difference as the *per capita* rate of $W \rightarrow Q$ aggression was still significantly higher than the *per capita* rate of $Q \rightarrow Q$ aggression (3.26 ± 0.69 versus 0.17 ± 0.14 s h^{-1} ; Wilcoxon signed-rank test: $Z = -5.11$, $n = 69$, $p < 0.001$).

Further analysis showed that the future reproductive state of queens over all colonies ('reproductive', $n = 22$; 'non-reproductive', $n = 47$) was predicted by the amount of worker aggression received (logistic regression: $\beta(\text{s.e.}) = -0.117(0.047)$, Wald = 6.32, d.f. = 1, $p = 0.01$, odds ratio (95% CI) = $0.89(0.812-0.975)$). On average, non-reproductive queens received over 100 times more $W \rightarrow Q$ aggression than reproductive queens (427.8 ± 92.8 versus 3.1 ± 1.2 s h^{-1} ; figure 2b; Mann-Whitney: $U = 91.0$, $n_1 = 22$, $n_2 = 47$, $p < 0.001$), and the level of aggression was often extreme with seven queens killed by workers (10% of queens, five colonies) and five permanently evicted (7% of queens, five colonies). In contrast, in 10 colonies, the reproductive queen received no worker aggression and in over 80 per cent of colonies (18/22) she received the least worker aggression among all colony queens (electronic supplementary material, S5). In the four colonies where a non-reproductive queen received less $W \rightarrow Q$ aggression than the reproductive queen, in two cases, the non-reproductive queen was permanently evicted from the nest before laying began, whereas in the other two colonies, the difference in aggression rate was minimal (4.4 versus 0 and 10 versus 8.4 s h^{-1}). In contrast to the high $W \rightarrow Q$ aggression, 91 per cent of queens received no aggression from other queens, and there was no difference in the level of $Q \rightarrow Q$ aggression received between reproductive and non-reproductive queens (zero versus

Figure 1. Worker aggression towards queens; example of workers spreading a queen that later did not reproduce (queen is in the centre).

Figure 2. Observed aggressive behaviour. (a) The rate (s h^{-1}) of aggression received by queens ($n = 69$) from other queens ($Q \rightarrow Q$) and from workers ($W \rightarrow Q$). The p -value was calculated using a Wilcoxon signed-rank test ($p < 0.001$). (b) The rate of $W \rightarrow Q$ aggression received by queens that became either reproductive ($n = 22$) or non-reproductive ($n = 47$). The p -value was calculated using a Mann-Whitney U -test ($p < 0.001$). Box-plots show the median (line), mean (dashed line), quartiles (box limits) and 10th and 90th percentiles (error bars).

0.81 ± 0.62 ; Mann-Whitney: $U = 472$, $n_1 = 22$, $n_2 = 47$, $p = 0.56$).

For $W \rightarrow Q$ aggression received by non-reproductive queens ($n = 47$), single bites contributed the least (0.95 ± 0.20 s h^{-1} , or 3.7 bites per queen, range = 0–15), followed by spreading (89.5 ± 25.5 s h^{-1}), then dragging

($130.1 \pm 27.4 \text{ s h}^{-1}$) and prolonged biting ($207.3 \pm 56.0 \text{ s h}^{-1}$) (electronic supplementary material, S7). In contrast, queens that later became reproductive ($n = 22$) were never subjected to spreading by workers and the average rates were low for bites ($0.12 \pm 0.06 \text{ s h}^{-1}$, or 0.5 bites per queen, range = 0–3), prolonged biting ($0.17 \pm 0.128 \text{ s h}^{-1}$) and dragging ($2.8 \pm 1.19 \text{ s h}^{-1}$). Comparisons between reproductive and non-reproductive queens for each of these aggressive interactions ($W \rightarrow Q$) separately were all significantly different (Mann–Whitney: all $U < 242$, all $n_1 = 22$, all $n_2 = 47$, all $p < 0.001$). We observed 38 per cent (18/47) of non-reproductive queens ($n = 15$ colonies) outside the nest for an average of $0.24 \pm 0.07 \text{ h}$ (range = 0.02–0.65), whereas reproductive queens were never seen outside the nest.

(ii) Grooming

The rate of non-aggressive $W \rightarrow Q$ ‘grooming’ behaviour (mean = $495.5 \pm 46.6 \text{ s h}^{-1}$) was significantly higher than the rate of $Q \rightarrow Q$ grooming (mean = $12.5 \pm 4.2 \text{ s h}^{-1}$; Wilcoxon sign rank test: $Z = 7.17$, $n = 69$, $p < 0.001$). $W \rightarrow Q$ grooming was also biased but in the opposite direction to that found for aggression, with future reproductive queens receiving a significantly higher rate of worker grooming than non-reproductive queens (667.7 ± 107.0 versus $415 \pm 42.9 \text{ s h}^{-1}$; Mann–Whitney: $U = 335$, $n_1 = 22$, $n_2 = 47$, $p = 0.019$; electronic supplementary material, S8). Furthermore, $W \rightarrow Q$ grooming predicted the future reproductive status of queens (logistic regression: $\beta(\text{s.e.}) = -0.002(0.001)$, Wald = 5.11, d.f. = 1, $p = 0.024$, odds ratio (95% CI) = $0.998(0.997\text{--}1.0)$). In contrast, there was no significant difference between reproductive and non-reproductive queens in the amount of $Q \rightarrow Q$ grooming received (means = 10.4 ± 5.6 versus $13.4 \pm 5.5 \text{ s h}^{-1}$; Mann–Whitney: $U = 478$, $n_1 = 22$, $n_2 = 47$, $p = 0.62$).

(c) Queen size

There was no significant difference in size between future reproductive and non-reproductive queens (mean = 0.52 mm, range = 0.44–0.59 versus mean = 0.51 mm, range = 0.43–0.56; Mann–Whitney: $U = 304$, $n_1 = 21$, $n_2 = 36$, $p = 0.22$). There was also no correlation between queen size and the rate of $W \rightarrow Q$ aggression received (Spearman’s rank correlation: $r = -0.24$, $p = 0.074$), $Q \rightarrow Q$ aggression carried out ($r = -0.03$, $p = 0.85$) or $Q \rightarrow Q$ received ($r = 0.115$, $p = 0.40$).

(d) Genetic analyses

(i) Sibship

Parentage analysis revealed that in 14/21 colonies a single resident queen had a genotype that matched the PMG of the majority fullsib family (referred to as ‘maternal colonies’). This queen was the previous year’s reproductive queen and the mother of almost all genotyped workers and larvae in the colony (electronic supplementary material, S9). In all 14 maternal colonies, it was this mother queen who received low aggression from workers and subsequently became reproductive during our observations. In non-maternal colonies ($n = 6$)—those colonies that did not have a previous year’s reproductive queen $W \rightarrow Q$ aggression was still found to be significantly biased towards queens that ended up

Figure 3. Observed $W \rightarrow Q$ aggression in non-maternal and maternal colonies. (a) In the six non-maternal colonies alone, the rate of $W \rightarrow Q$ aggression received by queens that became reproductive ($n = 6$) and non-reproductive ($n = 15$). The p -value was calculated using a Mann–Whitney U statistical test ($p = 0.008$). (b) The rate of $W \rightarrow Q$ aggression received by all queens in maternal ($n = 43$ queens in 14 colonies) and non-maternal colonies ($n = 21$ queens in six colonies). The p -value was calculated using a Mann–Whitney U statistical test ($p = 0.42$). Box-plots show the median (solid line), mean (dashed line), quartiles (box limits) and 10th and 90th percentiles (error bars).

non-reproductive (reproductive = $5.1 \pm 3.5 \text{ s h}^{-1}$ versus non-reproductive = $219.2 \pm 65.8 \text{ s h}^{-1}$; Mann–Whitney: $U = 12$, $n_1 = 6$, $n_2 = 15$, $p = 0.008$; figure 3a). As in maternal colonies, in five of six colonies, it was the queen who received the lowest level of aggression and remained in the nest that became the reproductive. There was no significant difference in the average rate of $W \rightarrow Q$ aggression between the 14 maternal colonies and the six non-maternal colonies (391 ± 103 versus $158 \pm 51.3 \text{ s h}^{-1}$; Mann–Whitney: $U = 395$, $n_1 = 43$, $n_2 = 21$, $p = 0.42$; figure 3b). Furthermore, there was no difference in the time until eggs appeared in the colony between maternal colonies (40.3 ± 3.8 days) and non-maternal colonies where no previous reproductive queen was present (33.6 ± 4.2 days; t -test = 1.03, d.f. = 15, $p = 0.32$). One colony (A01_1810) could not be classed as either maternal or non-maternal because no queen matched the PMG for any workers, but the reproductive queen did match the PMG for two larvae.

(ii) Relatedness

Average colony relatedness over all 22 colonies was high (0.68 ± 0.02 ; $n = 293$ individuals), and relatedness

among colony workers (0.74 ± 0.03 ; $n = 172$) and larvae (0.71 ± 0.04 ; $n = 63$ over 16 colonies) supports the sibship analysis that the majority of individuals within colonies belong to a single fullsib family (i.e. are full sisters). The average relatedness among colony queens was also high (0.57 ± 0.07 ; $n = 55$ over 18 colonies), and relatedness among non-reproductive queens (0.74 ± 0.05 ; $n = 30$ over 11 colonies) also suggests they are mostly full sisters.

4. DISCUSSION

We provide evidence that workers can play an influential role over skew among queens in social insects with morphologically distinct castes by showing that workers are influential over which queen becomes reproductive in functionally monogynous colonies of *L. acervorum*. Specifically, worker aggression towards queens predicted which queen reproduced in each colony, with the reproductive queen receiving low aggression (in many cases no aggression) and a high level of worker grooming. Indeed, worker aggression towards queens that did not reproduce was sometimes extreme, with workers killing and evicting queens. In contrast, we found no evidence of aggression-based hierarchies among queens as found in other ant species [26–31,42], as aggression among queens was particularly low and importantly neither queen aggression nor grooming predicted the future reproductive status of queens. These results show workers play an important role in regulating skew among queens and raises questions over the underlying cause of this behaviour:

- workers may act as queen ‘agents’, with worker aggression directed by the actions of the reproductive queen (a form of mutual queen and worker control). For example, reproductive queens could label other queens, either chemically or behaviourally, and so elicit worker aggression as seen in sting-smearing behaviour in *Dinoponera quadricaps* [32]. However, we saw no instances of sting smearing, or any sort of queen labelling, in more than 350 h of queen behaviour, and overall there were very few interactions, either aggressive or non-aggressive, between queens. The behaviour of *L. acervorum* workers does have parallels to the behaviour of *D. quadricaps*, however, as in *D. quadricaps* low-ranking females maintain the reproductive monopoly of alpha females by aggression towards beta females [32]. This similarity even stretches to particular behaviours, with ‘spreading’ behaviour in *L. acervorum* similar to ‘immobilization’ in *D. quadricaps* and also used for the same ends. However, one crucial difference is that in ponerine ants such as *D. quadricaps* and *Harpegnathos saltator* [33], where aggression limits the number of reproductives, low-ranking females have the potential, however small, to become reproductive. This contrasts our system where workers are physiologically incapable of replacing the queen and so worker aggression in *L. acervorum* cannot be motivated by the selfish desire of workers to reproduce directly;
- worker aggression towards queens might be a consequence of some queens being physiologically inferior and workers biasing their aggression towards less fecund queens. This idea has theoretical support

[43,44], and empirical studies have shown that workers are able to assess queen fecundity [45,46]. Implicit in this explanation is that queens differ in their fecundity, yet we found little evidence to support this. For example, previously reproductive queens (i.e. those in maternal colonies) were not faster to begin egg laying than those queens developing their ovaries for the first time (i.e. those in non-maternal colonies). Likewise, although fecundity in insects is often correlated with size [47,48], reproductive queens were not significantly larger than non-reproductive queens in this study. In addition, there is evidence showing no difference in egg-laying rates between newly laying queens (formerly non-reproductive) and established egg-laying queens when formerly non-reproductive queens develop their ovaries when separated along with a group of workers (R. J. Gill, R. L. Hammond & D. Coston 2008/2009, personal observation). The above measures of fecundity, however, are perhaps crude and further investigation of the role of fecundity variation would be valuable in testing this hypothesis further;

- the relationship between worker aggression and queen reproduction could be a causal one, with worker actions being the cause of variation in reproductive output and where worker interests prevail independent of queen interests (purely worker control). Such an explanation predicts that worker behaviour should increase worker fitness for example by workers favouring the queen that meets their genetic interest. In support, parentage analysis showed that when a mother queen is resident in the colony, workers always favoured her over other queens. This increases worker inclusive fitness as they raise more closely related fullsibs (average $r = 0.5$) than less related nieces/nephews ($r = 0.375$). A corollary of the causal hypothesis is that an absence of worker aggression towards queens should lead to all queens reproducing. In full support, in closely related polygynous populations of the same species [35], queens receive little or no aggression from workers [36,49] and tellingly all queens within MQ colonies reproduce (low skew; [36,50,51]).

These arguments are not mutually exclusive, however. For example, variation in fecundity among queens may be the cue that determines the direction of worker aggression, and worker aggression may, in turn, influence queen reproduction. Likewise, queen labelling and worker aggression may be co-selected if the interests of reproductive queens and workers coincide; a likely scenario if the reproductive queen is the most fecund and the mother of the workers. That said, the low level of queen–queen interactions and the lack of direct evidence of queen marking suggest workers operating as queen agents seems unlikely. Overall, the evidence does point to a causal role of worker behaviour in shaping skew among queens, although the cues that determine the direction of worker behaviour, especially aggression, remain open.

One thing is clear; worker preference for their mother maintains high relatedness within colonies. It is tempting to conclude that this selection of mothers over sisters by workers is an example of within-colony nepotism based on genetic cues [52], a behaviour often looked for but controversial [53]. We think that genetic-based nepotism

would be a premature conclusion for a number of reasons. First, this would require successful discrimination between very closely related mothers and sisters based purely on relatedness, which seems likely to be error-prone [52,54–56]. Second, in non-maternal colonies, relatedness cannot be the cue because worker aggression still predicts which workers reproduce even though relatedness is symmetrical both between workers and queens and among the queens workers are interacting with (queens and workers are full sisters). The similarity in worker behaviour in maternal and non-maternal nests shows that workers do not alter their patterns of aggression in response to a change in worker–queen relatedness asymmetry, which one would expect if workers were using relatedness-based cues [57]. We therefore suggest that worker behaviour towards queens is probably an evolved rule of thumb that maintains high colony relatedness but does not involve the direct discrimination of queens based on relatedness cues. Workers may simply favour a previous reproductive queen because she is likely to be their mother and hence rear full sisters.

Worker regulation over queen reproduction, in many senses, is a type of worker ‘policing’ behaviour akin to that found between queens and workers and among workers over male parentage [5]. Furthermore, worker regulation of queen reproduction extends the repertoire of reproductive conflicts over which workers are influential. In other social insects, excess queens are reared but then culled [58], and during pleometrotic events, (where MQs found a new colony) workers may eliminate all but one queen [59]. Although worker aggression in *L. acervorum* did sometimes result in queen death, the majority of *L. acervorum* queens were not killed and remained in the nest. This largely non-lethal aggression can be considered a conflict-resolving mechanism [5] that allows non-reproductive queens to remain in the nest as insurance against death of the reproductive queen [35], or perhaps for future budding [50,60] or solitary dispersal [61–64]. Social queuing—where queens wait for a reproductive opportunity [65,66]—could thus be potentially important in functionally monogynous *L. acervorum* colonies.

Worker regulation of queen reproduction leading to high skew in this functionally monogynous population makes sense as it provides a kin selective advantage. It is therefore intriguing that in other *L. acervorum* populations MQ colonies are polygynous, where MQs reproduce resulting in much lower colony relatedness [37,40,50,51,67], and behavioural observations have found a lack of worker aggression towards queens [36,49]. This contrast between worker aggression and high skew, and worker passivity and low skew, shows that the outcome of reproductive conflicts can vary even within a single species; something that has rarely been investigated [68]. Low skew cannot be because there is no available mechanism of control over skew, as this paper provides evidence that an effective mechanism does exist at least in one population. Given this, it seems likely that there is a benefit in MQs reproducing in polygynous populations that outweighs the dilution of relatedness [69,70]. This raises an important general point, namely that to understand more fully the ultimate explanations for difference in social organization, it is important to understand the benefit and cost components of Hamilton’s rule, not just how relatedness varies [71,72].

In terms of reproductive skew, transactional and compromise models assume that dominant and subordinate reproductive individuals determine skew, and in transactional models, group membership [25]. Our data support the influence of a non-reproductive party over both skew and, considering our observations of evictions and queen killing, also over group membership. This has important consequences for skew theory because workers cannot quit the colony and take up ‘outside options’ if skew among queens does not meet their fitness interest. Furthermore, differences among populations in skew in *L. acervorum* are likely to be genetically fixed [35], rather than facultative changes in behavioural time that also contradicts an important assumption of skew models [73]. Together, these findings throw doubt on the applicability of skew models, in particular transactional ones, and suggest that the testing of the fundamental assumptions of skew theory is critically important.

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