A Chemically-triggered Transition from Conflict to Cooperation in Burying Beetles

Bo-Fei Chen[†1,2,3], Mark Liu[†3], Dustin R. Rubenstein[4], Syuan-Jyun Sun[3], Jian-Nan Liu[3], Yu-Heng Lin[3] and Sheng-Feng Shen[1,2,3*]

[1]Department of Life Science, National Taiwan Normal University, Taipei, Taiwan 11677

[2]Biodiversity Program, Taiwan International Graduate Program, Academia Sinica and National Taiwan Normal University, Taipei, Taiwan 11529

[3]Biodiversity Research Center, Academia Sinica, Taipei, 11529, Taiwan

[4]Department of Ecology, Evolution and Environmental Biology, Columbia University, 1200 Amsterdam Avenue, New York, NY 10027 USA

[†]These authors contributed equally to this work

***Corresponding author:**

Sheng-Feng Shen

Address: Biodiversity Research Center, Academia Sinica, 11529, Taiwan

Email: shensf@sinica.edu.tw

Although interspecific competition has long been recognized as a major driver of trait divergence and adaptive evolution[1-3], relatively little effort has focused on how it influences the evolution of intraspecific cooperation[4-6]. 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*. In their natural environment in central Taiwan, *N. nepalensis* are typically aggressive to conspecifics and only cooperate with others of their own species at critical carcass resources in the presence of blowflies, their primary competitors[7]. We demonstrate that beetles form larger groups and are more cooperative in carcass preparation in warmer environments where the pressure of interspecific competition with blowflies is highest[8]. To test the hypothesis that the presence of blowflies promotes beetle cooperation and to identify the mechanism by which this occurs, we manipulated blowfly larvae on carcasses in the lab. 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 interspecific competition. This finding helps bridge the gap between the proximate and ultimate factors regulating the transition between cooperation and conflict and moves toward a more comprehensive understanding of the evolution of mechanisms governing intraspecific variation in social behaviour.

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[9-11]. Previous work has suggested that the key benefit of cooperation in the Asian burying beetle *N.*

67 *nepalensis* is that cooperative carcass preparation—including carcass cleaning,
68 shaping, and burial, as well as the elimination of competing species[9-12]—enables
69 beetles to outcompete their primary competitor, blowflies (family Calliphoridae),
70 particularly in warmer environments where blowflies are most abundant. By
71 experimentally manipulating burying beetle group size along an elevational
72 gradient, we showed that in cooler environments where the pressure of
73 interspecific competition is low, beetles in large groups are more aggressive
74 toward same-sex conspecifics and often engage in intense and even lethal fights
75 that result in a single individual monopolizing the carcass and having a higher
76 probability of breeding successfully than those in large groups[8]. In contrast, in
77 warmer environments where blowflies are more common, burying beetles
78 cooperate with conspecifics to more quickly bury carcasses and escape blowfly
79 competition[7], ultimately gaining greater reproductive success[8]. Although the
80 presence of blowflies at carcasses appears to facilitate a shift from competitive to
81 cooperative behaviour in *N. nepalensis*, it remains unclear what drives this
82 transition in beetle social behaviour and how individuals know to reduce conflict
83 and tolerate conspecifics.
84
85 To determine how ecology influences inter- and intraspecific social interactions
86 in natural burying beetle populations, we first quantified beetle social behaviour
87 and dynamics by video recording their breeding behaviours at 25 sites along two
88 elevational gradients in eastern and western Taiwan, each spanning more than
89 1000 m in elevation. We calculated the time that beetles spend on cooperative

carcass preparation (hereafter cooperative investment) both in terms of total investment (i.e. the cumulative time of the social group) and on a per capita basis for large (groups larger than the median size) and small groups (groups smaller than the median size), as well as in cool (<14.5°C) and warm environments (>14.5°C). We found that group size peaked at moderate temperatures (χ^2_1 = 5.52, P = 0.019, n = 245; Fig. 1a) and that per capita cooperative investment along the temperature gradient varied with group size (group size × temperature interaction, χ^2_1 = 11.20, P = 0.001, n = 89). Specifically, per capita cooperative investment increased with daily minimum temperature in large groups (χ^2_1 = 5.39, P = 0.02, n = 33), but not in small groups (χ^2_1 = 0.05, P = 0.83, n = 56; Fig 1b). Similarly, total cooperative investment increased with daily minimum temperature in large groups (χ^2_1 = 4.88, P = 0.03, n = 33), but not in small groups (χ^2_1 = 0.24, P = 0.60, n = 56; Fig. 1c). In contrast, per capita social conflict along the temperature gradient, measured as the number of intraspecific conflict events for each individual, varied with group size (mean group size × temperature interaction, χ^2_1 = 6.64, P < 0.01, n = 82, Fig. 2b), such that conflict increased with group size in cool environments (χ^2_1 = 11.24, P < 0.001, n = 40; Fig. 1d), but not in warm environments (χ^2_1 = 1.59, P = 0.2, n = 42; Fig. 1d).

To confirm that these patterns of social conflict and cooperation were the result of changes in social behaviour and not simply changes in activity associated with differences in ambient temperature, we further separated cooperative investment into (1) time spent simply walking on the carcass and (2) more

113 complex carcass-preparation behaviours, which are presumably more costly—
114 including maggot and rotten tissue removal, as well as carcass dragging,
115 depilation, and burial. We found that time spent on more complex carcass
116 preparation behaviours increased with increasing daily minimum temperature in
117 large groups ($\chi^2_1 = 5.39$, $P = 0.02$, n = 33; Fig. 2a), but not in small groups ($\chi^2_1 =$
118 0.17, $P = 0.68$, n = 56; Fig. 2a). However, there was no significant relationship
119 between walking time and daily minimum temperature in large ($\chi^2_1 = 0.24$, $P =$
120 0.60, n = 33; Fig. 2b) or small groups ($\chi^2_1 = 0.79$, $P = 0.37$, n = 56; Fig. 2b),
121 suggesting that the increase in total cooperative investment in warmer
122 environments was not simply the result of increased activity at warmer
123 temperatures.
124
125 Our field results demonstrate that *N. nepalensis* exhibits remarkably flexible
126 social behaviours along elevational and thermal gradients: beetles are normally
127 asocial and aggressive towards conspecifics in cooler environments but become
128 social and cooperate with conspecifics in warmer environments where the
129 competition for critical resources with other species is intense[7]. However, to
130 demonstrate experimentally that blowfly competition for carcasses drives the
131 transition from intraspecific competition to intraspecific cooperation, we
132 performed a series of laboratory experiments to directly manipulate the
133 presence or absence of blowflies at carcasses.
134

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 on the carcass. We found that more beetles cooperated ($t = 5.26$, $P < 0.001$; Fig. 3a), and that each individual beetle spent significantly more time cooperating, in the blowfly treatment than in the control treatment containing carcasses but no blowflies ($t = 3.27$, $P = 0.002$; Fig. 3b). 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). Although there was no difference in per capita social conflict between the blowfly and control treatments ($t = -0.33$, $P = 0.75$; Fig. 3d), after controlling for total investment time by dividing per capita social conflict by the total cooperative investment, the adjusted 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. S1). Thus, social conflict in burying beetles was lower and cooperation higher when blowflies were present on carcasses.

What is the mechanism driving the transition from intraspecific competition to intraspecific cooperation? Since blowfly species are diurnal but *N. nepalensis* is nocturnal, it is unlikely that the physical presence of blowflies influences *N. nepalensis* behaviour. Previous studies have demonstrated that sulfur-containing volatile organic compounds (S-VOCs) emitted from decomposing carcasses

158 attract burying beetles to this key resource[13,14]. Because GC-MS analysis showed
159 that dimethyl disulfide (DMDS) appeared earlier and was more abundant in the
160 blowfly treatment than in the control (Fig. 4a), we hypothesized that DMDS is the
161 key infochemical[15]—indicating not only the presence of a decaying carcass but
162 also the degree of interspecific competition at that carcass—that mediates the
163 transition between cooperative and competitive strategies in *N. nepalensis*.
164
165 To experimentally test this hypothesis, we injected DMDS into the body cavity of
166 mouse carcasses. We found that more individuals cooperated (t = -3.76, $P <$
167 0.001; Fig. 4b), and that each individual spent more time cooperating, in DMDS
168 treated carcasses relative to controls (t = -2.55, $P = 0.014$; Fig. 4c). Thus, there
169 was a higher total cooperative investment in the DMDS treatment than in the
170 control (t = -3.8, $P < 0.001$; Fig. 4d). These results were similar to those observed
171 in the blowfly treatment from the initial experiment. The only difference between
172 the DMDS and blowfly treatments was that there was marginally more social
173 conflict in the DMDS treatment than in the hexane control (t = -1.97, $P = 0.054$;
174 Fig. 4e), whereas this trend—while in the same direction—was not significant in
175 the blowfly treatment (t = -0.33, $P = 0.75$), presumably because there were no
176 real competitors that beetles need to remove in the DMDS treatment.
177
178 Our study shows that burying beetles transition from competitive to more
179 cooperative interactions as the pressure of interspecific competition increases.

180 Accumulating empirical evidence from other animals suggests that social conflict
181 in cooperative societies is often lower in adverse environments with strong
182 interspecific competition[5]. This pattern of reduced social conflict under strong
183 interspecific competition has largely been explained by the fact that the cost of
184 engaging in competitive interactions increases under adverse conditions[16]. Yet,
185 there is little empirical evidence demonstrating that social animals increase their
186 investment in cooperation under the threat of interspecific competition. One
187 exception comes from cooperatively breeding superb fairy-wrens (*Malurus*
188 *cyaneus*) who cooperate more in nest defence when exposed to a greater threat
189 of interspecific brood parasitism[17]. However, it remains unclear how intraspecific
190 conflict in fairy-wrens is influenced by the threat of interspecific competition.
191 Our study helps fill this knowledge gap by showing that cooperative carcass
192 preparation to reduce blowfly competition in warmer environments is critical for
193 predicting both the cooperative and competitive interactions among individuals
194 of the same species.
195
196 Furthermore, we show that the conditional cooperative and competitive
197 strategies[18,19] used by *N. nepalensis* to maximize their utility of carcasses are
198 mediated by a surprisingly simple chemical mechanism. DMDS is produced
199 during the decomposition process and acts as an indicator of the level of
200 competition from blowflies. Although interspecific competition has long been
201 recognized as a major ecological force that drives adaptive evolution[1-3], relatively
202 little effort has focused on how it influences intraspecific cooperation[4-6]. 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[15], and not as a pheromone produced by conspecifics[20,21]. Pheromones are often used for kin discrimination, and studying the olfactory sensory system and its genes have greatly advanced our understandings of the role that chemically-driven kin recognition has played in social evolution, especially in ants[22,23]. Here we demonstrate that interspecific chemical communication is also important to insect social evolution. Ultimately, by showing that chemically-mediated interspecific competition is a key driver of intraspecific cooperation and of social evolution more generally, our work demonstrates the value of integrating ultimate and proximate levels to study the evolution of cooperation[24].

Methods

Field Study. The field study was conducted in Taiwan from 2012 to 2015 along elevational gradients composed primarily of uncultivated forest in Nantou county (spanning 1688 m to 2650 m above sea level) and Hualien county (spanning 1193 m to 2720 m above sea level). A variety of social behaviours, including per capita social conflict and investment in cooperative carcass preparation (i.e. cooperative investment), were scored on the first night of video observation (from 19:00 to 05:00) using the Observer® XT 14 (Noldus). To quantify total cooperative investment, we estimated the cumulative time that each beetle spent depilating rat hair, cleaning rat carcasses by removing maggots, or dragging

carcasses during carcass burial and preparation. 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 three time periods (22:30 to 22:40; 01:30 to 01:40 and 04:30 to 04:40) during each video observation. Investment in cooperation was quantified as the duration of cumulative time sampled for a 10 mins observation period in each hour (i.e. 100 mins for each breeding experiment). In total, there were 89 breeding experiments (resulting in 8900 mins of video recordings) from which we were able to quantify total cooperative investment. Aggressive interactions were defined as 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 mins observation period. We measured per capita social conflict as the total number of aggressive interactions divided by the mean group size for each observation period. Conflict was quantified as the total number of aggressive interactions sampled for two 120 mins observation periods (from 19:30 to 21:30 and from 23:30 to 1:30). 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).

Collection and maintenance. Lab experiments were carried out 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 natural 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).

Experimental design and procedure. For each experimental replicate, three unrelated males and three unrelated females 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. The marking and weighing of beetles was done 2 hrs 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 random order

272 at the beginning of each experiment. Experimental boxes consisted of a smaller
273 plastic container (23 × 15.5 × 13.5 cm filled with 13.5 cm moist peat) located
274 inside a larger plastic container (45 × 34.5 × 25 cm filled with 13.5 cm moist
275 peat). A 4 cm high iron net with 2 cm^2 mesh was placed around the small
276 container to prevent beetles moving carcasses outside the field of view of the
277 digital cameras, but beetles could still move freely between the inner and outer
278 areas. A digital camera was fitted on the top of a 25 × 20 × 55 cm acrylic box,
279 which was fixed on the cap of the large container. To equalize the temperature of
280 the experimental apparatus, boxes were filled with moist peat and put into the
281 environmental chambers one day before the experiments began.
282 The blowfly treatment was conducted by exposing a 75 ± 5 g rat thawed
283 carcass to blowflies, oriental latrine flies (*Chrysomya megacephala*), in 32 × 32 ×
284 32 fly cages for 50 hrs before the start of each experiment. Fly cages contained
285 oriental latrine flies that had emerged from 10 g pupa and been kept in
286 environmental chambers at 26°C on a 14 L:10 D photoperiod. Except for maggot-
287 digested carcasses, all other carcasses in the same weight range were thawed at
288 4°C for 24 hrs before experiments began. Carcasses used in all treatments were
289 moved into the environmental chambers 8 hrs prior to the start of experiments
290 to equalize their temperatures. The hexane control and DMDS treatment used
291 thawed-only carcasses injected with 2 ml hexane or 0.01 M DMDS solution,
292 respectively, into abdominal cavities through the anus using 3 ml Terumo®
293 Syringes and needles 1 hr prior to the start of the experiment. The thawed-only
294 carcasses served as controls. The carcasses in controls and all treatments were

295 moved into the experimental boxes and put on the surface of peat in smaller
296 containers 1 hr before experiments began. Behavioural videos were recorded
297 either from 7 PM until the day and time at which a carcass was completely buried
298 into peat or for 72 hrs if the beetles did not completely bury the carcass (under
299 natural conditions, a carcass would be completely consumed by blowflies if
300 beetles did not completely bury it within 72 hrs). In total, 1020 hrs of videos
301 were analyzed from 23 blowfly control replicates, 23 blowfly treatment
302 replicates, 32 hexane control replicates, and 24 DMDS replicates. Social conflict
303 and cooperative investment behaviours were recorded in the first 10 hrs (7 PM
304 to 5 AM) of each experimental treatment using The Observer® XT 14 (Noldus).
305
306 **Gas chromatography-mass spectrometry (GC-MS) analysis.** The composition
307 of volatile organic compounds (VOCs) emitted by carcasses was determined from
308 two control and two blowfly treated carcasses prepared using the same
309 procedure described previously. The prepared carcasses were put on the peat
310 surface in glass vacuum desiccators (15 cm diameter × 22 cm tall) filled with 5
311 cm of moist peat. The stopcock and ground-glass rim of the desiccator lid were
312 greased with a thin layer of petroleum jelly to prevent the leakage of emitted
313 VOCs, as well as contamination from the atmosphere. The VOCs were sampled
314 using solid-phase micro-extraction (SPME)[25]. The SPME holder with CAR/PDMS
315 fiber (Supelco, previously desorbed for 5 mins in GC injection port heated to
316 200°C) was inserted through the hole of the stopcock into the atmosphere
317 surrounding the rat carcass. Immediately after exposing the fiber for 15 mins, the

318 sample was GC-MS-analyzed using a 6890N Network Gas Chromatograph
319 (Agilent Technologies) equipped with a HP-5ms column (Agilent J&W) and a
320 5975 Mass Selective Detector (Agilent Technologies). The GC oven was operated
321 at an initial temperature 40°C for 1 min and then ramped up at a rate 10°C per
322 min to 250°C (with a 10 mins hold). The temperatures of the GC inlet and
323 detector were set to 200°C and 260°C, respectively. The SPME samples were GC
324 analysed split-less. Helium (1 ml per min) was used as a carrier gas. Since the GC-
325 MS results showed DMDS was the major VOC emitted by the blowfly-treated
326 carcasses, DMDS was injected into carcasses in the further experiments. Two
327 DMDS-injected carcasses (also prepared using the same procedure described
328 previously) were used in GC-MS analyses (following the procedure described
329 above) to determine the composition of the VOCs they emitted.
330
331 **Statistical analyses.** Multivariate analyses were performed using generalized
332 linear models (GLMs) to determine statistical significance for differences
333 between controls and blowfly treatments or hexane controls and DMDS
334 treatments in mean group size, total and per capita cooperative investment, and
335 total and per capita social conflict. All statistical analyses were performed in R
336 using the packages stats, lme4, car, multcomp (http://cran.r-project.org/), and
337 glmmADMB (http://glmmadmb.r-forge.r-project.org/).

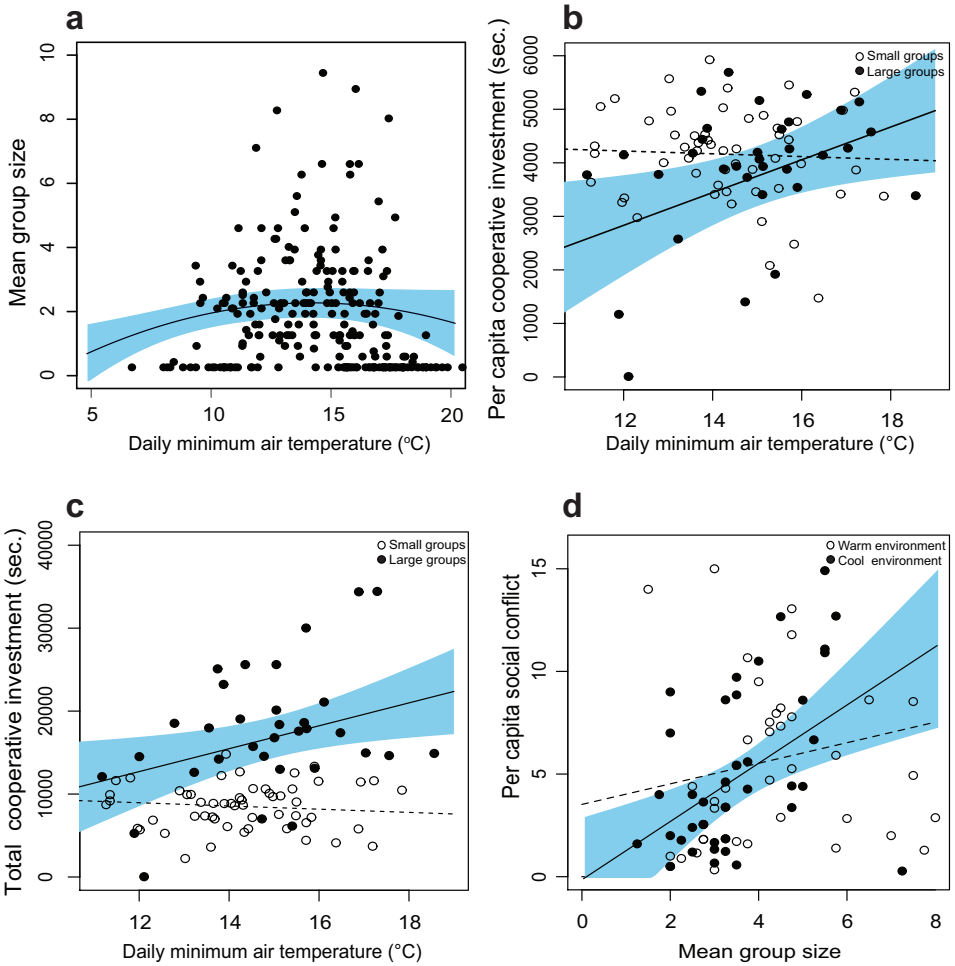


Figure 1 | Changes in *N. nepalensis* group size and social behaviours during carcass preparation along a temperature gradient. The relationship between daily minimum air temperature and **(a)** mean group size, **(b)** per capita cooperative investment, **(c)** total cooperative investment in large (closed circles) and small groups (open circles). Group size peaked at moderate temperatures, whereas per capita and total cooperative investment increased with daily minimum temperature in large but not small groups. Solid lines denote predicted relationships from GLMs, whereas dashed lines denote non-significant relationships. **(d)** Per capita social conflict increased with group size in cool environments (closed circles), but not in warm environments (open circles). Lines represent least-squared means (solid lines denote significant relationships and dotted lines non-significant relationships), and blue shaded areas represent 95% confidence intervals expected from GLMMs.

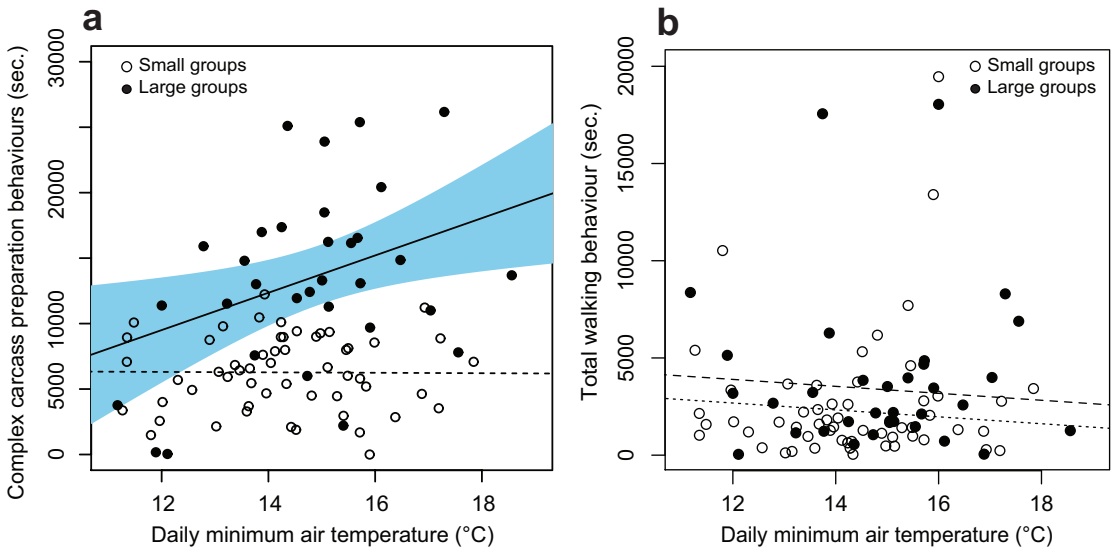


Figure 2 | Complex carcass preparation and simple walking behaviours during cooperative carcass preparation along the temperature gradient. The time that beetles spent on **(a)** complex carcass preparation behaviours and **(b)** walking on the carcass in relation to daily minimum air temperature in large and small groups. Compared to small groups (open circles), large groups (closed circles) spent more time on complex carcass preparation but not on walking as daily minimum air temperature increased, suggesting that the increase in total cooperative investment in warmer environments was not simply the result of increased activity at warmer temperatures. Lines represent least-squared means (solid lines denote significant relationships and dotted lines non-significant relationships), and the blue shaded area represent 95% confidence intervals expected from GLMMs.

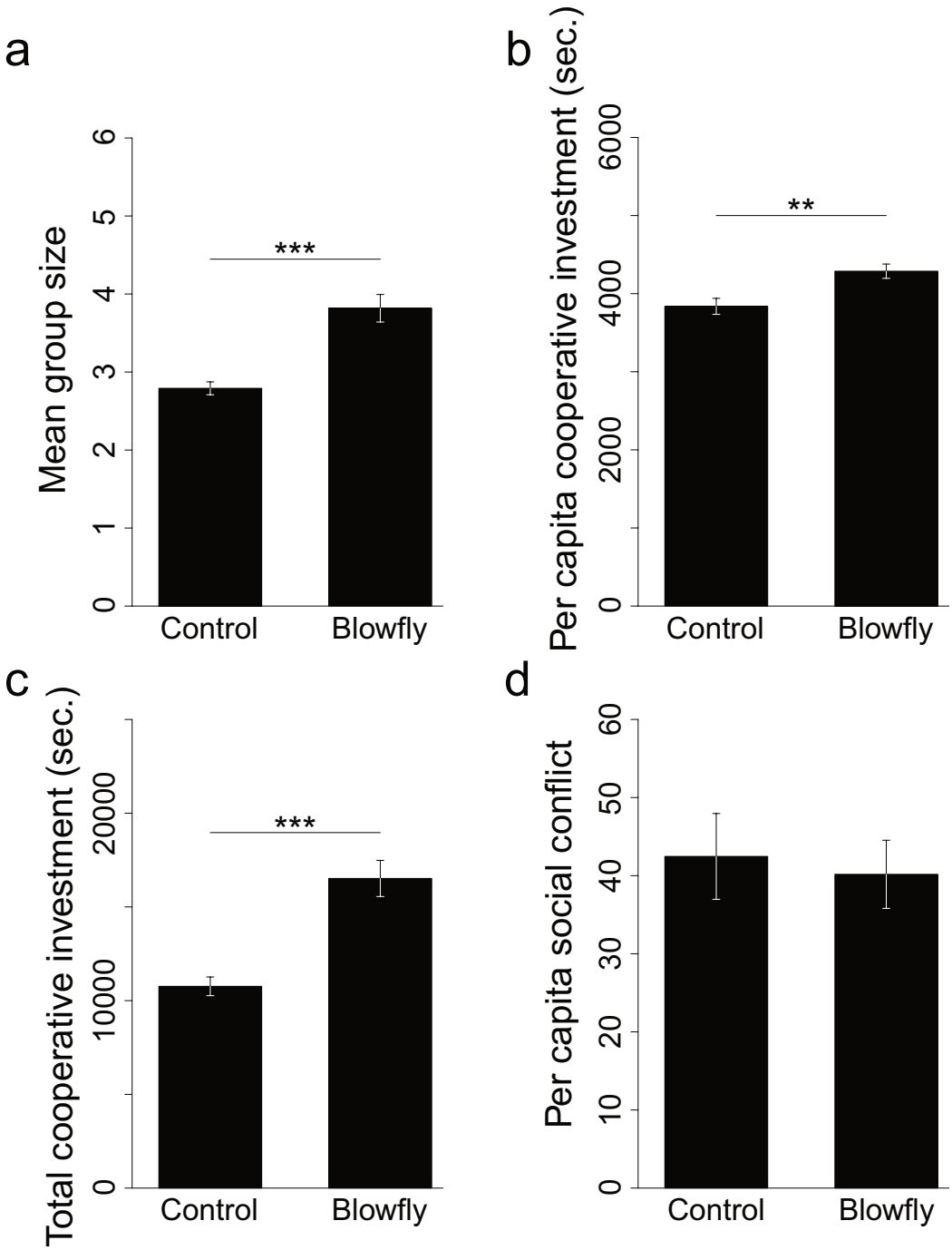


Figure 3 | *N. nepalensis* social behaviours in control and blowfly treatments. **(a)** Mean group size, **(b)** per capita cooperative investment, **(c)** total cooperative investment, and **(d)** per capita social conflict of burying beetles on carcasses. Beetles formed larger groups and had greater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

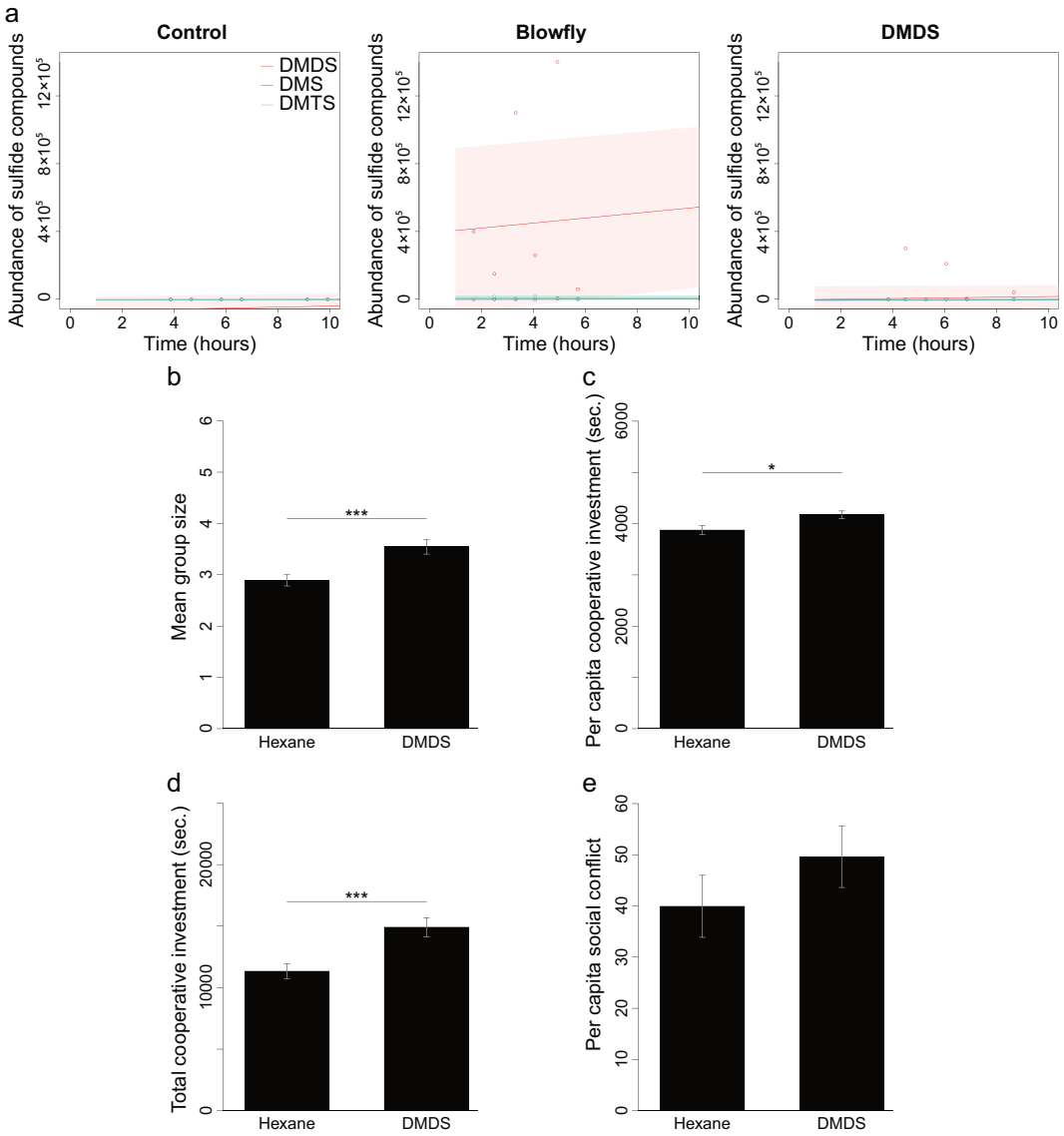


Figure 4 | Results of gas chromatography-mass spectrometry (GC-MS) analyses and *N. nepalensis* social behaviours in hexane and DMDS treatments. (a) GC-MS analyses showed an abundance of sulfide compounds, including dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) in control, blowfly, and DMDS treatments during the first 10 hrs. 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 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. * $P \leq 0.05$; *** $P \leq 0.001$.

Author contributions:

S.-F.S. conceived the idea for the study. B.-F.C., M.L., D.R.R. and S.-F.S. design the experiments. B.-F.C., M.L., S.-J.S. performed field experiment. B.-F.C. and J.-N.L. performed lab behavior experiments. B.-F.C. and Y.-H.L. did the GC-MS analysis. B.-F.C., M.L., D.R.R. and S.-F.S. analyzed the data and wrote the paper.

Acknowledgements

S.-F.S was supported by Career Development Award and Investigator Award, Academia Sinica and Ministry of Science and Technology of Taiwan (100-2621-B-001-001, 103-2621-B-001 -003 -MY3). D.R.R. was supported by the US National Science Foundation (IOS-1257530 and IOS-1656098).

Reference:

1 Bolnick, D. I. *et al.* 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 (2010).

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

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

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

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

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

7 Liu, M. *et al.* Ecological transitions in grouping benefits explain the paradox of environmental quality and sociality. (in review).

8 Sun, S.-J. *et al.* Climate-mediated cooperation promotes niche expansion in burying beetles. *eLife* **3**, e02440 (2014).

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

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

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

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

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

14 Podskalská, H., Růžička, J., Hoskovec, M. & Šálek, M. Use of infochemicals to attract carrion beetles into pitfall traps. *Entomol. Exp. Appl.* **132**, 59-64 (2009).

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

16 De Jaegher, K. & Hoyer, B. By-product mutualism and the ambiguous effects of harsher environments – A game-theoretic model. *J. Theor. Biol.* **393**, 82-97, doi:https://doi.org/10.1016/j.jtbi.2015.12.034 (2016).

17 Feeney, W. E. *et al.* Brood Parasitism and the Evolution of Cooperative Breeding in Birds. *Science* **342**, 1506-1508, doi:10.1126/science.1240039 (2013).

18 Gross, M. R. Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology & Evolution* **11**, 92-98, doi:http://dx.doi.org/10.1016/0169-5347(96)81050-0 (1996).

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

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

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

22 Trible, W. *et al.* Orco mutagenesis causes loss of antennal lobe glomeruli and impaired social behavior in ants. *Cell* **170**, 727-735. e710 (2017).

23 Yan, H. *et al.* An Engineered orco Mutation Produces Aberrant Social Behavior and Defective Neural Development in Ants. *Cell* **170**, 736-747.e739, doi:https://doi.org/10.1016/j.cell.2017.06.051 (2017).

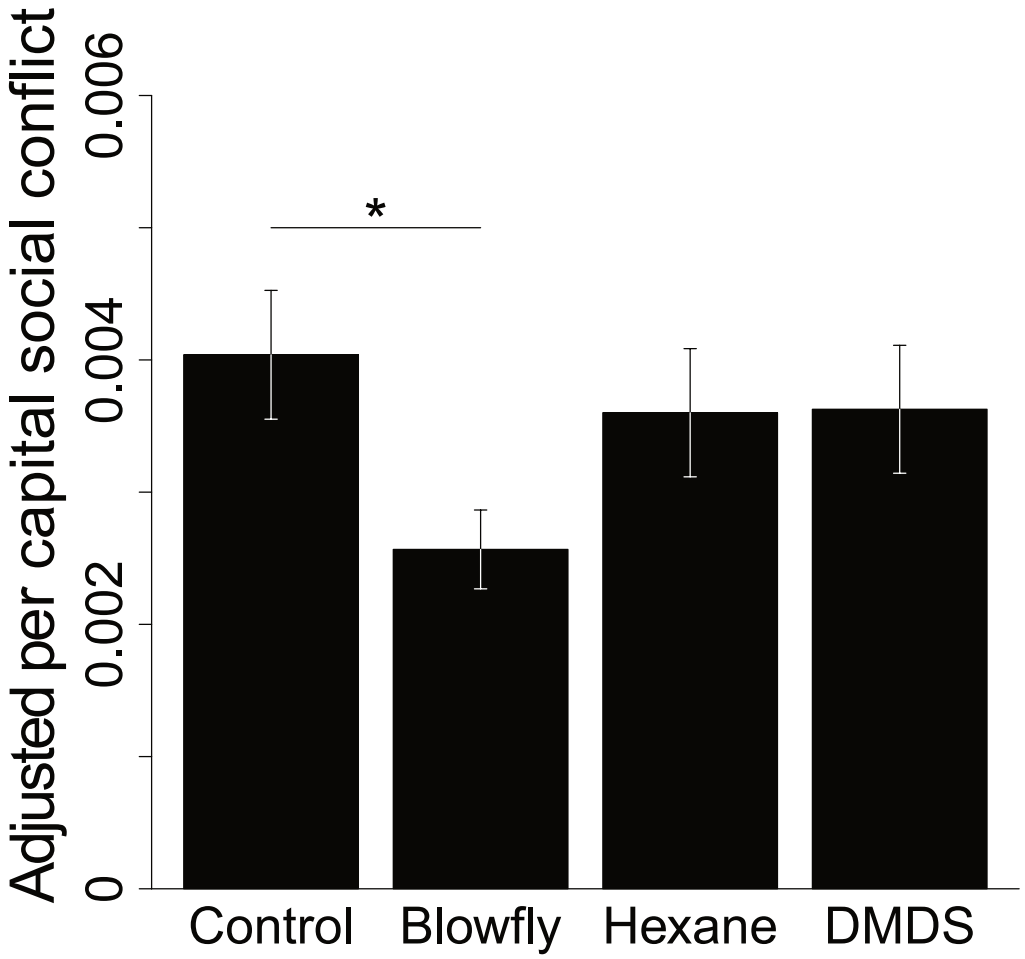
24 Hofmann, H. A. *et al.* An evolutionary framework for studying mechanisms

of social behavior. *Trends Ecol. Evol.* **29**, 581-589, doi:http://dx.doi.org/10.1016/j.tree.2014.07.008 (2014).

25 Vas, G. & Vékey, K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *J. Mass Spectrom.* **39**, 233-254, doi:doi:10.1002/jms.606 (2004).

462

463



464

465 **Figure S1 | Adjusted per capita social conflict in different experimental**
466 **treatments.** Adjusted per capita social conflict (i.e. per capita social conflict
467 divided by the total cooperative investment time) was lower in the blowfly than
468 control treatments. There was no difference in adjusted per capita social conflict
469 between the DMDS and hexane control treatments. * $P < 0.05$.