ECOLOGY LETTERS

Ecology Letters, (2020) **23**: 467–475 doi: 10.1111/ele.13445

LETTER

A chemically triggered transition from conflict to cooperation in burying beetles

Bo-Fei Chen,[1] Mark Liu,[1] Dustin R. Rubenstein,[2] Syuan-Jyun Sun,[1] Jian-Nan Liu,[1] Yu-Heng Lin[1] and Sheng-Feng Shen[1]*

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.13445

Abstract

Although interspecific competition has long been recognised as a major driver of trait divergence and adaptive evolution, relatively little effort has focused on how it influences the evolution of intraspecific cooperation. Here we identify the mechanism by which the perceived pressure of interspecific competition influences the transition from intraspecific conflict to cooperation in a facultative cooperatively breeding species, the Asian burying beetle *Nicrophorus nepalensis*. We not only found that beetles are more cooperative at carcasses when blowfly maggots have begun to digest the tissue, but that this social cooperation appears to be triggered by a single chemical cue – dimethyl disulfide (DMDS) – emitted from carcasses consumed by blowflies, but not from control carcasses lacking blowflies. Our results provide experimental evidence that interspecific competition promotes the transition from intraspecific conflict to cooperation in *N. nepalensis* via a surprisingly simple social chemical cue that is a reliable indicator of resource competition between species.

Keywords

conflict, cooperation, social behaviour, sociality.

Ecology Letters (2020) **23**: 467–475

INTRODUCTION

Unraveling the mechanisms that shift individuals from being competitive to cooperative is critical for understanding not only the evolution of sociality but also of biological organisation at many scales (Bourke 2011). Interspecific competition has long been recognised as a major driver of trait divergence and adaptive evolution (Hardin 1960; Pianka 1974; Bolnick *et al*. 2010), but its role in promoting intraspecific cooperation has been largely ignored outside of microorganisms (Cotter & Kilner 2010; Meunier 2015; Biedermann & Rohlfs 2017). Moreover, the proximate mechanisms affecting how individuals perceive the pressure of interspecific competition, which in turn shapes their cooperative and competitive strategies, remain poorly understood.

Burying beetles (*Nicrophorus spp*.) use small vertebrate carcasses as their sole resource for reproduction and often face intense intra- and interspecific competition for access to these precious but limiting resources (Pukowski 1933; Scott 1998; Rozen *et al*. 2008). Although primarily solitary and aggressive to conspecifics, some species of *Nicrophorus* beetles cooperate to bury carcass resources for reproduction (Eggert & Muller 1997; Scott 1998). Previous work has suggested that the key benefit of cooperation in the Asian burying beetle *N. nepalensis* is that cooperative carcass preparation—including carcass cleaning, shaping, and burial, as well as the elimination of competing species, behaviours that are also performed in other burying beetles species (Pukowski 1933; Scott 1998; Rozen *et al*. 2008; Cotter & Kilner 2010; Shukla *et al*. 2018) – enables beetles to outcompete their primary competitors, blowflies (family Calliphoridae) (Sun *et al*. 2014; Liu *et al*. in press). Temperature-dependent competition with blowflies occurs over carcass access with both adult blowflies, whose abundance and activity is higher at higher temperatures (Sun *et al*. 2014), and blowfly maggots, which grow faster and digest carcasses more quickly at higher temperatures (Donovan *et al*. 2006; Kotzé *et al*. 2016). By experimentally manipulating burying beetle group size along an elevational gradient, we showed previously that in cooler environments where the pressure of interspecific competition is low, beetles in large groups are more aggressive towards same-sex conspecifics and often engage in intense and even lethal fights that result in a single individual monopolising the carcass and having a higher probability of breeding successfully than those in large groups (Sun *et al*. 2014). In contrast, in hotter environments where blowflies are more common, burying beetles cooperate with conspecifics to more quickly bury carcasses and escape blowfly competition (Liu *et al*. in press), ultimately gaining greater reproductive success (Sun *et al*. 2014).

Although the presence of blowflies at carcasses appears to facilitate a shift from competitive to cooperative behaviour in *N. nepalensis* (Liu *et al*. in press), it remains unclear what drives this transition in beetle social behaviour and how individuals recognise the need to reduce social conflict and instead tolerate conspecifics. Here we experimentally test the hypothesis that the presence of blowflies promotes beetle cooperation and then identify the specific mechanism through which this occurs by manipulating the presence of blowfly larvae on

[1]*Biodiversity Research Center, Academia Sinica, Taipei 11529, Taiwan*
[2]*Department of Ecology, Evolution and Environmental Biology, Columbia University, 1200 Amsterdam Avenue, New York, NY 10027, USA*

**Correspondence: E-mail: shensf@sinica.edu.tw*

carcasses in the laboratory. We define cooperation as a behaviour that provides a benefit to another individual (recipient), and which is selected for at least partially because of this beneficial effect to the recipient (West *et al*. 2007). According to this definition, cooperation then includes both altruism, which has positive direct fitness effects on only the recipients, and mutual benefit, which has positive direct fitness effects on both actors and recipients (West *et al*. 2007). Since the exact fitness consequences of a behaviour for either an actor or a recipient are notoriously difficult to quantify empirically, cooperative behaviours of social animals are most often defined operationally as behavioural investment in common resources from which all group members benefit. In burying beetles, we define cooperative behaviour as investment in carcass preparation and defense, which is analogous to cooperatively breeding behaviour in vertebrates (i.e. when more than two individuals provide offspring care to young Emlen & Vehrencamp 1983; Cockburn 2006), in which feeding offspring in a communal domicile benefits the feeders as well other group members. To our knowledge, this is the first study to experimentally examine how interspecific competition influences the transition from intraspecific conflict to cooperation in a cooperatively breeding species. Moreover, we identify a surprisingly simple social chemical cue that is a reliable indicator of interspecific competition and that promotes cooperative behaviour.

MATERIALS AND METHODS

Social behaviour along elevational gradients

Our field study was conducted in Taiwan from 2012 to 2015 along elevational gradients composed primarily of uncultivated forest in Nantou (spanning 1688 to 2650 m above sea level) and Hualien counties (spanning 1193 to 2720 m above sea level). At both sites, temperature varies inversely with increasing elevation, and blowfly abundance increases with increasing temperature (Sun *et al*. 2014). To determine how temperature and blowfly abundance influence inter- and intraspecific social interactions in natural burying beetle populations, we first quantified beetle social behaviour and dynamics by video recording their breeding behaviour at 25 sites along the two elevational gradients. Our goal was to demonstrate quantitatively that facultative cooperative breeding behaviour occurs in *N. nepalensis*, a species better known for its parental care behaviour than its cooperative behaviour (Sun *et al*. 2014). We calculated the time that beetles spent on cooperative carcass preparation (hereafter cooperative investment; see below for description) both in terms of total investment (i.e. the cumulative time of social investment of the social group) and on a per capita basis for large groups (those larger than the median size) and small groups (those smaller than the median size) along the elevation and temperature gradients. We focused on studying cooperative and competitive behaviours during carcass preparation because this is the critically important stage that determines the breeding success of *N. nepalensis*, such that most carcasses that are successfully buried eventually produce offspring (64.2% of 399 cases). In contrast, those that failed to be buried were largely digested by blowfly maggots, presumably due to incomplete removal and cleaning of the larvae, and nearly all failed to produce any offspring.

A variety of social behaviours, including investment in cooperative carcass preparation (i.e. cooperative investment) and per capita social conflict were scored on the first night of video observation (from 19:00 to 05:00) using the Observer® XT 14 (Noldus). To thoroughly study cooperative behaviour in this species, we separated their behaviours into (1) time spent simply walking on the carcass and (2) time spent on more complex carcass-preparation behaviours, carcass cleaning (e.g. maggot removal [but not eating them] and carcass grooming), as well as carcass dragging, rolling, depilation, and burial (see supplementary material for detailed descriptions, illustrations, and videos of these behaviours). We only consider complex carcass preparation as a form of cooperative investment, and since the cumulative time that individuals spent on carcass preparation was positively correlated with total cooperative investment time (Fig. S1), we use the cumulative time that individuals spent on the carcass as our index of cooperative investment in both field and laboratory experiments. We measured per capita cooperative investment as the total cooperative investment divided by the mean group size, defined as the maximum number of beetles that stayed on or under carcasses averaged over 10 time intervals from 19:30 to 04:40 during each video observation. In general, more individuals participate in carcass preparation in larger groups. Investment in cooperation was quantified as the duration of the cumulative time sampled for a 10 min observation period in each hour (i.e. 100 mins for each breeding experiment). In total, there were 81 breeding experiments (resulting in 8100 mins of video recordings) from which we were able to quantify total cooperative investment. We also quantified the numbers of blowflies on carcasses during the first 24 h. For each experiment, we took snapshots of videos every 2 h and counted the number of blowflies on carcasses in the snapshots. We calculated the normalized mean blowfly number (using the z-score of mean blowfly number, or the average of the blowfly numbers from 12 snapshots) to represent the strength of interspecific competition in each experiment.

Four lines of evidence suggest that the behaviours we assumed to be cooperative (e.g. maggot removal and carcass grooming) were in fact related to cooperation and that individuals on the carcasses were mainly investing in carcass preparation and not simply in feeding on or around the carcasses. First, most carcass preparation behaviours can clearly be distinguished from feeding behaviour in our videos (Supplementary Material). Second, to determine whether beetles were mainly feeding on the carcasses rather than working to bury them for egg laying, we quantified the maximum food consumption by starving beetles for a week and then measured their food intake. We found that beetles ate on average 0.078 g (range: 0.01–0.124 g; $n = 39$) of maggots per day, which is only about 20% of their body weight (each maggot weighs 0.021 g). Although females can potentially eat more during breeding, given that the average weight of carcasses is more than 75 g (plus a large number of blowfly eggs and maggots are found on or in the carcass), we predicted that the time spent on a carcass would either decrease or not change over time if feeding is the primary reason that beetles spend

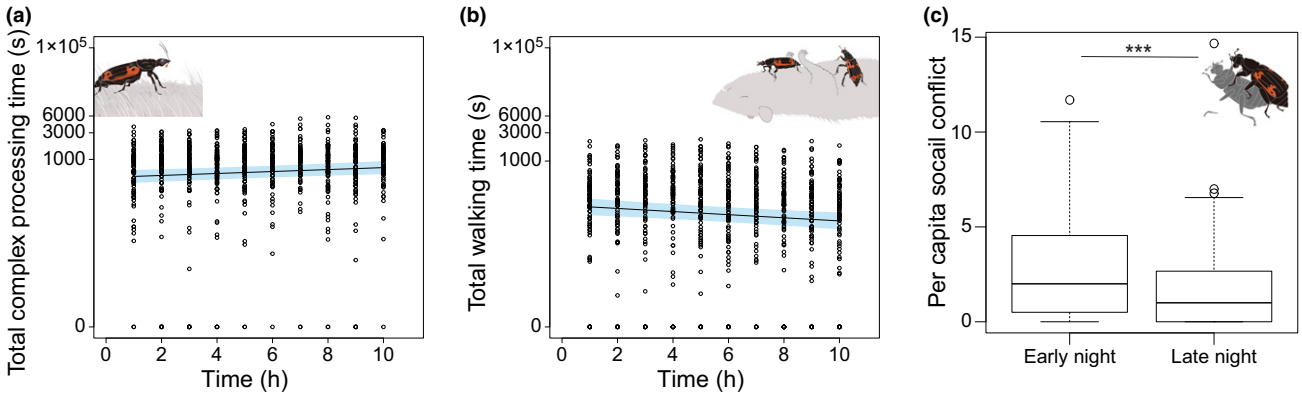

Figure 1 Changes in *N. nepalensis* social behaviour through time on the first night after the beetles' arrival. (a) Total complex carcass-preparation time on a log scale, (b) total walking time on a log scale, and (c) per capita social conflict after the beetles' arrival. Total complex carcass-preparation time increased over the course of the evening, whereas total walking time and per capita conflict decreased over the course of the evening. Lines represent least-squared means (solid lines denote significant relationships), and the blue shaded areas represent 95% confidence intervals expected from GLMMs. ***$P \leq 0.001$. Cartoons correspond to illustrations of social behaviours in the Supplemental Materials.

time on carcasses because they would become satiated. In contrast to this prediction, we found that a large proportion of the individual investment in complex carcass preparation behaviours *increased* over time throughout the evening ($\chi^2_1 = 10.85$, $P < 0.001$, $n = 847$, Fig. 1a), whereas walking time ($\chi^2_1 = 10.69$, $P = 0.01$, $n = 847$; Fig. 1b) and social conflict ($t = 3.95$, $P < 0.001$; Fig. 1c) both *decreased* over the course of the evening. Third, we found that beetles only spent 0.8% of their time eating maggots or carcasses. If feeding is the primary reason that beetles spend time on carcasses, we predicted that they would feed on maggots first because maggots are their preferred food source. In contrast, we found that most of the time they did not eat maggots—although maggots often left the carcasses when there were beetles walking on the carcass—and instead worked on carcass preparation (see Supplementary Figure and Video for walking behaviour). Finally, we found that time spent on more complex carcass preparation behaviours increased with increasing numbers of blowflies ($t = 3.74$, $P < 0.001$, $n = 81$; Fig. 2b and Fig. S2a). However, there was no significant relationship between walking time and the number of blowflies ($t = 0.15$, $P = 0.88$, $n = 81$; Fig. 2b, Fig. S2b), suggesting that the increase in total cooperative investment when blowfly pressure increased was not simply the result of increased feeding activity. Thus, beetles in our experiment appeared to be cooperatively preparing carcasses for burial and eventual egg laying rather than using them for feeding.

Aggressive interactions were defined as a form of social conflict if a beetle attacked, wrestled, chased, or escaped from another same-sexed individual (see below for definitions of each behaviour). We measured total social conflict as the total number of aggressive interactions over the 240 min observation period, and per capita social conflict as the total number of aggressive interactions divided by the mean group size for each observation period. Conflict behaviours were quantified as the total number of aggressive events sampled for two 120 min observation periods (from 19:30 to 21:30 [early night] and from 23:30 to 1:30 [late night]). We then divided per capita social conflict by the total investment time to control for the total activity time (*sensu* Johnson *et al.* 2004; Kaspersson *et al.* 2010). We used this per capita social conflict per unit time as an index of social conflict because if beetles spend more time on the carcass, there are more overall interactions with other individuals. We quantified aggressive behaviour differently than cooperative behaviour (i.e. individual events rather than a proportion of time) because aggressive interactions are infrequent and short in duration. In total, there were 82 breeding experiments (resulting in 19,680 mins of video recordings) from which we were able to quantify conflict behaviour. We determined the mean group size on the first night of each beetle's arrival in 245 breeding experiments (resulting in 7350 mins of video recordings). Given the difference in how cooperative and conflict behaviours were quantified (i.e. cumulated time vs. number of events), we did not measure cooperative and conflicting behaviours for the same duration of time.

Laboratory experiments to identify the impact of interspecific competition on cooperation

Experiments were conducted using *N. nepalensis* individuals from laboratory-reared strains that originated from Meifeng, Nantou County, Taiwan (24°5' N, 121°10'). Burying beetles were collected using hanging pitfall traps baited with 100 g rotten chicken breasts. Collected beetles were randomly paired and supplied with frozen and re-thawed 75 ± 5 g dead rats (*Rattus norvegicus*) in 23 × 15.5 × 16 cm plastic boxes filled with 10 cm moist peat for reproduction. The emerged beetles were housed individually in 7.3 × 7.3 × 3.5 cm plastic boxes filled with 2 cm moist peat and fed with dead superworms (*Zophobas morio*) once a week. All individuals were kept in environmental chambers at 13.2 – 19.7°C (to resemble the regular daily temperature fluctuation in their natural habitat) on a 14 L:10 D photoperiod. Experimental beetles were between 40 and 80 days of age, which is their optimal age for reproduction (individuals can live for over three months in the laboratory).

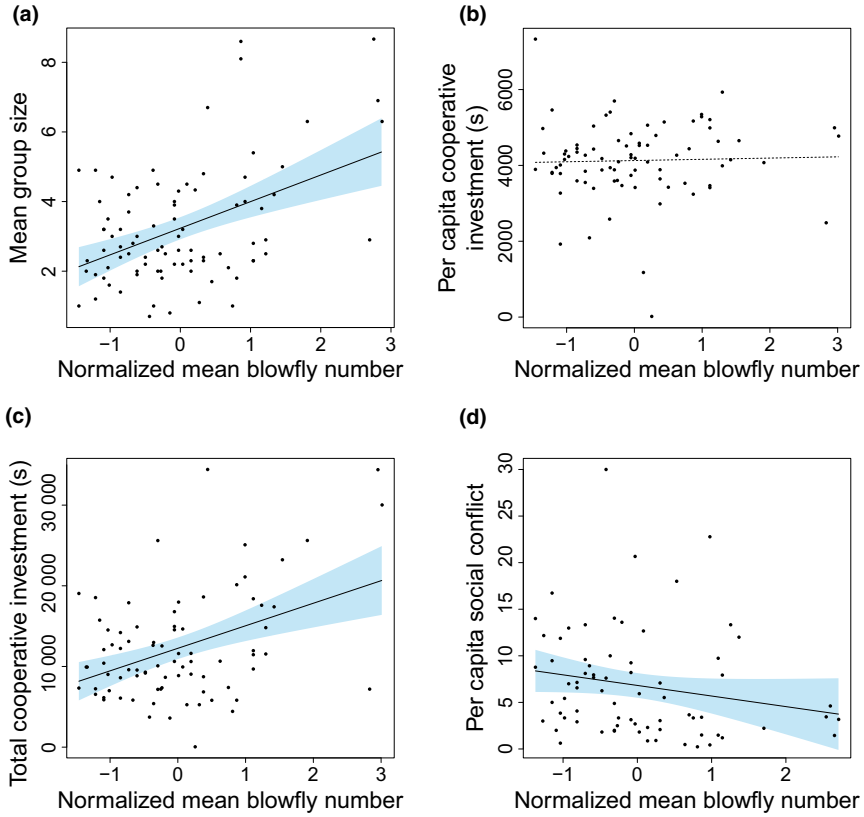

Figure 2 Changes in *N. nepalensis* group size and social behaviour during carcass preparation in relation to the pressure of interspecific competition. The relationship between mean blowfly number and (a) mean group size, (b) per capita cooperative investment, and (c) total cooperative investment. Group size increased with increasing blowfly numbers, but per capita cooperative investment did not. As consequence, total cooperative investment increased with increasing blowfly numbers. (d) Per capita social conflict decreased with increasing blowfly number. Solid lines denote predicted relationships of least-squared means from GLMs, whereas dashed lines denote non-significant relationships, and blue shaded areas represent 95% confidence intervals expected from GLMs. Normalized mean blowfly numbers were calculated by the Z-score of mean blowfly numbers on carcasses (see Methods)

For each experimental replicate, three unrelated male and three unrelated female beetles were randomly chosen from different families to avoid relatedness affecting their behaviours. Each individual was weighed to the nearest 0.1 mg and marked with a Uni POSCA paint marker on the elytra and coated with Scorch® Super GlueGel for individual identification in videos. Marking and weighing of beetles was done 2 h prior to beginning an experiment to ensure that all beetles would return to normal activity levels. All six marked beetles were placed into the experimental boxes in an arbitrary order at the beginning of each experiment. Experimental boxes consisted of a smaller plastic container (23 × 15.5 × 13.5 cm filled with 13.5 cm moist peat) located inside a larger plastic container (45 × 34.5 × 25 cm filled with 13.5 cm moist peat). A 4 cm high metal net with 2 cm^2 wire mesh was placed around the small container to prevent beetles moving carcasses outside the field of view of the digital cameras, but beetles could still move freely between the inner and outer areas. A digital camera (DS-IR507 S, HIK VISION) was fitted on the top of a 25 × 20 × 55 cm acrylic box and fixed to the cap of the larger container. To equalize the temperature of the experimental apparatus, boxes were filled with moist peat and put into the environmental chambers one day before the experiments began.

Since blowfly species are diurnal and *N. nepalensis* is nocturnal, it is more likely that the presence of maggots influences *N. nepalensis* behaviour than the presence of adults. The blowfly treatment was conducted by exposing a 75 ± 5 g rat thawed carcass (a previous study showed that 75 g is optimal for offspring production of a pair of *N. nepalensis*; Chan *et al.* 2019) to oriental latrine flies (*Chrysomya megacephala*) in 32 × 32 × 32 cm fly cages for 50 h before the start of each experiment. We chose oriental latrine flies because this species is one of the most abundant species of blowflies in the study region. After the flies laid eggs, the adult flies were removed and the carcasses used in all treatments were transferred into environmental chambers for 8 h prior to the release of beetles to equalise their temperatures in order to minimise the potential temperature influences on the behaviours of beetles. Importantly, beetles were only exposed to maggots and never to adult blowflies. Fly cages contained oriental latrine flies that had emerged from 10 g pupa and been kept in environmental chambers at 26°C on a 14 L:10 D photoperiod. Except for maggot-digested carcasses, all other carcasses in the same weight range were thawed at 4°C for 24 h before experiments began.

Laboratory experiments to identify the social chemical cue

We first used gas chromatography-mass spectrometry (GC-MS) analysis to determine the compositions of volatile organic compounds (VOCs) emitted by carcasses with and without blowflies from six control and six blowfly treated carcasses prepared using the same procedure described above. The prepared carcasses were put on the peat surface in glass vacuum desiccators (15 cm diameter × 22 cm tall) filled with 5 cm of moist peat. The stopcock and ground-glass rim of the desiccator lid were greased with a thin layer of petroleum jelly to prevent the leakage of emitted VOCs, as well as contamination from the atmosphere. The VOCs were sampled using solid-phase micro-extraction (SPME) (Vas & Vékey 2004). The SPME holder with CAR/PDMS fiber (Supelco, previously desorbed for 5 mins in GC injection port heated to 250°C) was inserted through the hole of the stopcock into the atmosphere surrounding the rat carcass. Immediately after exposing the fibre for 10 mins, the sample was GC-MS-analysed using a 6890N Network Gas Chromatograph (Agilent Technologies) equipped with a HP-5ms column (Agilent J&W) and a 5975 Mass Selective Detector (Agilent Technologies). The GC oven was operated at an initial temperature 40°C for 1 min and then ramped up at a rate 5°C per min to 250°C (with a 5 min hold). The temperatures of the GC inlet and MS detector were set to 200°C and 230°C, respectively. The SPME samples were GC analysed split at a flow of 60 mL per min. Helium (1 mL per min) was used as a carrier gas. The source temperature was 230°C. The data acquisition rate was 2.91 scans per second, along a mass range of 50–550 amu and a detector voltage of 1671 V. Since the GC-MS results showed DMDS was the major VOC emitted by the blowfly-treated carcasses, DMDS was injected into carcasses in the further experiments. Six DMDS-injected carcasses (also prepared using the same procedure described previously) were used in GC-MS analyses (following the procedure described above) to determine the composition of the VOCs they emitted.

To determine if DMDS is the social chemical cue that triggers cooperation in *N. nepalensis*, beetles were exposed to either a DMDS treatment or a hexane control. The methods for determining cooperative investment and social conflict were the same as those described in the laboratory experiments above. The treatment used thawed-only carcasses injected with 2 mL hexane or 0.01 M DMDS solution into abdominal cavities through the anus using 3 mL Terumo® syringes and needles 1 h prior to the start of the experiment. The thawed-only carcasses served as controls. Carcasses were moved into the experimental boxes and put on the surface of peat in smaller containers 1 h before experiments began. Behavioural videos were recorded either from 19:00 until the day and time at which a carcass was completely buried into peat or for 72 h if the beetles did not completely bury the carcass (under natural conditions, a carcass would be completely consumed by blowflies if beetles did not completely bury it within 72 h). In total, 1020 h of video were analysed from 23 blowfly control replicates, 23 blowfly treatment replicates, 32 hexane control replicates, and 24 DMDS replicates. Social conflict and cooperative investment behaviours were recorded in the first 10 h (19:00–05:00) of each experimental treatment using The Observer® XT 14 (Noldus).

Data analysis

Multivariate analyses were performed using general linear models (GLMs) to determine statistical significance for differences between controls and blowfly treatments or hexane controls and DMDS treatments in mean group size, total and per capita cooperative investment, and total and per capita social conflict. Due to the random effects of six individuals in each replicate, generalised linear mixed models (GLMMs) were used in the multivariate analyses of all individual comparisons between controls and blowfly treatments or hexane controls and DMDS treatments. If the random effect of repeated sampling at study sites was required, GLMMs were used. All statistical analyses were performed in R (R Core Team, 2019) using the packages *stats*, *lme4*, *car*, *multcomp* (http://cran.r-project.org), and *glmmADMB* (http://glmmadmb.r-forge.r-project.org/).

RESULTS

Cooperation in the field

In the field study, we found that group size increased with increasing blowfly numbers ($t = 3.45$, $P < 0.001$, $n = 81$; Fig. 2a). Although per capita cooperative investment did not vary with the number of blowflies ($t = 0.28$, $P = 0.78$, $n = 81$; Fig. 2b), total cooperative investment increased with increasing blowfly numbers due to the increased group size ($t = 3.32$, $P = 0.001$, $n = 81$; Fig. 2c). This result again demonstrates (see Methods) that cooperative investment in carcass preparation is not for feeding because per capita feeding time did not increase with increasing blowfly numbers ($t = -0.31$, $P = 0.76$, $n = 81$). In contrast, per capita social conflict decreased with increasing blowfly numbers ($t = -2.37$, $P = 0.02$, $n = 72$, Fig. 2d).

Experimental manipulation of interspecific competition in laboratory

Our field results demonstrate that *N. nepalensis* exhibits remarkably flexible social behaviour along thermal gradients: beetles are normally asocial and aggressive towards conspecifics in colder environments, but become social and cooperate with conspecifics in hotter environments where competition with blowflies for critical resources is intense (Liu *et al.* in press). However, to demonstrate experimentally that blowfly competition for carcasses is the mechanism that drives the transition from intraspecific competition to intraspecific cooperation, we performed a series of laboratory experiments to directly manipulate the presence or absence of blowflies at carcasses. Our first experiment introduced blowfly competition to burying beetles by exposing carcasses to adult blowflies in an incubator at 26°C for two days, conditions that match those in the field and are optimal for blowflies to lay eggs and for their maggots to partially consume the carcass. We then allowed six beetles (three males and three females) to breed

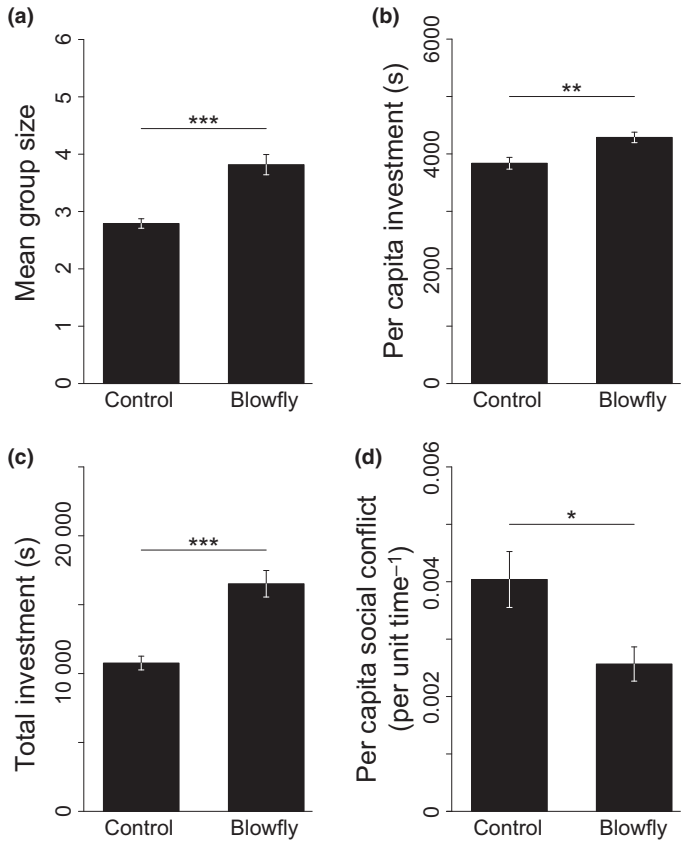

Figure 3 *N. nepalensis* social behaviours in control and blowfly treatments. (a) Mean group size, (b) per capita cooperative investment, (c) total cooperative investment, and (d) per capita social conflict per unit time of burying beetles on carcasses. Beetles formed larger groups and had greater per capital and total cooperative investment in carcass preparation in the presence of blowflies than in control treatments where blowflies were absent. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

on the carcass in the presence of blowfly larvae, but not adult blowflies. We found that more beetles cooperated (represented by mean group size; $t = 5.26$, $P < 0.001$; Fig. 3a) and that each individual beetle spent significantly more time cooperating (represented by the per capita cooperative investment; $t = 3.27$, $P = 0.002$; Fig. 3b) in the blowfly treatment (i.e. in the presence of blowfly maggots) than in the control treatment containing carcasses but no blowflies. As a consequence, the total cooperative investment was higher in the blowfly treatment than in the control treatment ($t = 5.37$, $P < 0.001$; Fig. 3c). Also as predicted, the per capita number of social conflicts per unit time was significantly lower in the blowfly treatment than in the control treatment ($t = -2.58$, $P = 0.013$; Fig. 3d). Thus, social conflict in burying beetles was lower and cooperation higher when blowfly maggots were present on carcasses.

Mechanism underlying the transition from intraspecific competition to intraspecific cooperation

Previous studies have demonstrated that sulfur-containing volatile organic compounds (S-VOCs) emitted from decomposing carcasses attract burying beetles to this key resource (Kalinova *et al.* 2009). Because our GC-MS analysis showed that dimethyl disulfide (DMDS) appeared earlier and was more abundant in the blowfly treatment than in the control (Fig. 4a), we hypothesised that DMDS is the key infochemical (Dicke & Sabelis 1988) – indicating not only the presence of a decaying carcass but also the degree of interspecific competition at that carcass – that mediates the transition between cooperative and competitive strategies in *N. nepalensis*. To experimentally test the hypothesis that DMDS promotes cooperative behaviour in *N. nepalensis*, we injected DMDS into the body cavity of mouse carcasses in the absence of blowflies. We found that more beetles cooperated (represented by mean group size; $t = -3.76$, $P < 0.001$; Fig. 4b) and that each individual spent more time cooperating in DMDS treated carcasses relative to controls (represented by the per capita cooperative investment; $t = -2.55$, $P = 0.014$; Fig. 4c). Thus, there was a higher total cooperative investment in the DMDS treatment than in the control ($t = -3.8$, $P < 0.001$; Fig. 4d). Importantly, these results were similar to those observed in the blowfly treatment from the initial experiment. The only difference between the DMDS and blowfly treatments was that there was marginally more social conflict in the DMDS treatment than in the control ($t = -1.97$, $P = 0.054$; Fig. 4e), whereas this trend – while in the same direction – was not significant in the blowfly treatment ($t = -0.33$, $P = 0.75$). Presumably, this difference was caused by the fact that there were no actual competitors (i.e. maggots, especially those inside the mouse body cavity) in the DMDS treatment that needed removing.

DISCUSSION

Our study shows that burying beetles transition from competitive to more cooperative interactions as the pressure of interspecific competition increases. Accumulating empirical evidence from other animals suggests that social conflict in cooperative societies is often lower in adverse environments with strong interspecific competition (Korb & Foster 2010). This pattern of reduced social conflict under strong interspecific competition has largely been explained by the fact that the cost of engaging in competitive interactions increases under adverse conditions (Shen *et al.* 2012; De Jaegher & Hoyer 2016). Yet, there is little empirical evidence demonstrating that social animals actually increase their investment in cooperation under the threat of interspecific competition, as we have shown here. One exception comes from cooperatively breeding superb fairy-wrens (*Malurus cyaneus*) that cooperate more in nest defense when exposed to a greater threat of interspecific brood parasitism (Feeney *et al.* 2013). However, it remains unclear how intraspecific conflict in fairy-wrens is influenced by the threat of interspecific competition. Our study helps fill this knowledge gap by showing that cooperative carcass preparation to reduce blowfly competition is critical for predicting both the cooperative and competitive interactions among individuals of the same species.

Furthermore, we show that the conditional cooperative and competitive strategies (Gross 1996; Tomkins & Hazel 2007) used by *N. nepalensis* to maximise their utility of carcasses for reproduction are mediated by a surprisingly simple chemical

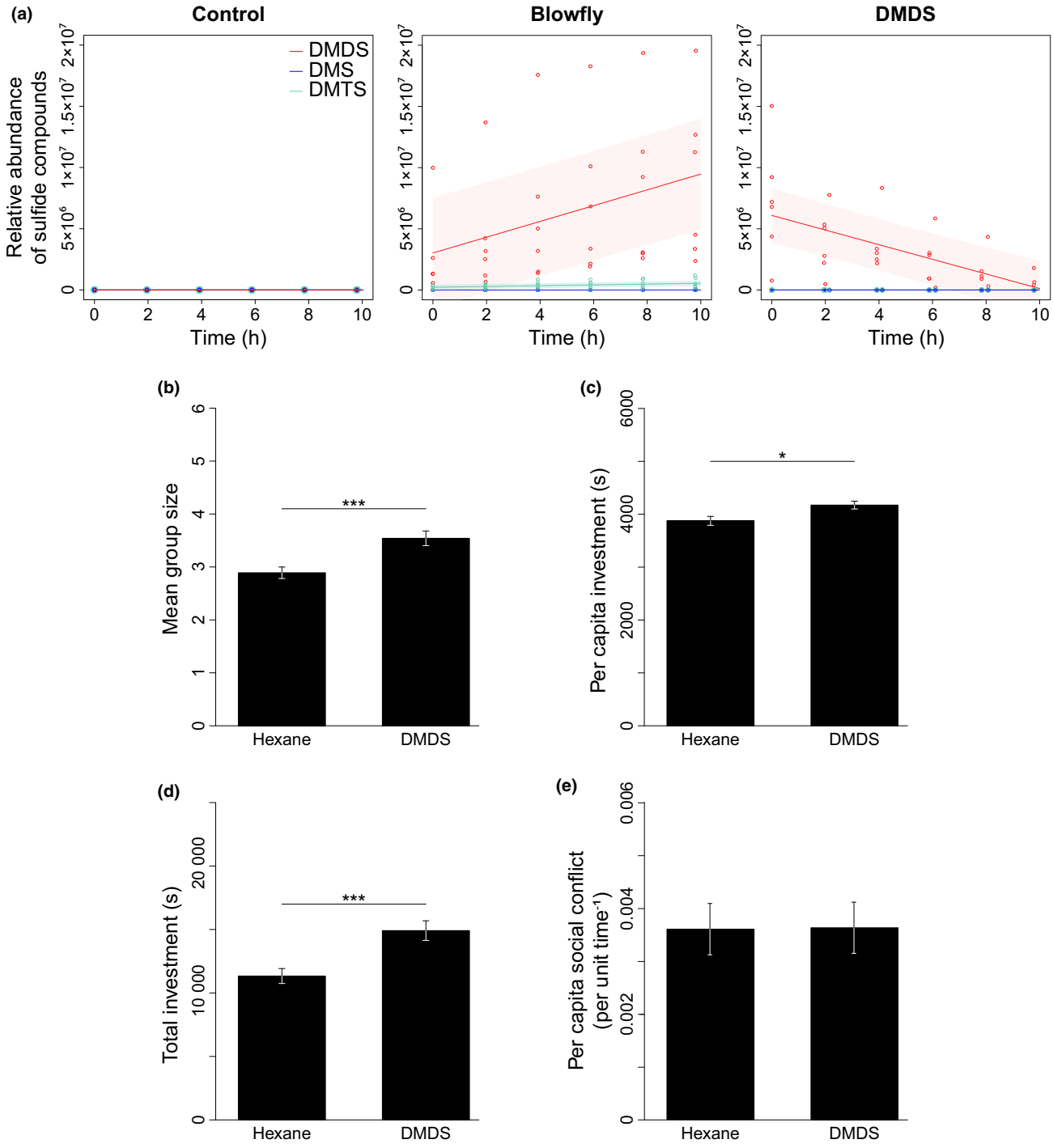

Figure 4 Results of gas chromatography-mass spectrometry (GC-MS) analyses and *N. nepalensis* social behaviours in hexane and DMDS treatments. (a) GC-MS analyses showed an abundance of sulfide compounds, including dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) in control, blowfly, and DMDS treatments during the first 10 h. DMDS was the major sulfide compound emitted by maggot-digested carcasses. Shaded areas represent 95% confidence intervals expected from GLMMs. (b) Mean group sizes, (c) per capita cooperative investment, (d) total cooperative investment, and (e) per capita social conflict per unit time of burying beetles on carcasses in DMDS and hexane control treatments. Beetles formed larger groups and had greater per capital and total cooperative investment on carcasses in the DMDS treatment compared to the hexane control treatment. $^{*}P \leq 0.05$; $^{***}P \leq 0.001$

mechanism. The presence of blowflies is known to accelerate the decomposition of carcasses and the emission of DMDS (Recinos-Aguilar *et al*. 2019) because maggots can enter the anterior part of a cephalopharyngeal skeleton, which allows them to tear the tissue to feed (El-Moaty & Kheirallah 2013) and to secrete different salivary substances that accelerate the liquefaction of tissues (Shiao & Yeh 2008). Maggots also facilitate suitable conditions for the growth of

some microorganisms, which further accelerates tissue degradation (Singh *et al.* 2015; Tomberlin *et al.* 2017). Thus, DMDS acts as an indicator of the level of competition from blowflies.

Although interspecific competition has long been recognized as a major ecological force that drives adaptive evolution (Hardin 1960; Pianka 1974; Bolnick *et al.* 2010), relatively little effort has focused on how it influences intraspecific cooperation (Korb & Foster 2010; Mitri *et al.* 2011; Celiker & Gore 2012). Our discovery of a novel social chemical cue provides unambiguous evidence that interspecific competition has shaped social evolution in *N. nepalensis*. DMDS acts as a kairomone because it is produced by heterospecifics (i.e. blowfly digestion), but benefits the receiver (Dicke & Sabelis 1988), and not as a pheromone produced by conspecifics to benefit the sender (Keller & Nonacs 1993; Vander Meer *et al.* 1998). Pheromones are often used for kin discrimination, and studying the olfactory sensory system and its genetic architecture has greatly advanced our understanding of the role that chemically driven kin recognition has played in social evolution, especially in ants (Trible *et al.* 2017; Yan *et al.* 2017). Here we demonstrate that interspecific chemical communication is also important to insect social evolution, including for rudimentary forms of cooperative behaviour. By showing that chemically-mediated interspecific competition is a key driver of intraspecific cooperation and possibly of social evolution more generally, our work demonstrates the value of integrating ultimate and proximate levels to study the evolution of cooperation (Hofmann *et al.* 2014). Ultimately, our findings suggest that the role of between–species competitive interactions driving within–species competitive and cooperative interactions are likely to have been important for the evolution of social behaviour in a number of animal species.

ACKNOWLEDGMENTS

S.-F.S was supported by Career Development Award and Investigator Award, Academia Sinica and Ministry of Science and Technology of Taiwan. D.R.R. was supported by the US National Science Foundation.

AUTHOR CONTRIBUTIONS

B-F Chen, M Liu, S-J Sun, J-N Liu, Y-H Lin conducted the field and lab work. B-F Chen and M Liu preformed data analysis. B-F Chen, M Liu, D Rubenstein and S-F Shen formulated the study and prepared the manuscript.

DATA AVAILABILITY STATEMENT

Supporting information and data will be available in Dryad.

Guide to files/metadata: https://doi.org/10.6084/m9.figshare.10316717.v1.

Dataset: https://doi.org/10.6084/m9.figshare.10316720.v1.

R Code: https://doi.org/10.6084/m9.figshare.10316732.v2; https://doi.org/10.6084/m9.figshare.10316729.v1; https://doi.org/10.6084/m9.figshare.10316741.v1.

REFERENCES

Biedermann, P.H. & Rohlfs, M. (2017). Evolutionary feedbacks between insect sociality and microbial management. *Current Opinion in Insect Science*, 22, 92–100.

Bolnick, D.I., Ingram, T., Stutz, W.E., Snowberg, L.K., Lau, O.L. & Paull, J.S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.*, 277, 1789–1797.

Bourke, A.F.G. (2011). *Principles of Social Evolution*. Oxford University Press, Oxford.

Celiker, H. & Gore, J. (2012). Competition between species can stabilize public-goods cooperation within a species. *Mol. Syst. Biol.*, 8, 621.

Chan, S.-F., Shih, W.-K., Chang, A.-Y., Shen, S.-F. & Chen, I.-C. (2019). Contrasting forms of competition set elevational range limits of species. *Ecol. Lett.*, 22, 1668–1679.

Cockburn, A. (2006). Prevalence of different modes of parental care in birds. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.*, 273, 1375–1383.

Cotter, S.C. & Kilner, R.M. (2010). Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *J. Anim. Ecol.*, 79, 35–43.

De Jaegher, K. & Hoyer, B. (2016). By-product mutualism and the ambiguous effects of harsher environments – A game-theoretic model. *J. Theor. Biol.*, 393, 82–97.

Dicke, M. & Sabelis, M.W. (1988). Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol.*, 2, 131–139.

Donovan, S., Hall, M., Turner, B. & Moncrieff, C. (2006). Larval growth rates of the blowfly, Calliphora vicina, over a range of temperatures. *Med. Vet. Entomol.*, 20, 106–114.

Eggert, A.K. & Muller, J.K. (1997). Biparental care and social evolution in burying beetles: lessons from the larder. In: *The Evolution of Social Behavior in Insects and Arachnids* (eds Choe, J.C. & Crespi, B.J.). Cambridge University Press Cambridge, pp. 216–236.

El-Moaty, Z.A. & Kheirallah, A.E.M. (2013). Developmental variation of the blow fly Lucilia sericata (Meigen, 1826)(Diptera: Calliphoridae) by different substrate tissue types. *J. Asia-Pacif. Entomol.*, 16, 297–300.

Emlen, S.T. & Vehrencamp, S.L. (1983). Cooperative breeding strategies among birds. In: *Perspectives in Ornighology* (eds Brush, A.H., & Clark, G.A.). Cambridge University Press, Cambridge, pp. 93–133.

Feeney, W.E., Medina, I., Somveille, M., Heinsohn, R., Hall, M.L., Mulder, R.A. *et al.* (2013). Brood parasitism and the evolution of cooperative breeding in birds. *Science*, 342, 1506–1508.

Gross, M.R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.*, 11, 92–98.

Hardin, G. (1960). The competitive exclusion principle. *Science*, 131, 1292–1297.

Hofmann, H.A., Beery, A.K., Blumstein, D.T., Couzin, I.D., Earley, R.L., Hayes, L.D. *et al.* (2014). An evolutionary framework for studying mechanisms of social behavior. *Trends Ecol. Evol.*, 29, 581–589.

Johnson, C.A., Grant, J.W.A. & Giraldeau, L. (2004). The effect of patch size and competitor number on aggression among foraging house sparrows. *Behavioral Ecology*, 15, 412–418.

Kalinova, B., Podskalska, H., Růžička, J. & Hoskovec, M. (2009). Irresistible bouquet of death—how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. *Naturwissenschaften*, 96, 889–899.

Kaspersson, R., Höjesjö, J. & Pedersen, S. (2010). Effects of density on foraging success and aggression in age-structured groups of brown trout. *Animal Behaviour*, 79, 709–715.

Keller, L. & Nonacs, P. (1993). The role of queen pheromones in social insects: queen control or queen signal? *Anim. Behav.*, 45, 787–794.

Korb, J. & Foster, K.R. (2010). Ecological competition favours cooperation in termite societies. *Ecol. Lett.*, 13, 754–760.

Kotzé, Z., Villet, M.H. & Weldon, C.W. (2016). Heat accumulation and development rate of massed maggots of the sheep blowfly, Lucilia cuprina (Diptera: Calliphoridae). *J. Insect Physiol.*, 95, 98–104.

Liu, M., Chan, S.-F., Rubensteinb, D.R., Sun, S.-J., Chen, B.-F. & Shen, S.-F. (in press). Ecological transitions in grouping benefits explain the paradox of environmental quality and sociality. *Am. Nat*.

Meunier, J. (2015). Social immunity and the evolution of group living in insects. *Philos. Trans. R. Soc. B.*, 370, 20140102.

Mitri, S., Xavier, J.B. & Foster, K.R. (2011). Social evolution in multispecies biofilms. *Proc. Natl. Acad. Sci. USA*, 108, 10839–10846.

Pianka, E.R. (1974). Niche overlap and diffuse competition. *Proc. Natl. Acad. Sci. USA*, 71, 2141–2145.

Pukowski, E. (1933). Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift fur Morphologie und Ökologie der Tiere*, 27, 518–586.

R Core Team (2019). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Recinos-Aguilar, Y.M., García-García, M.D., Malo, E.A., Cruz-López, L. & Rojas, J.C. (2019). The colonization of necrophagous larvae accelerates the decomposition of chicken carcass and the emission of volatile attractants for blowflies (Diptera: Calliphoridae). *J. Med. Entomol*., 56, 1590–1597.

Rozen, D., Engelmoer, D. & Smiseth, P. (2008). Antimicrobial strategies in burying beetles breeding on carrion. *Proc. Natl. Acad. Sci. USA*, 105, 17890–17895.

Scott, M.P. (1998). The ecology and behavior of burying beetles. *Annu. Rev. Entomol*., 43, 595–618.

Shen, S.-F., Vehrencamp, S.L., Johnstone, R.A., Chen, H.-C., Chan, S.-F., Liao, W.-Y., et al. (2012). Unfavourable environment limits social conflict in Yuhina brunneiceps. *Nature Communications*, 3.

Shiao, S.-F. & Yeh, T.-C. (2008). Larval competition of Chrysomya megacephala and Chrysomya rufifacies (Diptera: Calliphoridae): behavior and ecological studies of two blow fly species of forensic significance. *J. Med. Entomol*., 45, 785–799.

Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M. *et al*. (2018). Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proc. Natl Acad. Sci. USA*, 115, 11274–11279.

Singh, B., Crippen, T.L., Zheng, L., Fields, A.T., Yu, Z., Ma, Q. *et al*. (2015). A metagenomic assessment of the bacteria associated with Lucilia sericata and Lucilia cuprina (Diptera: Calliphoridae). *Appl. Microbiol. Biotechnol*., 99, 869–883.

Sun, S.-J., Rubenstein, D.R., Chen, B.-F., Chan, S.-F., Liu, J.-N., Liu, M. *et al*. (2014). Climate-mediated cooperation promotes niche expansion in burying beetles. *eLife*, 3, e02440.

Tomberlin, J.K., Crippen, T.L., Tarone, A.M., Chaudhury, M.F., Singh, B., Cammack, J.A. *et al*. (2017). A review of bacterial interactions with blow flies (Diptera: Calliphoridae) of medical, veterinary, and forensic importance. *Ann. Entomol. Soc. Am*., 110, 19–36.

Tomkins, J.L. & Hazel, W. (2007). The status of the conditional evolutionarily stable strategy. *Trends Ecol. Evol*., 22, 522–528.

Trible, W., Olivos-Cisneros, L., McKenzie, S.K., Saragosti, J., Chang, N.-C., Matthews, B.J. *et al*. (2017). Orco mutagenesis causes loss of antennal lobe glomeruli and impaired social behavior in ants. *Cell*, 170, e710.

Vander Meer, R.K., Breed, M.D., Winston, M. & Espelie, K. (1998). *Pheromone Communication in Social Insects*. Westview Press, Boulder CO.

Vas, G. & Vékey, K. (2004). Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *J. Mass Spectrom*., 39, 233–254.

West, S.A., Griffin, A.S. & Gardner, A. (2007). Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *J. Evol. Biol*., 20, 415–432.

Yan, H., Opachaloemphan, C., Mancini, G., Yang, H., Gallitto, M., Mlejnek, J. *et al*. (2017). An engineered orco mutation produces aberrant social behavior and defective neural development in ants. *Cell*, 170, 736–747, e739.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Vojtech Novotny
Manuscript received 24 September 2019
First decision made 5 November 2019
Manuscript accepted 21 November 2019