Author for correspondence:
Xuguo Zhou
e-mail: xuguozhou@uky.edu

THE ROYAL SOCIETY
PUBLISHING

Cooperative policing behaviour regulates reproductive division of labour in a termite

Qian Sun[1,2,†], Jordan D. Hampton[2,†], Austin Merchant[2], Kenneth F. Haynes[2] and Xuguo Zhou[2]

[1]Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
[2]Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY 40546-0091, USA

QS, 0000-0001-6341-6036; XZ, 0000-0002-2385-8224

Reproductive conflicts are common in insect societies where helping castes retain reproductive potential. One of the mechanisms regulating these conflicts is policing, a coercive behaviour that reduces direct reproduction by other individuals. In eusocial Hymenoptera (ants, bees and wasps), workers or the queen act aggressively towards fertile workers, or destroy their eggs. In many termite species (order Blattodea), upon the death of the primary queen and king, workers and nymphs can differentiate into neotenic reproductives and inherit the breeding position. During this process, competition among neotenics is inevitable, but how this conflict is resolved remains unclear. Here, we report a policing behaviour that regulates reproductive division of labour in the eastern subterranean termite, *Reticulitermes flavipes*. Our results demonstrate that the policing behaviour is a cooperative effort performed sequentially by successful neotenics and workers. A neotenic reproductive initiates the attack of the fellow neotenic by biting and displays alarm behaviour. Workers are then recruited to cannibalize the injured neotenic. Furthermore, the initiation of policing is age-dependent, with older reproductives attacking younger ones, thereby inheriting the reproductive position. This study provides empirical evidence of policing behaviour in termites, which represents a convergent trait shared between eusocial Hymenoptera and Blattodea.

1. Introduction

Eusocial insects exhibit reproductive division of labour between a few reproductive individuals and numerous sterile workers. However, potential conflicts arise in species where workers are capable of reproduction [1–4]. In addition to queen pheromones that regulate worker reproduction in many species [5–7], policing behaviour is an important solution to maintain reproductive harmony [2,8]. Queen policing, a term coined by Oster & Wilson in 1978, describes behaviours carried out by the queen to retain her reproductive dominance over workers [9]. Worker policing, which was named by Ratnieks in 1988, was used to describe the actions of workers that reduce the production of sons by workers in favour of the production of sons by the queen in honeybees [8]. The concept of 'policing behaviour' was expanded by Monnin & Ratnieks in 2001 to include all 'coercive actions that reduce direct reproduction by other individuals', which accommodates various forms of behavioural regulation observed in social insects [10]. In eusocial Hymenoptera (ants, bees and wasps), policing behaviour is performed via egg-eating or different forms of aggression, such as immobilization, biting and stinging [10–13]. Policing interactions may occur among workers [11], among reproductives [14,15], or between reproductives and workers [12,16–18]. The vast majority of investigations on policing behaviour have been focused on Hymenoptera, but little

is known about its occurrence or nature in termites (order Blattodea), a group of eusocial insects that evolved 50 Myr earlier than eusocial Hymenoptera [19].

Policing behaviour serves two functions that are not mutually exclusive, which are regulating genetic conflicts and improving colony efficiency [8,20,21]. Genetic conflicts arise between colony members due to relatedness asymmetries. For example, in social Hymenoptera with haplodiploid sex-determination, workers are often capable of laying unfertilized male eggs, and they are more closely related to their own sons than males produced by other individuals [2,8,22]. Moreover, policing behaviour contributes to colony efficiency even when little or no genetic conflict is present, as it optimizes the allocation of colony resources to reproduction [8,23], or maintains an adaptive colony-level phenotype [24].

Unlike social Hymenoptera, termite colonies are usually founded and dominated by a pair of primary reproductives (queen and king). Upon their death, neotenic reproductives of both sexes can differentiate either from workers, which become ergatoid reproductives that lack wing buds, or from nymphs, which become nymphoid reproductives that possess short wing buds [25]. Reproductive succession by neotenics has been reported in at least 13.4% of 'higher' termite genera (Termitidae) and 61.7% of 'lower' termite genera (all other termite families) [26]. In termites with diplodiploid sex determination, individuals are more closely related to their own offspring than that of their siblings, regardless of outbreeding or inbreeding. Therefore, genetic conflicts potentially exist and competition for reproduction between nest-mates is expected [27]. Moreover, given the fact that workers are reproductively totipotent in many species, colony-level efficiency can be compromised if excess neotenics differentiate, as it results in a reduced labour force and increased resource demand by the reproductives and their brood.

In the presence of fertile reproductives, neotenic formation is regulated through pheromones [6,28,29], and policing behaviour through overt aggression was considered rare in termites [28,30]. However, during the process of reproductive succession when colonies are orphaned and inhibitory pheromones are temporarily absent, production of excessive neotenic reproductives in the colony is expected [6,28,29]. In addition, cannibalism of neotenics was observed in several termite families including Termopsidae [31], Kalotermitidae [30,32–34] and Rhinotermitidae [35,36], suggesting the presence of policing behaviour that directly regulates reproduction in termites. Empirical studies of the process and causes of policing behaviour, however, are lacking. In this study, we conducted a series of experiments to understand whether the number of ergatoid neotenics is regulated behaviourally during reproductive succession, and how policing behaviour is performed in the eastern subterranean termite, *Reticulitermes flavipes*. Furthermore, we investigated a proximate factor that determines the succession of reproductives.

2. Material and methods

(a) Insect collection and maintenance

Field and laboratory colonies of *R. flavipes* were used in this study. Field colonies were collected from the Red River Gorge area, Daniel Boone National Forest (Slade, KY, USA) and the University of Kentucky Arboretum (Lexington, KY, USA). These colonies were obtained in summer using trapping stations filled with dampened cardboard rolls. Once captured termites were extracted from traps, and they were placed in Petri dishes (14.5 cm × 2.0 cm) with moistened unbleached paper towel as their only food source for 7–10 days before they were used in experiments. Only workers and soldiers were collected from the field. Laboratory colonies were established in 2010 by pairs of sibling alates from a dispersal flight in Lexington, Kentucky, and were kept in closed plastic boxes filled with moistened wood mulch and pinewood blocks in the laboratory for 5 years before use. All colonies were maintained in complete darkness (L : D = 0 : 24), at 27 ± 1°C, 80–99% RH.

(b) Orphaning assay to test ergatoid number restriction

This assay was used to simulate the reproductive replacement process after the death of primary reproductives. Groups of 100 workers were kept in Petri dishes (35 mm diameter) with moistened paper towel placed at the bottom. Two treatments ('removal' and 'non-removal' of ergatoids) were conducted. This experiment was specifically designed in a way to compare the number of ergatoids that can potentially differentiate and survive (removal) with that actually survived (non-removal). All termites were maintained at 27 ± 1°C and in complete darkness for 90 days. Each dish was checked by identifying the sex and counting the number of newly differentiated ergatoids. Each ergatoid was removed and replaced with a worker in the 'removal' treatment, but returned to the dish in the 'non-removal' treatment. Dishes in 'removal' treatments were checked every day, while dishes in 'non-removal' treatments were checked every 10 days to reduce stress to reproductives resulting from manipulations needed for sex identification. The number of remaining termites in each group was counted every 30 days, and mortality was calculated based on the difference between the numbers of initial and remaining individuals. Injured ergatoids were not counted. A total of 20 replications were made with five replications in each of the four colonies. Two field and two laboratory colonies were used in this experiment.

(c) Orphaning assay for observation of policing behaviour

This assay was designed to observe policing behaviour under orphaning conditions, which resembled the 'non-removal' treatment above. The dishes were incubated at 27 ± 1°C for a total of 90 days. Once a week, the dishes were checked for dryness, and water was added if the paper towels at the bottom appeared to be dry. Between 60 and 90 days, each dish was checked for the presence of ergatoids, and the dishes with ergatoids were selected to be video recorded for 6 days. Video cameras (Canon Vixia HF G20, Canon Inc., Tokyo, Japan) were used for recording and yielded high-quality images. The dishes and cameras were shaded under a piece of cardboard (1.5 m × 0.8 m) during recording. Ergatoids were identified as male or female and colour marked using enamel paint (Testor Corporation, Rockford, IL, USA) on their head capsule prior to recording. The colour codes for ergatoids were randomized, and we did not find influence of the colour mark on the termites or their subsequent behaviour. In addition, subterranean termites (with the exception of alates) do not develop functional eyes to recognize colour. During recording, the dishes were checked every day for missing ergatoids (which were cannibalized), and newly formed ergatoids were colour marked. When a marked ergatoid was missing, the video of the previous 24 h was quantitatively analysed.

We define the 'victim' as the ergatoid that was eventually cannibalized, the 'attacker' as the individual who attacked the victim, and 'bystanders' as the ergatoids who did not perform the first major attack. A major attack was recognized when the attacker visibly injured the victim such that the researcher could see the abdomen was torn, haemolymph was leaking and the victim
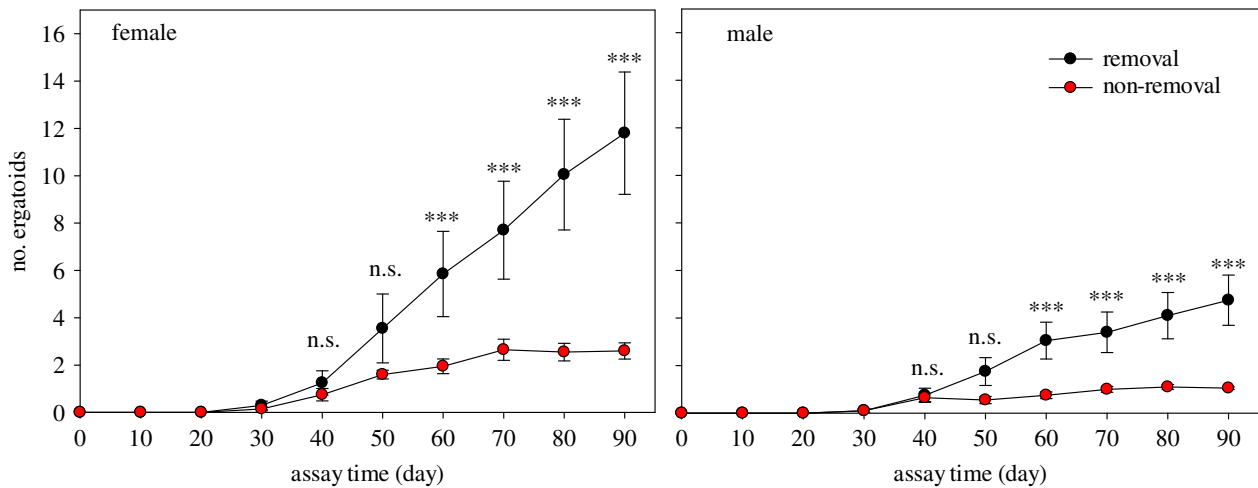
Figure 1. Restriction of ergatoid numbers in orphaning assay. Female and male ergatoids that formed from groups of 100 workers were documented for 90 days post establishment of the experiment. Cumulative number of differentiated ergatoids (removal treatment) and surviving number of ergatoids (non-removal treatment) are presented (mean ± s.e.) and compared within the same observation day. n.s., not significant; ***, $p < 0.001$; GLMM, Poisson family; $n = 20$ per treatment per observation day. (Online version in colour.)

quickly fled. The first major attack was designated as time '0', and the frequency of vibration and number of workers surrounding the victim were documented. A 1 min sample (30 s before and after the time point) was analysed for all these behaviours, with samples selected 5, 15, 20, 25, 30, 45 and 60 min before and after time '0'. Worker density near the victim was an indicator of cannibalism. The density was quantified by counting the number of workers and soldiers within a 4 mm radius from the centre of the victim (the radius approximately equals the body length of a worker). Only field-collected colonies were used in this experiment.

(d) Policing assay to test the effect of ergatoid age

This assay was used to determine if policing behaviour in R. flavipes is associated with the age of ergatoids. Each group of 50 workers was kept in a petri dish (55 mm diameter) lined with six layers of moistened paper towel. A pair of virgin ergatoids, one female and one male, was added to each dish on day 1. Female and male treatments were conducted, with a younger female or male ergatoid being added to the initial group every day. When added to the dish, the initial pair of ergatoids was 7 days post differentiation (7 days old) and became older over the course of the experiment, and subsequent ergatoids were no more than 7 days old. All of the ergatoids were colour marked as previously described, and no new ergatoids differentiated during this assay. The dishes were recorded until one of the ergatoids was missing. The video was then analysed to identify who attacked that ergatoid. In each replication, the workers, initial ergatoids and introduced ergatoids originated from the same colony. A total of 9 and 10 replications were made for female and male treatments, respectively; aggressively interacting pairs that were the same sex were analysed for their age differences. This experiment did not attempt to address sex-specificity, but it was designed for increased chance of sex-specific aggression, and only same-sex aggressions were analysed to eliminate confounding factors associated with sex. Only field-collected colonies were used in this experiment.

(e) Statistical analyses

Data were analysed using R (https://www.r-project.org) and Statistix 10 (Analytical Software, Tallahassee, FL, USA), and graphs were generated using SigmaPlot 13 (Systat Software Inc, Chicago, IL, USA). Data for ergatoid number and mortality in reproductive 'removal' and 'non-removal' assays were fitted to Poisson family generalized linear mixed models using R's *glmer*

function. In the model for ergatoid number, ergatoid sex and treatment group (removal versus non-removal) were coded as fixed effects, while colony of origin was coded as a random effect. An observation-level random effect was introduced to avoid overdispersion. In the model for mortality, treatment group was coded as a fixed effect, while colony of origin was coded as a random effect. In both cases, data were analysed separately for each 10-day interval. Data testing the influence of ergatoid age on aggressive interactions in the policing assay were analysed in Statistix using a Wilcoxon signed-rank test.

3. Results

(a) The number of ergatoids is restricted

In 'removal' treatment, significantly more female and male ergatoids differentiated than remained in 'non-removal' treatment within 90 days (figure 1; $\chi^2 = 137.14$, d.f. = 1, $p < 0.001$; GLMM, Poisson family; $n = 20$). At the end of day 90, 11.80 ± 2.59 (mean ± s.e.) female and 4.75 ± 1.06 male ergatoids differentiated when they were removed daily, compared with only 2.60 ± 0.34 female and 1.05 ± 0.05 male ergatoids if they were not removed. In addition, there was significantly higher overall mortality in 'non-removal' than in 'removal' treatments (electronic supplementary material, figure S1; $\chi^2 = 30.36$, d.f. = 1, $p < 0.001$; GLMM, Poisson family; $n = 20$). In 'non-removal' groups, we frequently observed injured ergatoids partially consumed by workers, along with other intact ergatoids. Between the two treatments, the mortality difference (8.6 individuals at day 60 and 10.2 at day 90) closely matched the difference in the number of ergatoids that differentiated and that survived (6.2 at day 60 and 12.9 at day 90), suggesting cannibalism of ergatoids in 'non-removal' treatments was primarily responsible for the difference in mortality.

(b) Ergatoids and workers cooperate in elimination of excessive ergatoids

A total of seven events were captured of the full behavioural process, which started with one ergatoid attacking another ergatoid and ended with the injured individual being
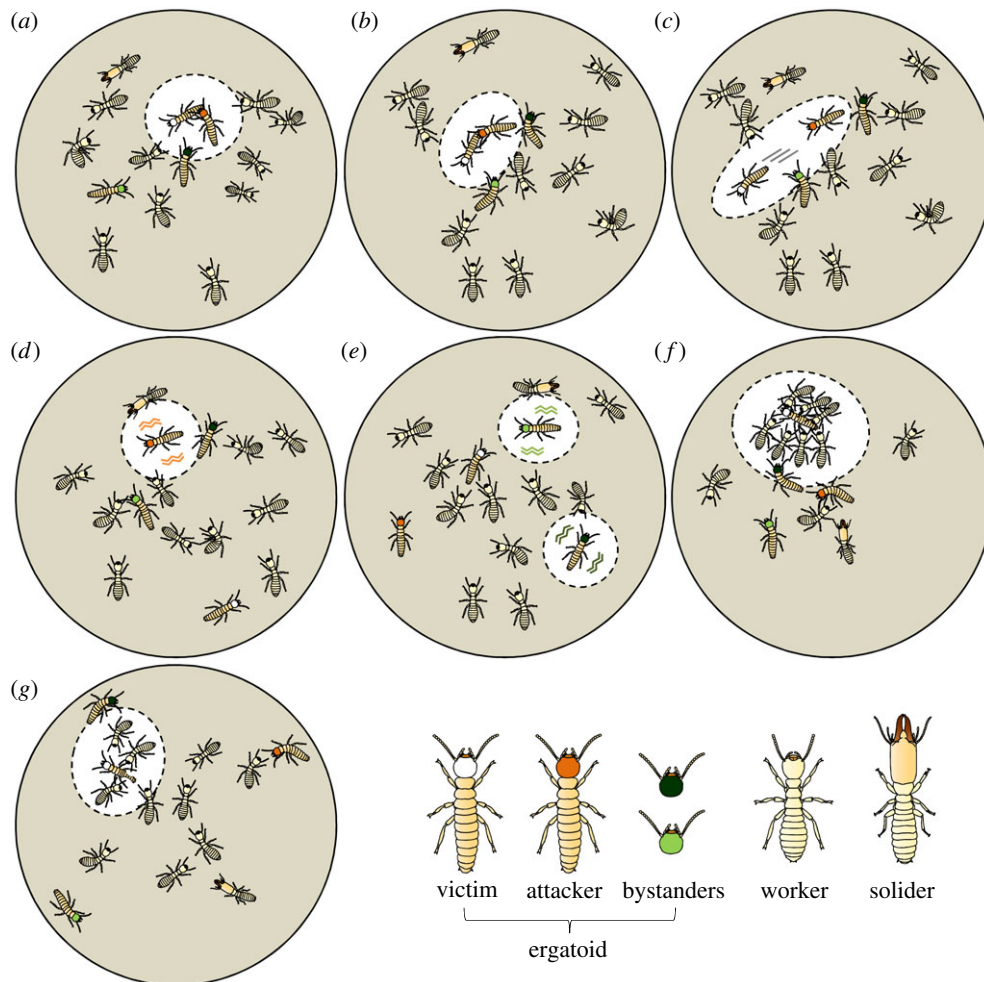
Figure 2. Behavioural process of cooperative policing. (*a*) An ergatoid attacker holds the victim using mouthparts (time = −10 s). (*b*) The attacker performs a major attack by biting the victim on its abdomen (time = 0). (*c*) The major attack causes the victim to quickly flee (time = 0). (*d*) The attacker displays alarm behaviour by vigorously vibrating its body towards multiple directions (time = 1 min). (*e*) Bystanders also display alarm behaviour in the same manner (time = 5 min). (*f*) Workers then surround and nibble the victim (time = 30 min). (*g*) Victim is partially cannibalized (time = 2 h). Attacker: ergatoid who performed the first major attack. Victim: ergatoid who received the attack and was later cannibalized. Bystander: other ergatoids. (Online version in colour.)

cannibalized by workers (figure 2; electronic supplementary material, table S1, movies S1–S4; movies S1–S4 were clips obtained from the same replication showing a representative behavioural response). In this behaviour, the attacker antennated the victim first, and used mouthparts to hold the abdomen or thorax of the victim before biting. The bite always caused the victim to leak haemolymph and quickly flee. Right after the attack, the attacker displayed alarm behaviour by vigorously vibrating the body in multiple directions; interestingly, the ergatoids who did not participate in the aggression (i.e. bystanders) also performed alarm behaviour after the attack, while the victim rarely engaged in vibration (figure 3*a*). Workers, on average, displayed little vibration (figure 3*a*). With the alarm of ergatoids, workers rapidly began to surround the injured victim, biting and consuming it while it was still alive, and the cannibalism reached a peak 30 min after the attack (figure 3*b*). As the cannibalism began to decline following consumption of the victim, the frequency of vibration by the ergatoid attacker and bystanders began to reduce (45–60 min after the attack) (figure 3*a*). Soldiers were produced via differentiation from workers in all seven replications by the time of policing behaviour (electronic supplementary material, table S1), but they did not participate in alarm behaviour or cannibalism (figure 3*a,b*).

(c) Ergatoid elimination is age-dependent

The majority of aggressive interactions involved an ergatoid attacking a same-sex ergatoid (15 out of 19), while the others involved either a male ergatoid attacking a female (one case in the female treatment), or a group of workers nibbling and consuming an ergatoid (1 and 2 cases in female and male treatments, respectively). In the same-sex aggressive interactions, the attacker was always older than the victim in both female and male treatments (figure 4; female: $Z = −2.3664$, $p < 0.01$, $n = 7$; male: $Z = −2.5205$, $p < 0.01$, $n = 8$; Wilcoxon signed-rank test, one-tailed). The median age difference between the pair of attacker and victim was 5 and 2 days for females and males, respectively.

4. Discussion

(a) Cooperative effort and justification of 'policing behaviour'

Overall, this study reveals a behavioural mechanism regulating reproductive division of labour during reproductive succession in termites. Unlike butting behaviour, an indicator of reproductive dominance reported in a drywood termite,
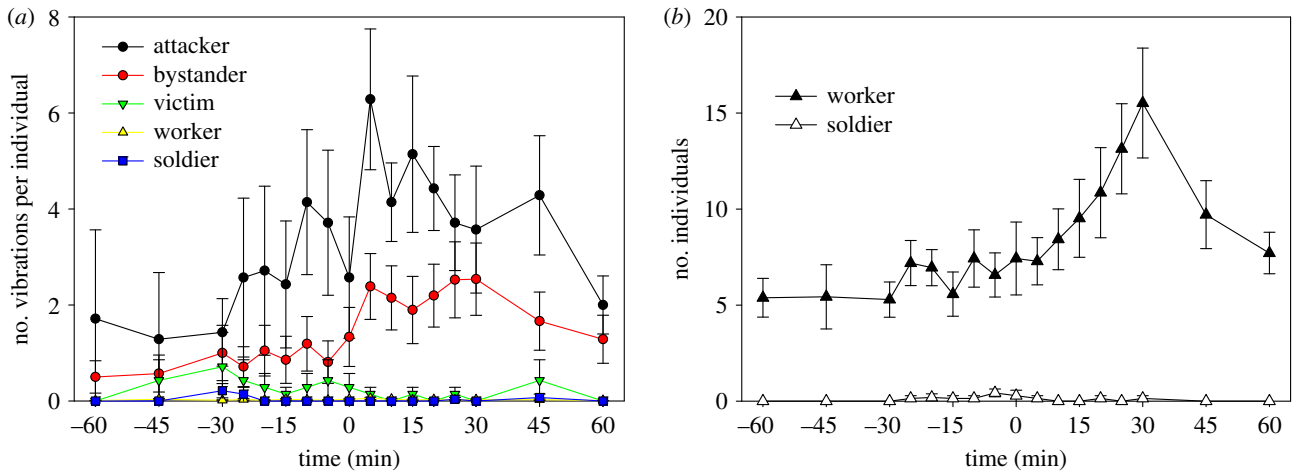
Figure 3. Participation of different castes in policing behaviour. (*a*) Figure shows the number of vibrations per minute performed by each individual (mean ± s.e., $n = 7$). time '0' was the time of the first major attack. A major attack was recognized when an ergatoid visibly injured another ergatoid. A 1 min sample (30 s before and after) was analysed for each time point. (*b*) The number of individuals surrounding the victim before and after the attack (mean ± s.e., $n = 7$). The increased density of workers reflects cannibalism. (Online version in colour.)
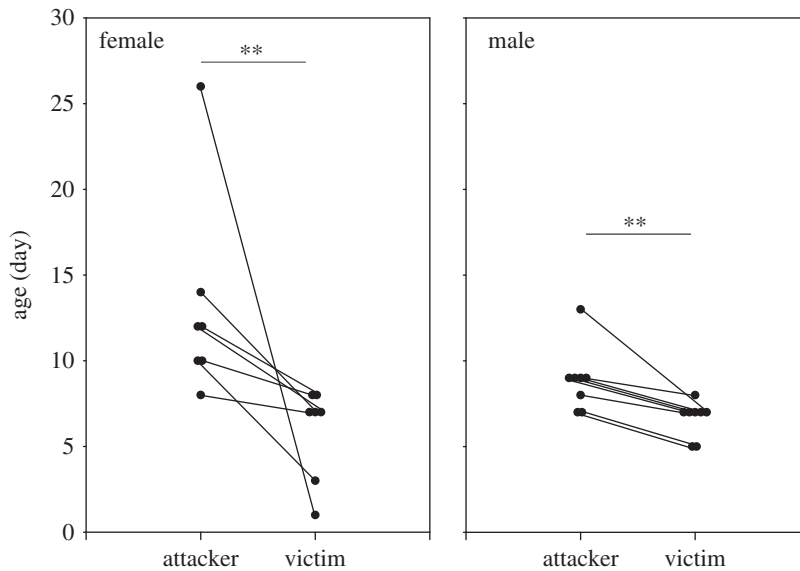

Figure 4. Age of interacting ergatoids in policing behaviour. Each dot represents an individual attacker or victim, and lines connect the attacker to the corresponding victim. The negative slope of the lines in both graphs shows that an older ergatoid attacked a younger ergatoid in every replication, and the age differences are significant (female: $Z = -2.3664$, $p < 0.01$, $n = 7$; male: $Z = -2.5205$, $p < 0.01$, $n = 8$; Wilcoxon signed-rank test, one-tailed; **, $p < 0.01$).

Cryptotermes secundus, and a dampwood termite, *Zootermopsis nevadensis* [37,38], this behaviour in *R. flavipes* directly acts to eliminate reproductive individuals. The attack and cannibalism reflect a coercive action by older reproductives and workers towards younger reproductives, and the elimination reduces direct reproduction of policed individuals; therefore, this behaviour satisfies the definition of policing [10].

Policing in *R. flavipes* is a sequential behaviour cooperatively performed by older reproductives in concert with workers. This is comparable to the sting smearing behaviour in a queenless ant, *Dinoponera quadriceps*, a textbook example of policing behaviour [39]. In this ant, the alpha female chemically marks a low-ranking challenger using her stinger, causing workers to immobilize the marked individual [40]. Such a cooperative effort allows the alpha to inflict punishment indirectly and maintain her dominant status without fighting. Similarly, in *R. flavipes*, the ergatoid attacker does not kill its rival directly; rather, it induces haemolymph exposure of the victim by

biting, and proceeds with an alarm behaviour. Workers eventually eliminate the injured individual. The workers are probably recruited by the vibrational signals, and the cannibalistic behaviour is potentially caused by chemical cues in the haemolymph, which remain to be investigated. Policing represents another social context in which vibratory alarm behaviour is displayed by termites, in addition to previously documented contexts such as colony defense [41], undertaking behaviour [42], pathogen avoidance [43], reproductive recognition [44] and physical colony disturbance in general [45].

(b) Caste fate conflict
In the absence of reproductives and their inhibitory pheromones, excess ergatoid production in *R. flavipes* is an expression of caste fate conflict, which also occurs in social Hymenoptera. For example, in the *Melipona* stingless bees, caste is self-determined, and immature females selfishly

develop into queens to maximize direct reproduction [46]. Such excess queen production causes depletion of the workforce and poses a cost to the colony, leading to a situation known as 'tragedy of the commons' [47]. The policing behaviour in *R. flavipes* provides an effective solution that prevents unsustainable reproduction upon the loss of former reproductives, and supports colony efficiency. Uncontrolled reproduction causes increased resource demand by the reproductive individuals and their brood, and a balanced ratio of reproductives and workers is often optimal to the colony. For example, in the parthenogenic ant *Platythyrea punctata*, worker to brood ratio is limited, and colonies are incapable of rearing brood produced by additional reproductives [23]. Similarly, an optimal allocation to reproduction is required in the Japanese subterranean termite *R. speratus*, and increased number of queens does not lead to additional reproductive output [48].

In social insects, policing often inhibits reproduction of the focal individual without killing it. In Hymenoptera, policing behaviour often enforces workers to stop reproducing and cooperate in brood care. In subterranean termites, however, worker-reproductive differentiation is an irreversible process achieved through at least one molt [25]. Ergatoid reproductives are a non-foraging caste that depends on workers to provide food [49], and the presence of excess ergatoids and the subsequent brood are costly for the colony. In *R. flavipes*, it is likely that this policing behaviour also occurs among nymphoids, another type of neotenic reproductives commonly found in subterranean termites. For instance, in a congeneric species, *R. speratus*, and in the Asian subterranean termite, *Coptotermes gestroi*, it was observed that excess nymphoids were cannibalized by workers after colony orphaning [35,36]. Elimination of additional neotenics avoids future colony investment; cannibalism, in addition, allows the colony to recycle nutrients from the policed individuals and partially rescue the cost that has already occurred upon their differentiation.

(c) Reproductive competition

The aggressive interaction between ergatoids in termites reflects competition among colony members in inheriting the breeding position after the death of primary reproductives. This form of policing might be common in termite species where workers or nymphs retain reproductive potential. Indeed, a similar behaviour has been observed in a drywood termite, *Kalotermes flavicollis*, where neotenic reproductives attack each other and injured individuals are cannibalized by workers and nymphs [34]. In another drywood termite, *Pterotermes occidentis*, workers and nymphs were observed to mutilate the wind buds of their nest-mates upon the loss of the royal pair, and mutilated individuals stopped or delayed their molt into reproductives [50]. However, it was argued that wing bud mutilation might be an artefact of experimental handling, as this behaviour was only observed in disturbed colonies in the drywood species *C. secundus* [51]. In addition, reproductive competition also occurs upon fusion of neighbouring conspecific colonies. In *Z. nevadensis*, reproductives of encountering colonies engage in agonistic behaviour, leading to a reduction in their numbers [52]. Moreover, reproductive conflict among unrelated queens happens in species where colonies are founded by pleometrosis (i.e. colony founding via multiple queens). In a fungus-growing termite, *Macrotermes michaelseni*, the mutilation of queen antennae indicates aggression between primary reproductives that are

co-founders, and this behaviour may influence queen number [53]. These findings suggest that aggressive interaction between reproductives is widespread in termites under diverse social contexts.

(d) Age-dependent elimination

The age of ergatoids is an important factor that influences the outcome of policing behaviour in *R. flavipes*. This result is consistent with the dampwood termite *Porotermes adamsoni*, in which neotenic reproductives that develop earlier have higher survivorship than those that differentiate later [54]. Age is also positively correlated with dominance rank in the naked mole-rat *Heterocephalus glaber* [55], in which younger individuals receive more aggression than older ones [56]. In termites, older ergatoids have the first chance to use resources from the colony, such as food provided by workers and mating opportunities with existing reproductives. These factors may contribute to the maturity of ergatoids in terms of gonad development [57], body weight and mandible sclerotization, thus allowing them to outcompete the younger ones. In this study, younger individuals were subsequently introduced to each group to simulate the ergatoid differentiation process in a colony, and a residency effect was possibly associated with the policing outcome. The ergatoids introduced later potentially interacted with the existing group less frequently, which might be an additional contributing factor to the observed results. In *C. secundus*, compared with neotenics that were eventually eliminated, surviving neotenics interacted more frequently with workers through proctodeal trophallaxis after their differentiation [30]. This behaviour is possibly associated with age, but it is yet to be tested if neotenics perform trophallaxis more frequently as they become older.

The elimination of younger ergatoids in termites is also similar to the selective elimination of small queens in the stingless bee *Schwarziana quadripunctata*, where large fecund queens are favoured and dwarf queens tend to be killed by workers [58]. With policing behaviour that strongly acts against small queens, small females should be less likely to develop into queens, a situation indicated by a theoretical study based on inclusive fitness theory [58]. In *R. flavipes*, while a few ergatoids were formed and eliminated, the majority of workers did not molt into ergatoids. This suggests that the same theory may apply to termites: in the presence of older ergatoids, rather than developing into reproductives and being killed, workers can gain indirect fitness benefits by not differentiating.

(e) Evolution of policing behaviour

Agonistic behaviour has been observed in both worker and queen policing in social Hymenoptera [10–13], as well as in termites. Agonism might represent a conserved component of policing in social insects, which evolved from their non- or sub-social ancestors. Agonistic interactions in competitions for resources and/or mates are common in non-social insects, and the pattern of 'older wins' has been demonstrated in many species, such as the fruit fly (*Drosophila melanogaster*) [59], a burying beetle (*Nicrophorus orbicollis*) [60] and a parasitoid wasp (*Nasonia vitripennis*) [61]. Interestingly, this phenomenon is also observed in cockroaches, which are close relatives to termites in the order Blattodea. In the cinereous cockroach, *Nauphoeta cinerea*, adult males form dominance hierarchies and older individuals win contests via elaborate agonistic interactions [62]. This implies that neotenic agonism in termites

might have evolved from similar behaviours displayed in dominance contests in their cockroach-like ancestors.

In termites, neotenic reproduction has been considered an important condition for the evolution of eusociality [26]. Reproduction without dispersal would enable individuals to inherit the established nest and resources, and this may have promoted selection for further diversification of helper castes such as workers and soldiers [26,52]. Agonistic behaviour among neotenics might have played a role in enforcing nest-mate altruism during the early evolution of eusociality in termites. Enforced altruism by policing has been demonstrated in social Hymenoptera, with evidence showing that fewer workers reproduce when the effectiveness of policing increases [63]. With the evolution of a true worker caste in termites, the policing behaviour, as seen in *R. flavipes*, would then evolve to involve worker participation to eliminate neotenics that lose in the competition. Further investigation is warranted to test this 'enforced altruism to elimination' scenario.

(f) Future directions

Several questions remain to be addressed in regard to the pheromonal mechanisms underlying policing behaviour, including the dynamic change of reproductive pheromones with age and the chemical cues inducing cannibalism of erga-toids by workers. Importantly, policing behaviour, in a broad sense, may be more widespread in termites than previously considered. In addition to a number of convergent traits in social Hymenoptera and Blattodea, such as suicidal colony defence [64], collective foraging using trail pheromones [65] and undertaking behaviour [66], policing behaviour represents another important behaviour that independently evolved in both eusocial groups. Comparative studies on proximate and ultimate aspects of policing behaviour among social insect taxa will provide important insights into the evolution of eusociality in insects.

References

1. Robinson GE. 1992 Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**, 637–665. (doi:10.1146/annurev.en.37.010192.003225)

2. Ratnieks FLW, Foster KR, Wenseleers T. 2006 Conflict resolution in insect societies. *Annu. Rev. Entomol.* **51**, 581–608. (doi:10.1146/annurev.ento.51.110104.151003)

3. Hart AG, Monnin T. 2006 Conflict over the timing of breeder replacement in vertebrate and invertebrate societies. *Insectes Soc.* **53**, 375–389. (doi:10.1007/s00040-005-0895-4)

4. Korb J. 2018 Chemical fertility signaling in termites: idiosyncrasies and commonalities in comparison with ants. *J. Chem. Ecol.* **44**, 818–826. (doi:10.1007/s10886-018-0952-2)

5. Conte YL, Hefetz A. 2008 Primer pheromones in social Hymenoptera. *Annu. Rev. Entomol.* **53**, 523–542. (doi:10.1146/annurev.ento.52.110405.091434)

6. Matsuura K, Himuro C, Yokoi T, Yamamoto Y, Vargo EL, Keller L. 2010 Identification of a pheromone regulating caste differentiation in termites. *Proc. Natl Acad. Sci. USA* **107**, 12 963–12 968. (doi:10.1073/pnas.1004675107)

7. Holman L, Jørgensen CG, Nielsen J, d'Ettorre P. 2010 Identification of an ant queen pheromone regulating worker sterility. *Proc. R. Soc. B* **277**, 3793–3800. (doi:10.1098/rspb.2010.0984)

8. Ratnieks FLW. 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat.* **132**, 217–236. (doi:10.1086/284846)

9. Oster GF, Wilson EO. 1978 *Caste and ecology in the social insects*. Princeton, NJ: Princeton University Press.

10. Monnin T, Ratnieks FLW. 2001 Policing in queenless ponerine ants. *Behav. Ecol. Sociobiol.* **50**, 97–108. (doi:10.1007/s002650100351)

11. Ratnieks FLW, Visscher PK. 1989 Worker policing in the honeybee. *Nature* **342**, 796–797. (doi:10.1038/342796a0)

12. Wenseleers T, Tofilski A, Ratnieks FLW. 2005 Queen and worker policing in the tree wasp *Dolichovespula sylvestris*. *Behav. Ecol. Sociobiol.* **58**, 80–86. (doi:10.1007/s00265-004-0892-4)

13. Foster KR, Ratnieks FLW. 2001 Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proc. R. Soc. Lond. B* **268**, 169–174. (doi:10.1098/rspb.2000.1346)

14. Bourke AFG. 1991 Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. *Anim. Behav.* **42**, 295–310. (doi:10.1016/S0003-3472(05)80561-5)

15. Medeiros FNS, Lopes LE, Moutinho PRS, Oliveira PS, Hölldobler B. 1992 Functional polygyny, agonistic interactions and reproductive dominance in the Neotropical ant *Odontomachus chelifer* (Hymenoptera, Formicidae, Ponerinae). *Ethology* **91**, 134–146. (doi:10.1111/j.1439-0310.1992.tb00857.x)

16. Adams ES, Balas MT. 1999 Worker discrimination among queens in newly founded colonies of the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **45**, 330–338. (doi:10.1007/s002650050568)

17. Loope K. 2015 Queen killing is linked to high worker–worker relatedness in a social wasp. *Curr. Biol.* **25**, 2976–2979. (doi:10.1016/j.cub.2015.09.064)

18. Monnin T, Peeters C. 1997 Cannibalism of subordinates' eggs in the monogynous queenless ant *Dinoponera quadriceps*. *Naturwissenschaften* **84**, 499–502. (doi:10.1007/s001140050433)

19. Misof B et al. 2014 Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763–767. (doi:10.1126/science.1257570)

20. Wenseleers T, Ratnieks FLW. 2006 Comparative analysis of worker reproduction and policing in eusocial Hymenoptera supports relatedness theory. *Am. Nat.* **168**, E163–E179. (doi:10.1086/508619)

21. Walter B, Brunner E, Heinze J. 2011 Policing effectiveness depends on relatedness and group size. *Am. Nat.* **177**, 368–376. (doi:10.1086/658396)

22. Ratnieks FLW, Reeve HK. 1992 Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol.* **158**, 33–65. (doi:10.1016/S0022-5193(05)80647-2)

23. Hartmann A, Wantia J, Torres JA, Heinze J. 2003 Worker policing without genetic conflicts in a clonal ant. *Proc. Natl Acad. Sci. USA* **100**, 12 836–12 840. (doi:10.1073/pnas.2132993100)

24. Teseo S, Kronauer DJC, Jaisson P, Châline N. 2013 Enforcement of reproductive synchrony via policing in a clonal ant. *Curr. Biol.* **23**, 328–332. (doi:10.1016/j.cub.2013.01.011)

25. Korb J, Hartfelder K. 2008 Life history and development: a framework for understanding developmental plasticity in lower termites. *Biol. Rev.* **83**, 295–313. (doi:10.1111/j.1469-185X.2008.00044.x)

26. Myles TG. 1999 Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology* **33**, 1–43.

27. Bartz SH. 1979 Evolution of eusociality in termites. *Proc. Natl Acad. Sci. USA* **76**, 5764–5768. (doi:10.1073/pnas.76.11.5764)

28. Matsuura K. 2012 Multifunctional queen pheromone and maintenance of reproductive harmony in termite colonies. *J. Chem. Ecol.* **38**, 746–754. (doi:10.1007/s10886-012-0137-3)

29. Sun Q, Haynes KF, Hampton JD, Zhou X. 2017 Sex-specific inhibition and stimulation of worker-reproductive transition in a termite. *Sci. Nat.* **104**, 79. (doi:10.1007/s00114-017-1501-5)

30. Hoffmann K, Korb J. 2011 Is there conflict over direct reproduction in lower termite colonies? *Anim. Behav.* **81**, 265–274. (doi:10.1016/j.anbehav.2010.10.017)

31. Lenz M. 1985 Is inter-and intraspecific variability of lower termite neotenic numbers due to adaptive thresholds for neotenic elimination? Considerations from studies on *Porotermes adamsoni* (Froggatt) (Isoptera: Termopsidae). In *Caste differentiation in social insects*, Vol. 3 (eds JAL Watson, BM Okot-Kotber, C Noirot), p. 125. Oxford, UK: Pergamon Press.

32. Lenz M, McMahan EA, Williams ER. 1982 Neotenic production in *Cryptotermes brevis* (Walker): influence of geographical origin, group composition, and maintenance conditions (Isoptera: Kalotermitidae). *Insectes Soc.* **29**, 148–163. (doi:10.1007/BF02228748)

33. Lüscher M. 1952 Die produktion und elimination von ersatzgeschlechtstieren bei der termite *Kalotermes flavicollis* Fabr. *J. Comp. Physiol. A* **34**, 123–141. (doi:10.5962/bhl.part.75627)

34. Ruppli E. 1969 Die Elimination Überzähliger Ersatzgeschlechtstiere bei der Termite *Kalotermes Flavicollis* (Fabr.). *Insectes Soc.* **16**, 235–248. (doi:10.1007/BF02223411)

35. Costa-Leonardo AM, Arab A, Casarin FE. 2004 Neotenic formation in laboratory colonies of the termite *Coptotermes gestroi* after orphaning. *J. Insect Sci.* **4**, 10. (doi:10.1673/031.004.1001)

36. Miyata H, Furuichi H, Kitade O. 2004 Patterns of neotenic differentiation in a subterranean termite, *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Entomol. Sci.* **7**, 309–314. (doi:10.1111/j.1479-8298.2004.00078.x)

37. Korb J, Weil T, Hoffmann K, Foster KR, Rehli M. 2009 A gene necessary for reproductive suppression in termites. *Science* **324**, 758. (doi:10.1126/science.1170660)

38. Penick CA, Trobaugh B, Brent CS, Liebig J. 2013 Head-butting as an early indicator of reproductive disinhibition in the termite *Zootermopsis nevadensis*. *J. Insect Behav.* **26**, 23–34. (doi:10.1007/s10905-012-9332-x)

39. Rubenstein DR, Alcock J. 2019 *Animal behavior: an evolutionary approach*, 11th edition. Sunderland, MA: Sinauer Associates.

40. Monnin T, Ratnieks FLW, Jones GR, Beard R. 2002 Pretender punishment induced by chemical signalling in a queenless ant. *Nature* **419**, 61. (doi:10.1038/nature00932)

41. Tian L, Preisser EL, Haynes KF, Zhou X. 2017 Social buffering in a eusocial invertebrate: termite soldiers reduce the lethal impact of competitor cues on workers. *Ecology* **98**, 952–960. (doi:10.1002/ecy.1746)

42. Sun Q, Haynes KF, Zhou X. 2013 Differential undertaking response of a lower termite to congeneric and conspecific corpses. *Sci. Rep.* **3**, 1650. (doi:10.1038/srep01650)

43. Rosengaus RB, Jordan C, Lefebvre ML, Traniello JFA. 1999 Pathogen alarm behavior in a termite: a new form of communication in social insects. *Naturwissenschaften* **86**, 544–548. (doi:10.1007/s001140050672)

44. Funaro CF, Böröczky K, Vargo EL, Schal C. 2018 Identification of a queen and king recognition pheromone in the subterranean termite *Reticulitermes flavipes*. *Proc. Natl Acad. Sci. USA* **115**, 3888–3893. (doi:10.1073/pnas.1721419115)

45. Crosland MWJ, Traniello JFA. 1997 Behavioral plasticity in division of labor in the lower termite *Reticulitermes fukienensis*. *Naturwissenschaften* **84**, 208–211. (doi:10.1007/s001140050380)

46. Wenseleers T, Ratnieks FLW, Billen J. 2003 Caste fate conflict in swarm-founding social Hymenoptera: an inclusive fitness analysis. *J. Evol. Biol.* **16**, 647–658. (doi:10.1046/j.1420-9101.2003.00574.x)

47. Wenseleers T, Ratnieks FLW. 2004 Tragedy of the commons in *Melipona* bees. *Proc. R. Soc. Lond. B* **271**, S310–S312. (doi:10.1098/rsbl.2003.0159)

48. Yamamoto Y, Matsuura K. 2011 Queen pheromone regulates egg production in a termite. *Biol. Lett.* **7**, 727–729. (doi:10.1098/rsbl.2011.0353)

49. Eggleton P. 2011 An introduction to termites: biology, taxonomy and functional morphology. In *Biology of termites: a modern synthesis* (eds DE Bignell, Y Roisin, N Lo), pp. 1–26. Dordrecht, The Netherlands: Springer Netherlands.

50. Zimmerman RB. 1983 Sibling manipulation and indirect fitness in termites. *Behav. Ecol. Sociobiol.* **12**, 143–145. (doi:10.1007/BF00343205)

51. Korb J. 2005 Regulation of sexual development in the basal termite *Cryptotermes secundus*: mutilation, pheromonal manipulation or honest signal? *Naturwissenschaften* **92**, 45–49. (doi:10.1007/s00114-004-0589-6)

52. Thorne BL, Breisch NL, Muscedere ML. 2003 Evolution of eusociality and the soldier caste in termites: influence of intraspecific competition and accelerated inheritance. *Proc. Natl Acad. Sci. USA* **100**, 12 808–12 813. (doi:10.1073/pnas.2133530100)

53. Brandl R, Hacker M, Bagine R, Kaib M. 2001 Geographic variation of polygyny in the termite *Macrotermes michaelseni* (Sjöstedt). *Insectes Soc.* **48**, 134–137. (doi:10.1007/PL00001755)

54. Mensa-Bonsu A. 1976 The production and elimination of supplementary reproductives in *Porotermes adamsoni* (Froggatt) (Isoptera, Hodotermitidae). *Insectes Soc.* **23**, 133–153. (doi:10.1007/BF02223847)

55. Clarke FM, Faulkes CG. 1997 Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. *Proc. R. Soc. Lond. B* **264**, 993–1000. (doi:10.1098/rspb.1997.0137)

56. O'Riain J. 1996 Pup ontogeny and factors influencing behavioural and morphological variation in naked mole-rats, *Heterocephalus glaber* (Rodentia, Bathyergidae). PhD, University of Cape Town.

57. Brent CS, Traniello JFA. 2001 Social influence of larvae on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*. *Physiol. Entomol.* **26**, 78–85. (doi:10.1046/j.1365-3032.2001.00221.x)

58. Wenseleers T, Hart AG, Ratnieks FLW. 2004 When resistance is useless: policing and the evolution of reproductive acquiescence in insect societies. *Am. Nat.* **164**, E154–E167. (doi:10.1086/425223)

59. Hoffmann AA. 1990 The influence of age and experience with conspecifics on territorial behavior in *Drosophila melanogaster*. *J. Insect Behav.* **3**, 1–12. (doi:10.1007/BF01049191)

60. Trumbo ST. 2012 Contest behavior and other reproductive efforts in aging breeders: a test of residual reproductive value and state-dependent models. *Behav. Ecol. Sociobiol.* **66**, 1511–1518. (doi:10.1007/s00265-012-1406-4)

61. Tsai YJJ, Barrows EM, Weiss MR. 2014 Why do larger and older males win contests in the parasitoid wasp *Nasonia vitripennis*? *Anim. Behav.* **91**, 151–159. (doi:10.1016/j.anbehav.2014.03.010)

62. Schal C, Bell WJ. 1983 Determinants of dominant-subordinate interactions in males of the cockroach *Nauphoeta cinerea*. *Biol. Behav.* **8**, 117–139.

63. Wenseleers T, Ratnieks FLW. 2006 Enforced altruism in insect societies. *Nature* **444**, 50. (doi:10.1038/444050a)

64. Shorter J, Rueppell O. 2012 A review on self-destructive defense behaviors in social insects. *Insectes Soc.* **59**, 1–10. (doi:10.1007/s00040-011-0210-x)

65. Czaczkes TJ, Grüter C, Ratnieks FLW. 2015 Trail pheromones: an integrative view of their role in social insect colony organization. *Annu. Rev. Entomol.* **60**, 581–599. (doi:10.1146/annurev-ento-010814-020627)

66. Sun Q, Haynes KF, Zhou X. 2018 Managing the risks and rewards of death in eusocial insects. *Phil. Trans. R. Soc. B* **373**, 20170258. (doi:10.1098/rstb.2017.0258)