Plant metabolites modulate animal social networks and lifespan

Pragya Singh[1,*], Leon Brueggemann[1], Steven Janz[1], Yasmina Saidi[1], Gaurav Baruah[2] and Caroline Müller[1]

[1]Chemical Ecology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

[2]Theoretical Biology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

*Corresponding Author:

Email: pragya.singh42019@gmail.com

ORCiD: https://orcid.org/0000-0002-7411-3206

Acknowledgements

This study was funded by the German Research Foundation (DFG) as part of the SFB TRR 212 (NC³), project number 396777467 (granted to CM). We thank Karin Djendouci for her help in conducting the experiments and Rohit Sasidharan for help with collecting sawflies.

Author Contributions

Conceptualization and experimental design: PS; data collection: YS for experiment 2, SJ for experiment 3, SJ, PS and LB for experiment 4; chemical analysis: LB; data validation and analysis: PS and GB for network analysis, PS for other experiments; data visualisation: PS; writing original draft: PS, CM; reviewing and editing: PS, CM, LB, GB; funding acquisition: CM.

Data availability

The datasets are available on the Zenodo repository (https://doi.org/10.5281/zenodo.10969832).

Abstract

Social interactions influence disease spread, information flow, and resource allocation across species, yet heterogeneity in social interaction frequency and its fitness consequences remain poorly understood. Additionally, animals can utilize plant metabolites for purposes beyond nutrition, but whether that shapes social networks is unclear. Here, we investigated how non-nutritive plant metabolites impact social interactions and the lifespan of the turnip sawfly, *Athalia rosae*. Adult sawflies acquire neo-clerodane diterpenoids ('clerodanoids') from non-food plants, showing intraspecific variation in natural populations and laboratory-reared individuals. Clerodanoids can also be transferred between conspecifics, leading to increased agonistic social interactions. Network analysis indicated increased social interactions in sawfly groups where some or all individuals had prior access to clerodanoids. Social interaction frequency varied with clerodanoid status, with fitness costs including reduced lifespan resulting from increased interactions. Our findings highlight the role of intraspecific variation in the acquisition of non-nutritional plant metabolites in shaping social networks, with fitness implications on individual social niches.

Keywords

pharmacophagy, specialized plant metabolites, non-nutritional plant-insect interactions, mating behaviour, aggressive behaviour, network dynamics, social network analysis

Introduction

Social interactions are widespread in animals and lead to the emergence of diverse social networks, from nominally to highly interactive (Frank 2007; Krause *et al.* 2015). Such interactions can range from mating to agonistic. A common aspect of networks constructed on social interactions, i.e. animal social networks, is skewness in the number of interactions individuals have (Fisher *et al.* 2019; Gartland *et al.* 2022; Krause *et al.* 2015). Some individuals have frequent social interactions while others have few. Consequently, this could have ecological ramifications. For instance, individuals with a higher frequency of interactions may overly affect disease transmission (Lloyd-Smith *et al.* 2005), which in turn may shape evolution of social systems (Udiani & Fefferman 2020). Furthermore, more or few social interactions may have associated benefits or costs for an individual (Koto *et al.* 2023; Ruan & Wu 2008; Snyder-Mackler *et al.* 2020). For example, increased agonistic social interactions can lead to injuries or even mortality (Archer 1988), although such costs are more widely documented in species that have teeth, claws or other armaments than in unarmed species. Even in unarmed species, there can be costs of frequent social interactions, such as depletion of energy reserves (Briffa & Sneddon 2007), such as reduced lipid or carbohydrate reserves.

Social interactions can depend on various biotic and abiotic factors (Candolin & Wong 2012; Fisher *et al.* 2021). One of these biotic factors may be plant-animal associations, but their role in explaining the observed skew in social interactions is only little explored. Studies have shown that several specialised plant metabolites are taken up by certain animal species independently of nutrition, and stored (sequestered), for example, for use in mating or as anti-predation defence (Beran & Petschenka 2022; Nishida 2014; Opitz & Müller 2009). This phenomenon is called pharmacophagy (Boppré 1984). Remarkably, such non-nutritive plant metabolites or their derivatives, can also be acquired from other animals, either conspecifics

71 (Paul *et al.* 2021; Singh *et al.* 2022) or heterospecifics (Tea *et al.* 2021). The uptake of such
72 non-nutritive plant metabolites can have direct effects on the behaviour of the individuals
73 (Amano *et al.* 1999; Conner *et al.* 2000; Gonzalez *et al.* 1999). This individual-level variation
74 in behaviour and how the individuals interact with each other can in turn lead to the
75 emergence of complex patterns in animal social networks (Krause *et al.* 2015). Thus, these
76 sequestered plant metabolites can impact social interactions, and consequently influence the
77 fitness of the individual animals (Formica *et al.* 2012; Royle *et al.* 2012; Silk *et al.* 2003) and
78 their individualised social niche (Kaiser *et al.* 2024; Trappes *et al.* 2022). Moreover, taking up
79 plant metabolites may entail other costs, such as potential toxicity, if the substances are
80 harmful (Kortbeek *et al.* 2019).

81 Interestingly, many animal species show intraspecific variation in the presence and quantity
82 of sequestered plant metabolites (Liu *et al.* 2023; Mattila *et al.* 2021; Opitz & Müller 2009;
83 Speed *et al.* 2012). Such variation can result from variation in the plant metabolite
84 concentration per se, potential costs related to metabolite acquisition by the animal or factors
85 such as age, sex or immunological status of the individuals (Dimarco & Fordyce 2017;
86 Massad *et al.* 2011; Moore *et al.* 2014; Smilanich *et al.* 2009; Zvereva & Kozlov 2015). This
87 raises the question of whether intraspecific variability in sequestered plant metabolites can
88 influence social interactions, and if these metabolites, directly or through modified social
89 interactions, impact fitness indicators such as lifespan. Additionally, the transfer of plant
90 metabolites between individuals is likely to influence the social interactions of both the donor
91 and the recipient. However, despite the known impact of sequestered plant metabolites on
92 individual behaviour (Boppré 1984; Nishida 2014), our understanding of how these effects
93 translate into broader implications for social interactions remains limited.

94 To target these open questions, we examined the effect of intraspecific variation in
95 sequestered plant metabolites on social interactions and lifespan in the turnip sawfly, *Athalia*

96 *rosae* (Hymenoptera: Tenthredinidae). The adults of *A. rosae* feed on floral nectar, usually
97 from Apiaceae plants. In addition, they visit non-food plant species such as *Ajuga reptans*
98 (Lamiaceae), on which they exhibit pharmacophagy by taking up specialised metabolites,
99 neo-clerodane diterpenoids (hereafter called 'clerodanoids'), which they further metabolise
100 slightly (Brueggemann *et al.* 2023). These (metabolised) clerodanoids can serve as a resource
101 for *A. rosae* adults in multiple ways (Paul *et al.* 2022). Access to clerodanoids increases their
102 mating success (Amano *et al.* 1999) and also provides chemical defence against predators
103 (Nishida & Fukami 1990; Singh *et al.* 2022). Moreover, individuals can obtain clerodanoids
104 via agonistic interactions like nibbling on conspecifics that have had access to plant
105 clerodanoids (Paul & Müller 2021; Singh *et al.* 2022). Such agonistic interactions can
106 potentially be costly, as suggested by the transcriptional upregulation of metabolic pathways
107 in sawflies that nibbled on conspecifics (Paul *et al.* 2021). Additionally, the uptake of
108 clerodanoids may also directly pose fitness costs, such as a reduced lifespan (Zanchi *et al.*
109 2021). However, laboratory cultures of *A. rosae* are usually maintained without access to
110 clerodanoids, indicating that clerodanoids are not essential for individual survival.

111 In this study, we initially assessed intraspecific variation in clerodanoid levels within natural
112 *A. rosae* populations. Subsequently, we investigated how access to clerodanoids, either
113 directly through *A. reptans* leaves or indirectly via conspecifics, influenced behavioral
114 interactions in laboratory-reared individuals, and analysed their clerodanoid levels. Notably,
115 intraspecific variation in clerodanoid content was observed in both wild-caught and
116 laboratory-reared individuals. To further understand the impact of clerodanoid variation on
117 social interactions, we established treatment groups with different levels of clerodanoid
118 access, using network analysis to quantify individual and group social interaction metrics
119 (Wey *et al.* 2008). Lastly, we explored the fitness consequences of clerodanoid acquisition by
120 examining its effects on lifespan and metabolic profiles, i.e. lipid and carbohydrate content,

employing treatment groups with varying clerodanoid levels. Through these analyses, we elucidated the ecological significance of clerodanoids in shaping social networks and individual fitness in *A. rosae* populations. We expected intraspecific variation in adult clerodanoid levels. Groups lacking clerodanoid access were expected to exhibit minimal social interactions, whereas asymmetric clerodanoid access would promote increased social interactions. Individuals without direct access were predicted to adjust social interaction frequency based on the clerodanoid status of conspecifics. Furthermore, increased social interactions for clerodanoid acquisition were expected to incur fitness costs, resulting in a reduced lifespan and a greater depletion of lipid and carbohydrate reserves.

Materials and Methods

Maintenance of study organism

Adults of *A. rosae* used in the experiments (other than wild-caught adults) were taken from a laboratory stock population that had been established from sawflies collected in and around Bielefeld, Germany, and annually supplemented with field-caught individuals (see Singh *et al.*, 2023 for laboratory rearing details). The individuals were provided a 2% (v/v) honey-water solution on paper that was refreshed every alternate day. The laboratory population was maintained in multiple mesh cages (each 60 x 60 x 60 cm) at room temperature (15-25°C) with a 16:8 h light:dark cycle and approximately 60% relative humidity.

Experiment 1. Chemical analysis for clerodanoid content of wild-caught sawflies

In total eighteen female and eight male adult *A. rosae* were collected in the wild at three locations (SI.1). For analysis of clerodanoid amounts, the insects were individually frozen, lyophilised, homogenised and extracted in ethyl acetate (LC-MS grade, VWR, Leuven, Belgium), using ultrasonication. After centrifugation, supernatants were dried and resolved in

100 µl of methanol with mefenemic acid (Sigma-Aldrich GmbH, Taufkirchen, Germany) as internal standard. Samples were filtered and analysed using an ultra-high performance liquid chromatograph (UHPLC; Dionex UltiMate 3000, Thermo Fisher Scientific, San José, CA, USA) equipped with a Kinetex XB-C18 column (1.7 µm, 150 × 2.1 mm, with guard column, Phenomenex) kept at 45 °C, coupled to a quadrupole time of flight mass spectrometer (QTOF-MS/MS; compact, Bruker Daltonics, Bremen, Germany) in negative electrospray ionisation mode, following Brueggemann *et al.* (2023; for details see Supplement).Samples were separated with a gradient from 0.1% formic acid (p.a., eluent additive for LC-MS, ~98%, Sigma-Aldrich) in deionised water (eluent A) to 0.1% formic acid in acetonitrile (LC-MS grade, Fisher Scientific, Loughborough, UK; eluent B) at a flow rate of 0.5 ml min^{-1}, starting eluent B at 2%, increasing to 30% within 20 min, and then to 75% within 9 min, followed by column cleaning and equilibration. The settings for the MS mode were capillary voltage 3000 V, end plate offset 500 V, nebuliser (N_2) pressure 3 bar, dry gas (N_2, 275 °C) flow 12 L min^{-1}, quadrupole ion energy 4 eV and collision energy 7 eV. Line spectra were captured at 6 Hz, with a range of 50-1300 *m/z*. For recalibration of the *m/z* axis, a Na(HCOO)-solution was injected prior to each sample. Blanks without animal material were prepared in the same way and measured under the same conditions.

Mass axis recalibration and peak picking with the T-ReX 3D algorithm including spectral background subtraction were performed in Metaboscape 2021b (Bruker Daltonics), with an intensity threshold of 1000 counts and minimum peak length of 9 spectra. The allowed ion types for bucket generation were: $[M-H]^-$, $[M-H2O-H]^-$, $[M+Cl]^-$, $[M+HCOOH-H]^-$, $[M+CH_3COOH-H]^-$ and $[2M-H]^-$. From each bucket only the feature with the highest intensity was used. We focussed on two metabolites which are likely taken up from *A. reptans* and metabolised by the sawflies (Brueggemann *et al.* 2023), putative clerodanoid 1 eluting at 16.3 min (*m/z* 529.229 $[M+HCOOH-H]^-$) and putative clerodanoid 2 eluting at 18.2 min (*m/z*

527.214 [M+HCOOH-H]$^-$), with the monoisotopic masses of 484.231 Da (proposed sum formula $C_{24}H_{36}O_{10}$) and 482.214 Da ($C_{24}H_{34}O_{10}$), respectively. Peak intensities of these target metabolites were related to the peak intensity of mefenemic acid for semi-quantitative analysis.

Experiment 2. Effect of direct and indirect clerodanoid acquisition on social interactions

We investigated the effects of no clerodanoid access (C-), indirect clerodanoid access from a conspecific (C+ from conspecific), or direct clerodanoid access from a plant (C+ from plant) by females on social interactions with C- males. Note that 'C+ from conspecific' has been previously referred to as AC+ (Paul *et al.* 2021; Singh *et al.* 2022). For the 'C+ from plant' treatment, freshly eclosed females were provided individually an *A. reptans* leaf disc (~1 cm^2) for 48 h, while 'C+ from conspecific' treatment females were kept with a C+ conspecific (2-4 days old female that had previously contact to *A. reptans*) for 48 h. Males (6- 10 days post-eclosion) were kept individually in Petri dishes for 48 h prior to the assays. The males were mated to a non-focal C- female (~3 days old) 24 h prior to the experiment to avoid any differences arising due to first mating, as observed in other species (Torres-Vila & Jennions 2005). For the behavioural assays, one female was set up with one male in a clean Petri dish (5.5 cm diameter) and recorded (Sony HDR-CX410VE camcorder, AVCHD – 1920 × 1080 − 25 fps) for 25 minutes (16 replicates per treatment combination). One to two replicates of each treatment were set up simultaneously in a trial. An observer scored the number of occurrences of agonistic behaviour, including frontlimb battling, fighting and nibbling events (see Paul & Müller, 2021 for detailed behavioural descriptions), and mating behaviour (number of mating, latency until first mating) in each movie replicate using the software BORIS v7.13.9 (Friard & Gamba 2016), being blind to the treatment. After the behavioural assays, five females from each treatment were chosen randomly, frozen and analysed for their clerodanoid content as in experiment 1.

195 ***Experiment 3. Effect of intraspecific variation in clerodanoids on social networks***

196 We found intraspecific variation in clerodanoid content in both wild-caught and lab-reared
197 sawflies (see Results). To test the effect of clerodanoid acquisition status on social networks,
198 we set up three group types of two males and two females each, in which either no individual
199 had prior access to clerodanoids (C- group, symmetric), only one female and one male had
200 prior access to clerodanoids (Mixed group, asymmetric) or all individuals had prior access to
201 clerodanoids from *A. reptans* leaves (C+ group, symmetric). We set up 15 replicates per
202 group type. Two hours before the behavioural assay, each individual was colour-marked
203 (green, red, white, or yellow; Uni POSCA marker pens, Japan) on its thorax and wings to
204 enable identification during behaviour scoring. The colours were alternated between sexes
205 and treatments across replicates to avoid any colour effects. In each trial, one to two replicates
206 per group type were set up simultaneously in Petri dishes and recorded for 25 minutes.
207 Agonistic and mating interactions of each individual in a movie replicate were scored by an
208 observer as in experiment 2.

209 For the social network analysis, we analysed group as well as individual measures of the
210 network. Therefore, we constructed an undirected weighted network for the social interactions
211 (sum of agonistic and mating interactions) in each replicate. We calculated the network
212 weights as the total number of interactions between each pair of individuals (dyad). We
213 normalised the weights at the replicate level and at the dyadic level in each replicate, by
214 dividing by the maximum number of interactions across all replicates and all dyads,
215 respectively. At the group level, we calculated the density of social interactions in each
216 replicate, as following:

217 $$Density = \frac{sum\ of\ weights}{[number\ of\ vertices * (number\ of\ vertices - 1) * 0.5},$$

218 where vertices is the number of individuals in a replicate. Moreover, at the group level, we
219 counted the number of distinct dyads that exhibited social interactions in each replicate. At
220 the individual level, we calculated the strength (Barrat *et al.* 2004) and harmonic centrality
221 (Marchiori & Latora 2000). The strength is a measure of how strongly an individual is
222 directly connected to other individuals in the network. Harmonic centrality calculates the
223 'average' distance of an individual to the other individuals in the network. We used harmonic
224 centrality as we had disconnected networks in some replicates.

225 ***Experiment 4. Costs of direct and indirect clerodanoid acquisition on lifespan, lipid and***
226 ***carbohydrate content***

227 To examine fitness costs of clerodanoid acquisition and effects on metabolism, we measured
228 the lifespan and lipid and carbohydrate content of individuals from different groups. Similar
229 to experiment 3, freshly eclosed *A. rosae* adults were colour-marked and assigned to C- or
230 C+, offering the latter *A. reptans* leaves for 48 hours. The adults were then used to construct
231 three group types consisting of two males and two females, as in experiment 3 (C- group,
232 Mixed group and C+ group). We set up 10 replicates per group type. After four days of being
233 in a group, the adults were separated and one male and one female were taken to score their
234 survivorship while the other male and female were taken to measure their lipid and
235 carbohydrate content. Note that some individuals died while being in a group, they were
236 included for survivorship and metabolic trait analysis.

237 Lipid and carbohydrate contents were determined for each individual using a method adapted
238 from Cuff *et al.* (2021). Individuals were isolated in Eppendorf tubes (2 ml) and frozen at -
239 80°C, lyophilised for 48 hours and weighed. Afterwards, each individual was soaked in 0.5
240 ml of a 1:12 chloroform:methanol solution for 24 hours. From the supernatant 250 µl was
241 collected for lipid analysis, followed by additional soaking of the adult in 0.25 ml of 1:12
242 chloroform:methanol to remove residual lipids for later carbohydrate analysis. The solvent

with residuals was discarded. Lipid analysis was conducted with 50 µl of supernatant in a 96-well plate to which 10 µl of concentrated sulfuric acid was added. The plate was heated in at 100°C for 10 min and then 240 µl of 0.2 mg/ml vanillin in phosphoric acid was added, followed by photometric analysis at 490 nm. A dilution series of cricket oil (Acheta Cricket Oil, Thailand Unique) in 1:12 chloroform:methanol was measured on the same plate for calibration. For carbohydrate analysis, the remaining individuals were homogenised with glass beads, treated with 0.5 ml of 0.1 M NaOH, incubated in a shaker (250 rpm for 30 min at 80 °C) and subsequently allowed to stand at room temperature for 16 hours. Carbohydrate analysis was conducted with 40 µl of the supernatant in a 96-well plate, adding 160 µl of anthrone reagent, and heating the plate at 92°C for 10 min, before photometric analysis at 620 nm. A dilution seried of 1:1 trehalose:glycogen (trehalose: 98%, Roth; glycogen: AppliChem) in water was measured on the same plate for calibration of carbohydrates. Body lipid and carbohydrate content were determined as percentage relative to dry body mass.

We also conducted a similar experiment constructing pairs of females with no access, asymmetric access or symmetric access to clerodanoids (see SI.2), which allowed us to confirm that our effects of direct and indirect clerodanoid acquisition on lifespan (see Results) were not stemming only from increased mating interactions in Mixed and C+ groups but also from increase agonistic interactions in these group types.

Statistical analyses

All statistical analyses were conducted using R version 4.2.1 (R Core Team 2022). We used Poisson-distributed generalised linear mixed models (GLMM) with trial as a random effect to assess the effect of female treatment on agonistic and mating interactions in Experiment 2 (package ‘lme4’ version 1.1-30, package ‘lmerTest’ version 3.1-3; Bates *et al.* 2015; Kuznetsova *et al.* 2017). Effect of female treatment on mating latency was examined using a linear mixed model (LMM) with trial as a random effect. Differences in clerodanoid 1 and 2

among treatment groups were assessed using Kruskal-Wallis tests followed by Dunn tests with Holm correction ('FSA' package version 0.9.4, Ogle *et al.* 2023). In Experiment 3, we ran LMMs for density and binomial GLMMs for the number of dyads with social interactions, with group type as a fixed effect and trial as a random effect. Effect of group type, sex and their interaction on individual-level network measures were analyzed separately for C- and C+ sawflies using poisson-distributed GLMMs for strength and LMMs for harmonic centrality ('igraph' package version 1.5.1, Csárdi *et al.* 2023), with replicate ID and trial as random effect. For Experiment 4, individuals were classified based on both their individual and group treatments, yielding four composite treatment categories: "C- from C- group", "C- from Mixed group", "C+ from Mixed group", and "C+ from C+ group". LMM were used to assess the impact of sex, composite treatment, and their interaction on lifespan, lipid, and carbohydrate content, with replicate ID as random effect. Model validity was confirmed by examining residual distributions. If the interaction terms were non-significant, they were dropped to test the significance of the lower-order terms. Post-hoc analyses were conducted using the 'multcomp' package version 1.4-20 (Hothorn *et al.* 2008) for LMMs and GLMMs.

Results

Intraspecific variation in clerodanoids in natural A. rosae populations

Both females and males showed intraspecific variation in the amount of putative clerodanoid 1 and clerodanoid 2 across the three locations from which they were collected (Figure 1). Some individuals had no clerodanoids, while others had relatively high quantities.

Clerodanoid uptake affects sawfly behaviour and varied levels observed in lab-reared sawflies

There was a significant effect of female treatment on number of agonistic interaction occurrences ($\chi^2 = 48.56$, df = 2, $P < 0.001$, Figure 2a), but not on mating occurrences ($\chi^2 =$

292 1.76, df = 2, $P = 0.41$, Figure 2b). C- and 'C+ from conspecific' treatment females had
293 significantly fewer agonistic interactions than 'C+ from plant' treatment females (SI.3a).
294 There was a significant effect of female treatment on mating latency ($\chi^2 = 9.90$, df = 2, $P =$
295 0.007, Figure 2c) with C- females having a significantly longer latency until mating than 'C+
296 from plant' females, while 'C+ from conspecific' females showed an intermediate mating
297 latency (SI.3b).

298 There was a significant difference between C-, 'C+ from conspecific' and 'C+ from plant'
299 females in the amount of clerodanoid 1 ($\chi^2 = 8.11$, df = 2, $P = 0.017$, Figure 2d) and
300 clerodanoid 2 ($\chi^2 = 8.10$, df = 2, $P = 0.017$, Figure 2e). No clerodanoids were detected in C-
301 females, while there was intraspecific variation in clerodanoids in both 'C+ from conspecific'
302 and 'C+ from plant' females, which did not differ significantly from each other (SI.3c, SI.3d).

303 ***Intraspecific variation in clerodanoid access modulates social networks***

304 The group types varied in their social networks, with the C- group having fewer interactions
305 than the Mixed and the C+ group (Figure 3a). Our results showed that there was a significant
306 effect of group type on density ($\chi^2 = 27.98$, df = 2, $P < 0.001$, Figure 3b) and number of dyads
307 with social interactions ($\chi^2 = 41.29$, df = 2, $P < 0.001$, Figure 3c). Mixed groups had a
308 significantly higher density and more dyads that had interacted than C- and C+ groups (SI.4a,
309 SI.4b). Together this suggests that access to clerodanoids increases social interactions with
310 more individuals networking, particularly in the Mixed group.

311 For C- sawflies, there was a significant effect of group type ($\chi^2 = 63.55$, df = 1, $P < 0.001$) on
312 strength (Figure 4a) such that C- sawflies in Mixed groups had a higher strength, i.e., had
313 more social interactions, than adults in C- groups (SI.5a). In contrast, there was a significant
314 effect of sex ($\chi^2 = 25.14$, df = 1, $P < 0.001$) on strength of interactions (Figure 4b) for C+
315 sawflies, with males having a lower strength than females across Mixed and C+ groups

316 (SI.5a). For harmonic centrality, there was a significant effect of group type (χ^2 = 6.88, df = 1,
317 P = 0.008) in C- sawflies (Figure 4c), such that those in Mixed groups had higher harmonic
318 centrality values. There was no significant effect of any predictor variable on harmonic
319 centrality in C+ sawflies (Figure 4d, SI.5c).

320 ***C+ from Mixed group adults have a shorter lifespan but no effect on lipid and carbohydrate***
321 ***content***

322 For lifespan, there was a significant effect of composite treatment (χ^2 = 18.74, df = 3, P <
323 0.001) such that "C+ from Mixed group" sawflies had a shorter lifespan than "C- from C-
324 group", "C- from Mixed group", and "C+ from C+ group" (Figure 5a, SI.6a,7). Additionally,
325 sex (χ^2 = 12.52, df = 1, P < 0.001) had a significant effect on lifespan with males having a
326 shorter lifespan than females. There was no significant effect of sex, composite treatment or
327 their interaction on body lipid content (SI.6b). Only sex had a significant effect on body
328 carbohydrate content (χ^2 = 13.09, df = 1, P < 0.001; SI.6c), with females having higher
329 carbohydrate content than males. We obtained qualitatively similar results for the effect of
330 direct and indirect clerodanoid acquisition on lifespan using female pairs (see SI.2). Together,
331 this suggests that social interactions, especially agonistic, to obtain clerodanoids may have
332 fitness costs in terms of lifespan.

333 ## *Discussion*

334 We observed variation in clerodanoid amounts in wild-caught *A. rosae* adults of both sexes
335 and from all locations. A capture release study had shown that most *A. rosae* adults could be
336 recaptured from within 100 m of their release, suggesting limited dispersal in this species
337 (Nagasaka 1992). Thus, our field populations were likely separated, indicating that
338 intraspecific variation in sequestered compounds may be common in natural populations of
339 herbivorous insects that are able to sequester plant metabolites. Moreover, we observed

340 intraspecific variation in clerodanoid amounts of lab-reared sawflies with both direct (via
341 plant leaves) and indirect (via nibbling on conspecifics) access to clerodanoids, similar to
342 previous studies (Singh *et al.* 2022). Such intraspecific variation in sequestered plant
343 chemicals in animals is widely observed (Opitz & Müller 2009; Speed *et al.* 2012). While this
344 variation may result from environmental stochasticity, e.g., variation in metabolite
345 concentrations across plants, it could also have an evolutionarily basis, e.g., some individuals
346 of an animal species may be chemically defended while others are not (Brower *et al.* 1970;
347 Speed *et al.* 2012). Our study shows that intraspecific variation in sequestered plant chemicals
348 can impact social networks and have fitness costs.

349 The presence and quantity of clerodanoids can be considered as important parts of the
350 chemical phenotype of individuals, which could play a key role in shaping the individualised
351 niche, as has been argued for plants (Müller & Junker 2022). While effects of food plants on
352 the phenotype and behaviour of herbivorous individuals are widely documented (Geiselhardt
353 *et al.* 2012; Jarrett & Miller 2024; Müller & Müller 2017; Singh *et al.* 2020), we showed here
354 that plant chemicals sequestered from plants that do not serve as food plants can also
355 modulate social interactions. Our results demonstrated that social networks comprising Mixed
356 groups exhibited the highest density and number of interacting dyads, followed by C+ groups.
357 The asymmetric access to clerodanoids in Mixed groups may lead to an increase in social
358 interactions, potentially due to C- trying to obtain clerodanoids from C+ individuals.
359 Moreover, C- individuals in Mixed groups could acquire clerodanoids from C+ individuals,
360 which may then increase their social interactions, as it will change their clerodanoid
361 acquisition status. Individuals in C- groups had the least density and number of interacting
362 dyads, suggesting that when all individuals lack clerodanoids, they have only few intra-and
363 inter-sex social interactions.

364 The social interactions of the individuals depended not only on their own clerodanoid
365 acquisition status but also on that of other conspecifics in the group. For example, C-
366 individuals had a higher number of social interactions in Mixed than in C- groups (see Figure
367 4a,4c), suggesting that even for the same clerodanoid-holding status, an individual may have
368 low or high frequencies of social interactions, depending on the group composition. Similar
369 effects of conspecifics on an individual's behaviour have been shown in an aquatic insect,
370 *Notonecta irrorata*, where the propensity to disperse depended on the behavioural type of
371 conspecifics present (Kitchen & Chalcraft 2020). Indeed, studies across various animal
372 species have shown that the behaviour of a focal individual can be shaped by both internal
373 factors, such as personality traits, and external factors, such as social context and interactions
374 with conspecifics (Webster & Ward 2011). Given the intraspecific variation in clerodanoid
375 content that we observed in wild-caught individuals of *A. rosae*, we expect social interactions
376 in natural populations to range from low, for example, if no *A. reptans* plants are around from
377 which clerodanoids can be sequestered, to high, when some insects could sequester
378 clerodanoids, leading to a skewed distribution of social interaction frequency.

379 Social interactions may accrue over time, and influence fitness of individuals through
380 different ways, such as injury or mortality resulting from agonistic interactions, variation in
381 mating frequency or disease progression (Alwash & Levine 2019; Dawson *et al.* 2018; Guo &
382 Dukas 2020; Stroeymeyt *et al.* 2018). We observed a reduced lifespan in C+ adults from
383 Mixed groups. In experiment 2, we saw that C+ females paired with C- males had the most
384 agonistic interactions. Similarly, in Mixed groups, we found more frequent interactions than
385 in C- or C+ groups. Thus, we can expect that C+ adults in Mixed groups had more social,
386 especially agonistic, interactions with their conspecifics that may have nibbled on the C+
387 individual to get access to clerodanoids. These increased agonistic interactions may have led
388 to a decreased lifespan for C+ adults in Mixed groups. Such costs of aggression have also

been shown in the fruit fly, *Drosophila melanogaster*, where flies that were engaged in aggressive behaviour had a reduced survival despite not showing any differences in wing damage from no-aggression treatment flies (Guo & Dukas 2020). This particular study hypothesised that such costs could arise from physiological and metabolic changes triggered by stress and aggressive conflicts. Indeed, a previous study (Paul *et al.* 2021) demonstrated transcriptional upregulation of metabolic pathway genes in *A. rosae* sawflies engaging in conspecific nibbling, indicating potential costs of aggressive interactions. However, we found no significant impact on body lipid and carbohydrate content in the present study.

Along with chemical defence against predation and increased mating success, sequestered plant metabolites may also lead to benefits for the offspring (Eisner *et al.* 2000; Eisner & Meinwald 1995; Gonzalez *et al.* 1999). However, at least under controlled laboratory conditions, no significant effects of parental clerodanoid status on offspring was found in *A. rosae* (Paul *et al.* 2022). Thus, while clerodanoid acquisition may be associated with benefits such as enhanced defence against predation (Singh *et al.* 2022) and mating success (Amano *et al.* 1999; Paul & Müller 2021), there may be a trade-off with costs in terms of a reduced lifespan due to agonistic interactions. This in turn may maintain intraspecific variation in clerodanoids in natural populations of *A. rosae*. Understanding the effects of variation in these sequestered plant metabolites is essential because these ecological effects can scale up from the individual level to factors that shape animal social interaction networks.

References

Alwash, N. & Levine, J.D. (2019). Network analyses reveal structure in insect social groups. *Current Opinion in Insect Science*, 35, 54–59.

Amano, T., Nishida, R., Kuwahara, Y. & Fukami, H. (1999). Pharmacophagous acquisition of clerodendrins by the turnip sawfly (*Athalia rosae ruficornis*) and their role in the mating behavior. *Chemoecology*, 9, 145–150.

Archer, J. (1988). *The behavioural biology of aggression*. Cambridge, UK: Cambridge University Press.

Barrat, A., Barthélemy, M., Pastor-Satorras, R. & Vespignani, A. (2004). The architecture of complex weighted networks. *Proceedings of the National Academy of Sciences*, 101, 3747–3752.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Beran, F. & Petschenka, G. (2022). Sequestration of plant defense compounds by insects: from mechanisms to insect–plant coevolution. *Annu. Rev. Entomol.*, 67, 163–180.

Boppré, M. (1984). Redefining "pharmacophagy." *Journal of Chemical Ecology*, 10, 1151–1154.

Briffa, M. & Sneddon, L.U. (2007). Physiological constraints on contest behaviour. *Functional Ecology*, 21, 627–637.

Brower, L.P., Pough, F.H. & Meck, H. (1970). Theoretical investigations of automimicry, I. Single trial learning. *Proceedings of the National Academy of Sciences*, 66, 1059–1066.

Brueggemann, L., Tewes, L.J. & Müller, C. (2023). Characterisation and localisation of plant metabolites involved in pharmacophagy in the turnip sawfly. *PLoS ONE*, 18, e0291180.

Candolin, U. & Wong, B.B. (2012). *Behavioural responses to a changing world: mechanisms and consequences*. Oxford University Press.

Conner, W.E., Boada, R., Schroeder, F.C., Gonzalez, A., Meinwald, J. & Eisner, T. (2000). Chemical defense: Bestowal of a nuptial alkaloidal garment by a male moth on its mate. *Proceedings of the National Academy of Sciences*, 97, 14406–14411.

Csárdi, G., Nepusz, T., Traag, V., Horvát, S., Zanini, F., Noom, D., *et al.* (2023). *igraph: Network Analysis and Visualization in R*.

Cuff, J.P., Wilder, S.M., Tercel, M.P.T.G., Hunt, R., Oluwaseun, S., Morley, P.S., *et al.* (2021). MEDI: Macronutrient extraction and determination from invertebrates, a rapid, cheap and streamlined protocol. *Methods in Ecology and Evolution*, 12, 593–601.

Dawson, E.H., Bailly, T.P.M., Dos Santos, J., Moreno, C., Devilliers, M., Maroni, B., *et al.* (2018). Social environment mediates cancer progression in *Drosophila*. *Nature Communications*, 9, 3574.

Dimarco, R.D. & Fordyce, J.A. (2017). Not all toxic butterflies are toxic: high intra- and interspecific variation in sequestration in subtropical swallowtails. *Ecosphere*, 8.

Eisner, T., Eisner, M., Rossini, C., Iyengar, V.K., Roach, B.L., Benedikt, E., *et al.* (2000). Chemical defense against predation in an insect egg. *Proceedings of the National Academy of Sciences*, 97, 1634–1639.

Eisner, T. & Meinwald, J. (1995). The chemistry of sexual selection. *Proceedings of the National Academy of Sciences*, 92, 50–55.

Fisher, D.N., Kilgour, R.J., Siracusa, E.R., Foote, J.R., Hobson, E.A., Montiglio, P., *et al.* (2021). Anticipated effects of abiotic environmental change on intraspecific social interactions. *Biological Reviews*, 96, 2661–2693.

Fisher, D.N., Rodríguez-Muñoz, R. & Tregenza, T. (2019). Dynamic networks of fighting and mating in a wild cricket population. *Animal Behaviour*, 155, 179–188.

Formica, V.A., Wood, C.W., Larsen, W.B., Butterfield, R.E., Augat, M.E., Hougen, H.Y., *et al.* (2012). Fitness consequences of social network position in a wild population of forked fungus beetles (*Bolitotherus cornutus*). *Journal of Evolutionary Biology*, 25, 130–137.

Frank, S.A. (2007). All of life is social. *Current Biology*, 17, R648–R650.

Friard, O. & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7, 1325–1330.

Gartland, L.A., Firth, J.A., Laskowski, K.L., Jeanson, R. & Ioannou, C.C. (2022). Sociability as a personality trait in animals: methods, causes and consequences. *Biological Reviews*, 97, 802–816.

Geiselhardt, S., Otte, T. & Hilker, M. (2012). Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. *Ecology Letters*, 15, 971–977.

Gonzalez, A., Rossini, C., Eisner, M. & Eisner, T. (1999). Sexually transmitted chemical defense in a moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences*, 96, 5570–5574.

Guo, X. & Dukas, R. (2020). The cost of aggression in an animal without weapons. *Ethology*, 126, 24–31.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50, 346–363.

Jarrett, B.J.M. & Miller, C.W. (2024). Host plant effects on sexual selection dynamics in phytophagous insects. *Annual Review of Entomology*, 69, annurev-ento-022823-020258.

Kaiser, M.I., Gadau, J., Kaiser, S., Müller, C. & Richter, S.H. (2024). Individualized social niches in animals: Theoretical clarifications and processes of niche change. *BioScience*, biad122.

Kitchen, A.J. & Chalcraft, D.R. (2020). An individual's propensity to disperse is dependent on the behavioral type of its peers but not its own behavioral type. *Oecologia*, 194, 403–413.

Kortbeek, R.W.J., Van Der Gragt, M. & Bleeker, P.M. (2019). Endogenous plant metabolites against insects. *European Journal of Plant Pathology*, 154, 67–90.

Koto, A., Tamura, M., Wong, P.S., Aburatani, S., Privman, E., Stoffel, C., *et al.* (2023). Social isolation shortens lifespan through oxidative stress in ants. *Nature Communications*, 14, 5493.

Krause, J., James, R., Franks, D.W. & Croft, D.P. (2015). *Animal social networks*. Oxford University Press, USA.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1–26.

Liu, Y., Heath, J.J., Zhang, S., Van Wijk, M., Wang, G., Buellesbach, J., *et al.* (2023). A mosaic of endogenous and plant-derived courtship signals in moths. *Current Biology*, 33, 3529-3535.e4.

Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E. & Getz, W.M. (2005). Superspreading and the effect of individual variation on disease emergence. *Nature*, 438, 355–359.

Marchiori, M. & Latora, V. (2000). Harmony in the small-world. *Physica A: Statistical Mechanics and its Applications*, 285, 539–546.

Massad, T.J., Fincher, R.M., Smilanich, A.M. & Dyer, L. (2011). A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants. *Arthropod-Plant Interactions*, 5, 125–139.

Mattila, A.L.K., Jiggins, C.D., Opedal, Ø.H., Montejo-Kovacevich, G., Pinheiro de castro, É.C., McMillan, W.O., *et al.* (2021). Evolutionary and ecological processes influencing chemical defense variation in an aposematic and mimetic *Heliconius* butterfly. *PeerJ*, 9, e11523.

Moore, B.D., Andrew, R.L., Külheim, C. & Foley, W.J. (2014). Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytologist*, 201, 733–750.

Mueller, T. & Mueller, C. (2017). Host plant effects on the behavioural phenotype of a Chrysomelid. *Ecological Entomology*, 42, 336–344.

Müller, C. & Junker, R.R. (2022). Chemical phenotype as important and dynamic niche dimension of plants. *New Phytologist*, 234, 1168–1174.

Nagasaka, K. (1992). Movement patterns of three *Athalia* sawflies in relation to the spatio-temporal distributions of their habitats. *Population Ecology*, 34, 1–14.

Nishida, R. (2014). Chemical ecology of insect–plant interactions: ecological significance of plant secondary metabolites. *Bioscience, Biotechnology, and Biochemistry*, 78, 1–13.

Nishida, R. & Fukami, H. (1990). Sequestration of distasteful compounds by some pharmacophagous insects. *Journal of Chemical Ecology*, 16, 151–164.

Ogle, D.H., Doll, J.C., Wheeler, A.P. & Dinno, A. (2023). *FSA: Simple Fisheries Stock Assessment Methods*.

Opitz, S.E.W. & Müller, C. (2009). Plant chemistry and insect sequestration. *Chemoecology*, 19, 117–154.

Paul, S.C., Dennis, A.B., Tewes, L.J., Friedrichs, J. & Müller, C. (2021). Consequences of pharmacophagous uptake from plants and conspecifics in a sawfly elucidated using chemical and molecular techniques (preprint). BioRxiv. *https://doi.org/10.1101/2021.02.09.430406*.

Paul, S.C. & Müller, C. (2021). Fighting over defense chemicals disrupts mating behavior. *Behavioral Ecology*, arab117.

Paul, S.C., Singh, P., Dennis, A.B. & Müller, C. (2022). Intergenerational effects of early life starvation on life-history, consumption, and transcriptome of a holometabolous insect. *The American Naturalist*, 719397.

R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Royle, N.J., Pike, T.W., Heeb, P., Richner, H. & Kölliker, M. (2012). Offspring social network structure predicts fitness in families. *Proceedings of the Royal Society*, 279, 4914–4922.

Ruan, H. & Wu, C.-F. (2008). Social interaction-mediated lifespan extension of *Drosophila* Cu/Zn superoxide dismutase mutants. *Proceedings of the National Academy of Sciences*, 105, 7506–7510.

Silk, J.B., Alberts, S.C. & Altmann, J. (2003). Social bonds of female baboons enhance infant survival. *Science*, 302, 1231–1234.

Singh, P., van Bergen, E., Brattström, O., Osbaldeston, D., Brakefield, P.M. & Oostra, V. (2020). Complex multi-trait responses to multivariate environmental cues in a seasonal butterfly. *Evolutionary Ecology*, 34, 713–734.

Singh, P., Grone, N., Tewes, L.J. & Müller, C. (2022). Chemical defense acquired via pharmacophagy can lead to protection from predation for conspecifics in a sawfly. *Proceedings of the Royal Society*, 289, 20220176.

Singh, P., Wolthaus, J., Schielzeth, H. & Müller, C. (2023). State dependency of behavioural traits is a function of the life stage in a holometabolous insect. *Animal Behaviour*, 203, 29–39.

Smilanich, A.M., Dyer, L.A., Chambers, J.Q. & Bowers, M.D. (2009). Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecology Letters*, 12, 612–621.

Snyder-Mackler, N., Burger, J.R., Gaydosh, L., Belsky, D.W., Noppert, G.A., Campos, F.A., *et al.* (2020). Social determinants of health and survival in humans and other animals. *Science*, 368, eaax9553.

Speed, M.P., Ruxton, G.D., Mappes, J. & Sherratt, T.N. (2012). Why are defensive toxins so variable? An evolutionary perspective. *Biological Reviews*, 87, 874–884.

Stroeymeyt, N., Grasse, A.V., Crespi, A., Mersch, D.P., Cremer, S. & Keller, L. (2018). Social network plasticity decreases disease transmission in a eusocial insect. *Science*, 362, 941–945.

Tea, Y., Soong, J.W., Beaver, E.P. & Lohman, D.J. (2021). Kleptopharmacophagy: Milkweed butterflies scratch and imbibe from *Apocynaceae*-feeding caterpillars. *Ecology*.

Torres-Vila, L.M. & Jennions, M.D. (2005). Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners? *Behavioral Ecology and Sociobiology*, 57, 318–326.

Trappes, R., Nematipour, B., Kaiser, M.I., Krohs, U., Van Benthem, K.J., Ernst, U.R., *et al.* (2022). How individualized niches arise: defining mechanisms of niche construction, niche choice, and niche conformance. *BioScience*, 72, 538–548.

Udiani, O. & Fefferman, N.H. (2020). How disease constrains the evolution of social systems. *Proceedings of the Royal Society*, 287, 20201284.

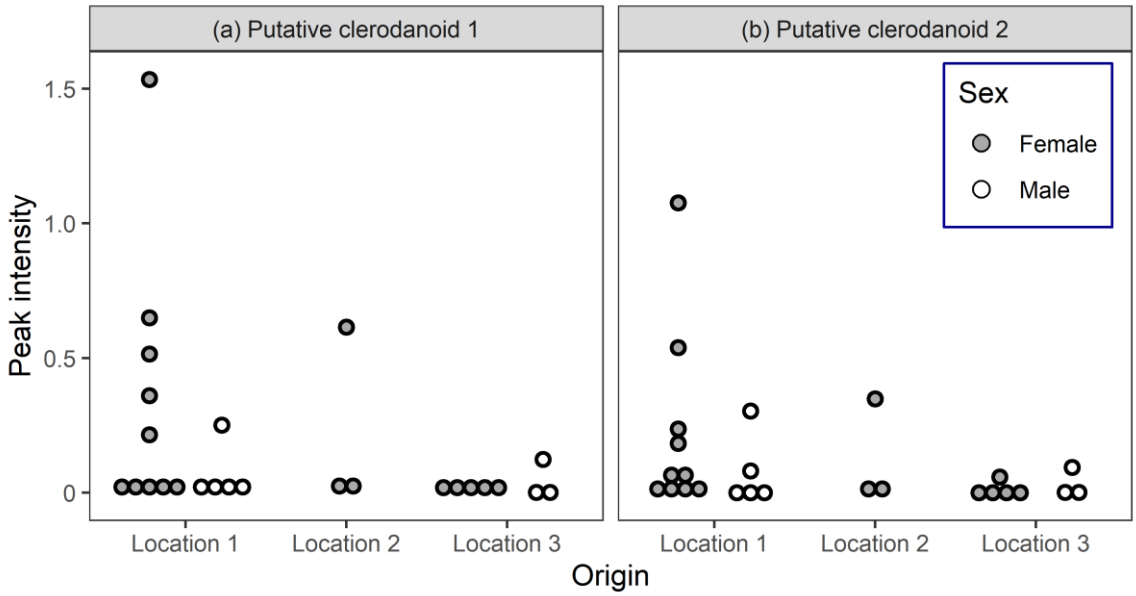
Webster, M.M. & Ward, A.J.W. (2011). Personality and social context. *Biological Reviews*, 86, 759–773.

Wey, T., Blumstein, D.T., Shen, W. & Jordán, F. (2008). Social network analysis of animal behaviour: a promising tool for the study of sociality. *Animal Behaviour*, 75, 333–344.

Zanchi, C., Lo, L.K., R, R., Moritz, I., Kurtz, J. & Müller, C. (2021). Survival of the sawfly *Athalia rosae* upon infection by an entomopathogenic fungus and in relation to clerodanoid uptake. *Frontiers in Physiology*, 12, 637617.

Zvereva, E.L. & Kozlov, M.V. (2015). The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. *Ecological Monographs*, 15-0911.1.

591 ***Figures***



592

593 Figure 1. Intraspecific variation in peak intensities of putative (a) clerodanoid 1 and (b)

594 clerodanoid 2 per individual in wild-caught female (n=18) and male (n=8) *Athalia rosae*

595 sawflies from three locations.

596

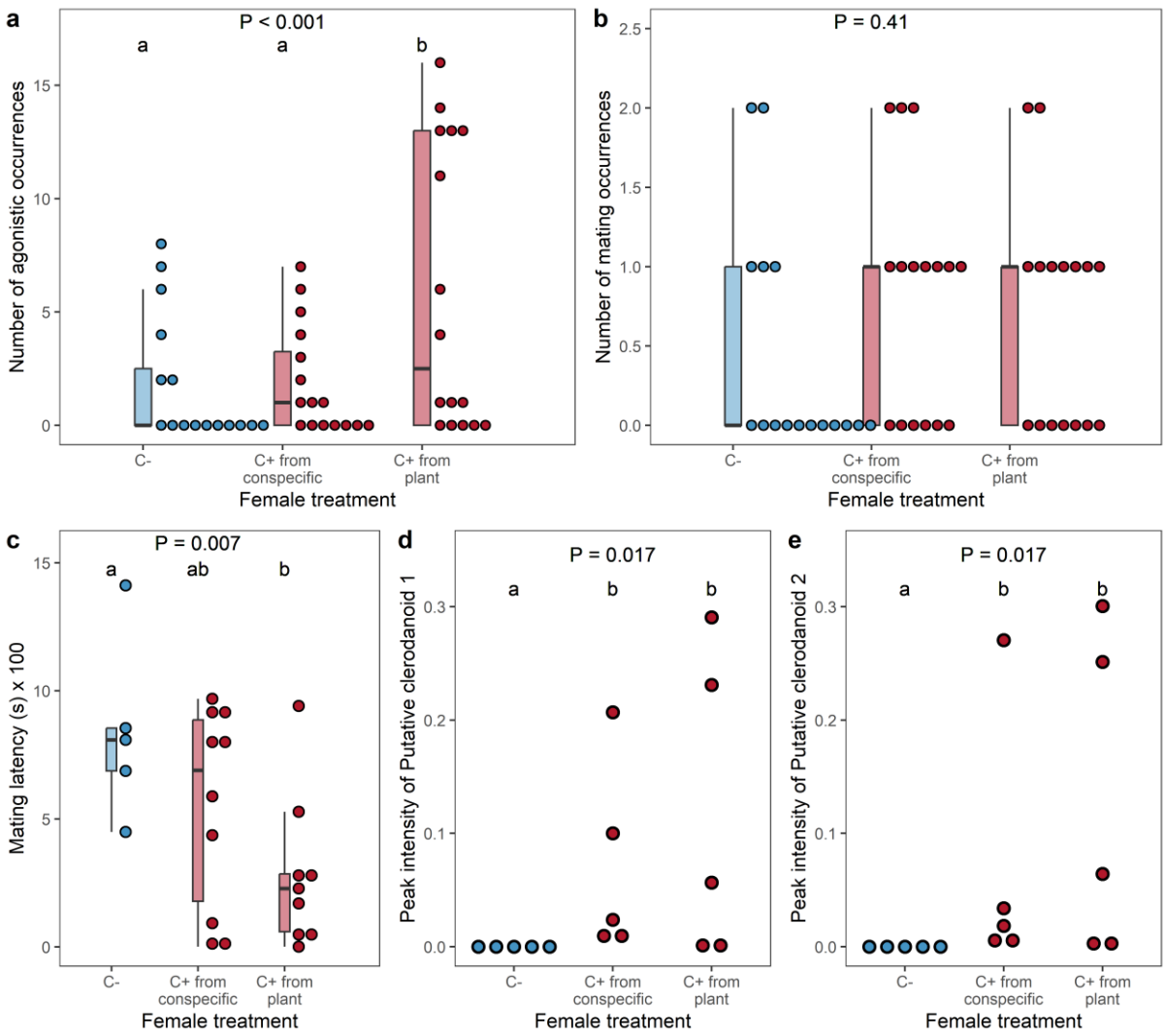


Figure 2. Effect of female treatment on the number of occurrences of (a) agonistic and (b) mating interactions, as well as (c) mating latency with C- males of *Athalia rosae*. Additionally, the effect of female treatment on the peak intensity of putative (d) clerodanoid 1 and (e) clerodanoid 2 in lab-reared female sawflies is shown. Treatments include no access (C-), indirect access (C+ from conspecific: prior contact with other females that had access to *Ajuga reptans* leaf), or direct access (C+ from plant: prior access to *A. reptans* leaf) to clerodanoids. Boxplots display the median, 25th, and 75th percentiles, with whiskers indicating values within 1.5 times the interquartile range. Coloured circles represent individual data points. *P*-values are derived from GLMM (a,b), LMM (c), or Kruskal-Wallis

607 (d,e) tests. Different lowercase letters indicate significant differences from post-hoc tests ($P <$

608 0.05).

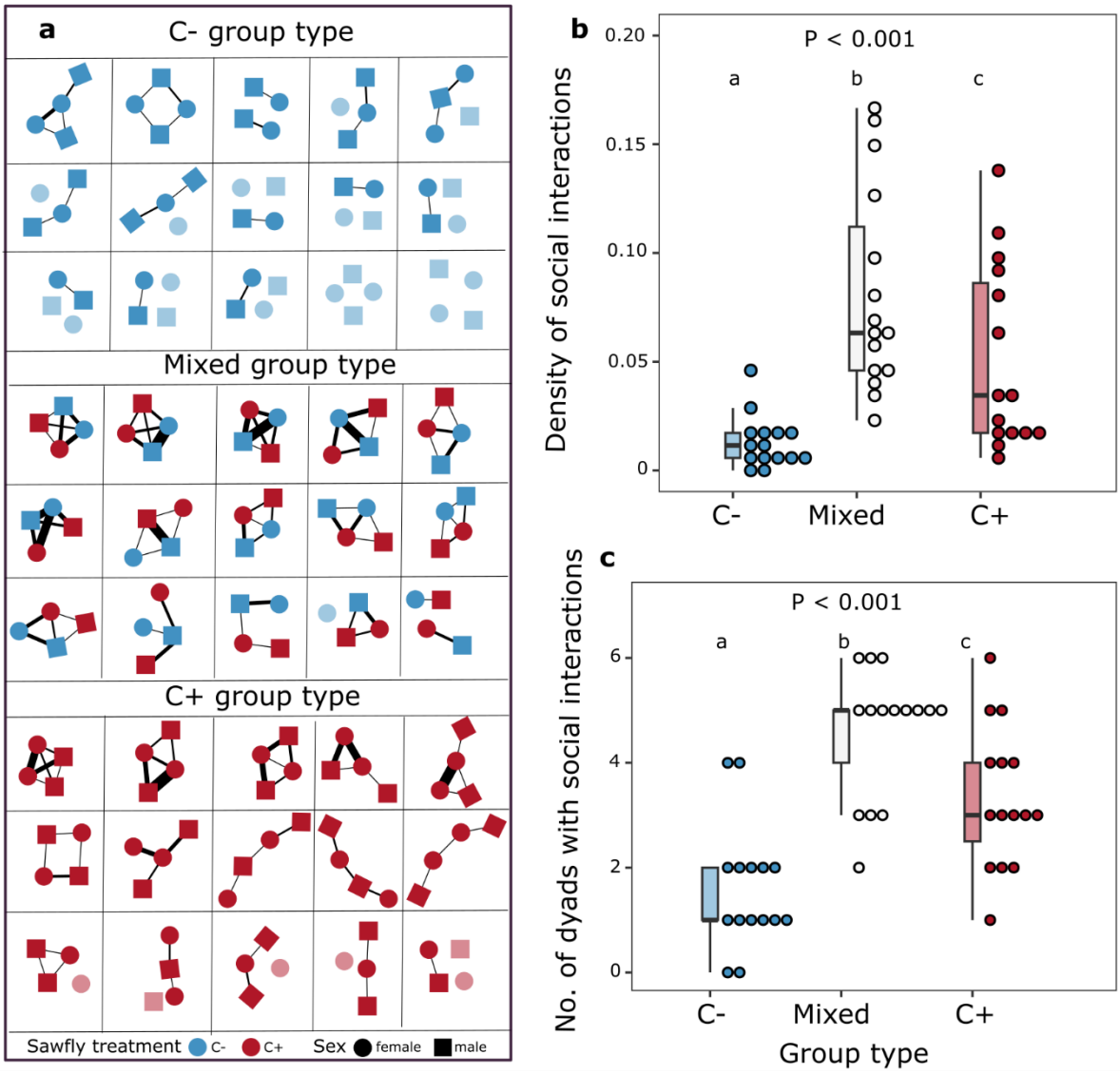
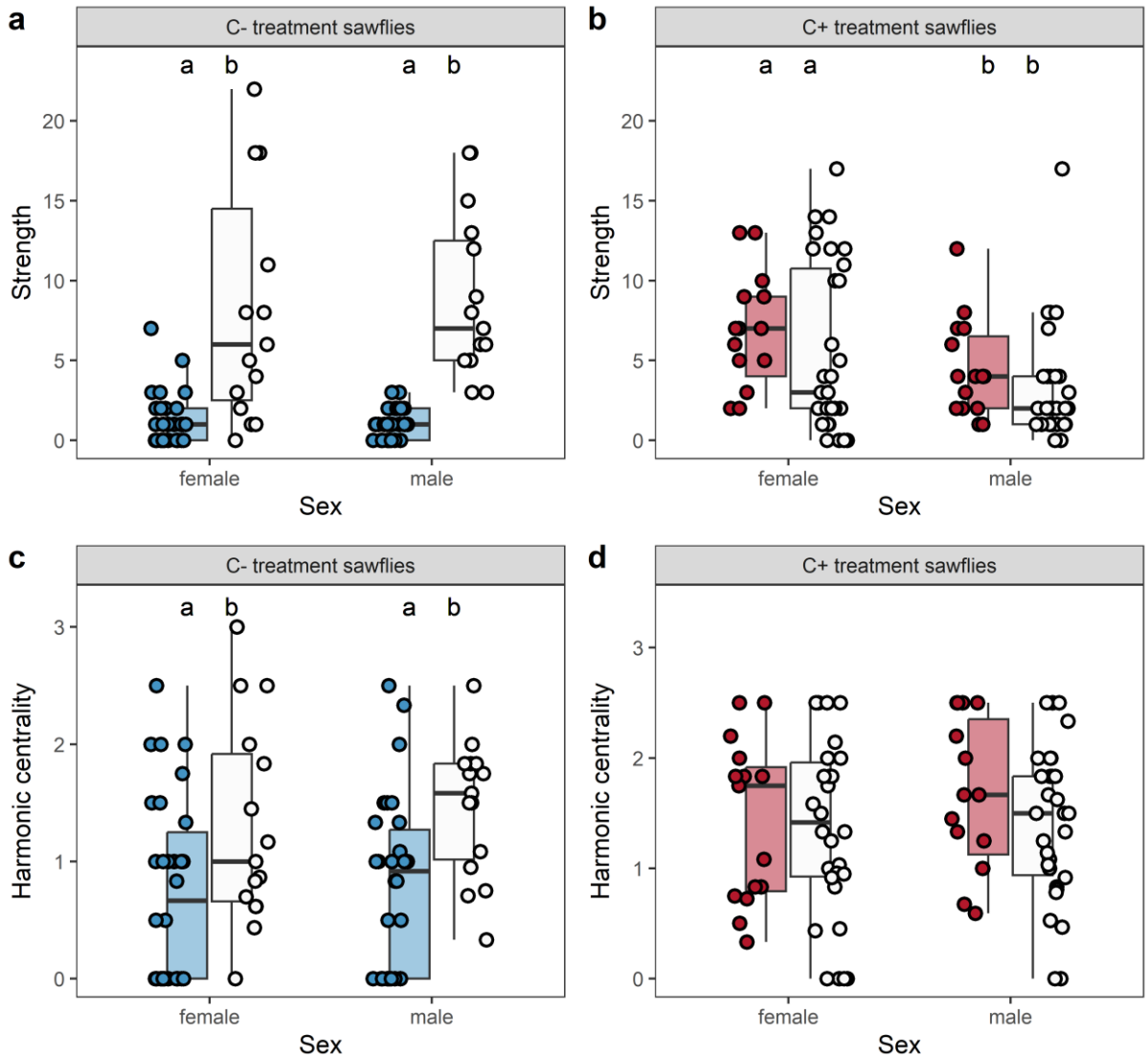


Figure 3. (a) Network representations of C-, Mixed and C+ groups (n=15 per group type). Each cell in the grid corresponds to one replicate and consisted of two female (circular nodes) and two male (square nodes) adults of *Athalia rosae*. Sawflies had either no access (C-, red nodes) or prior access to *Ajuga reptans* (C+, blue nodes). Social interactions between dyads are depicted by lines, with line thickness indicating interaction frequency. Lighter nodes indicate individuals with no social interactions during the observation period. Effect of group type on (b) density of social interactions and (c) number of dyads with social interactions. Boxplots display the median, 25th, and 75th percentiles, with whiskers indicating values within 1.5 times the interquartile range. Coloured circles represent individual data points. *P*-

619 values are derived from LMM (b) or GLMM (c) models. Different lowercase letters denote

620 significant differences from post-hoc tests ($P < 0.05$).

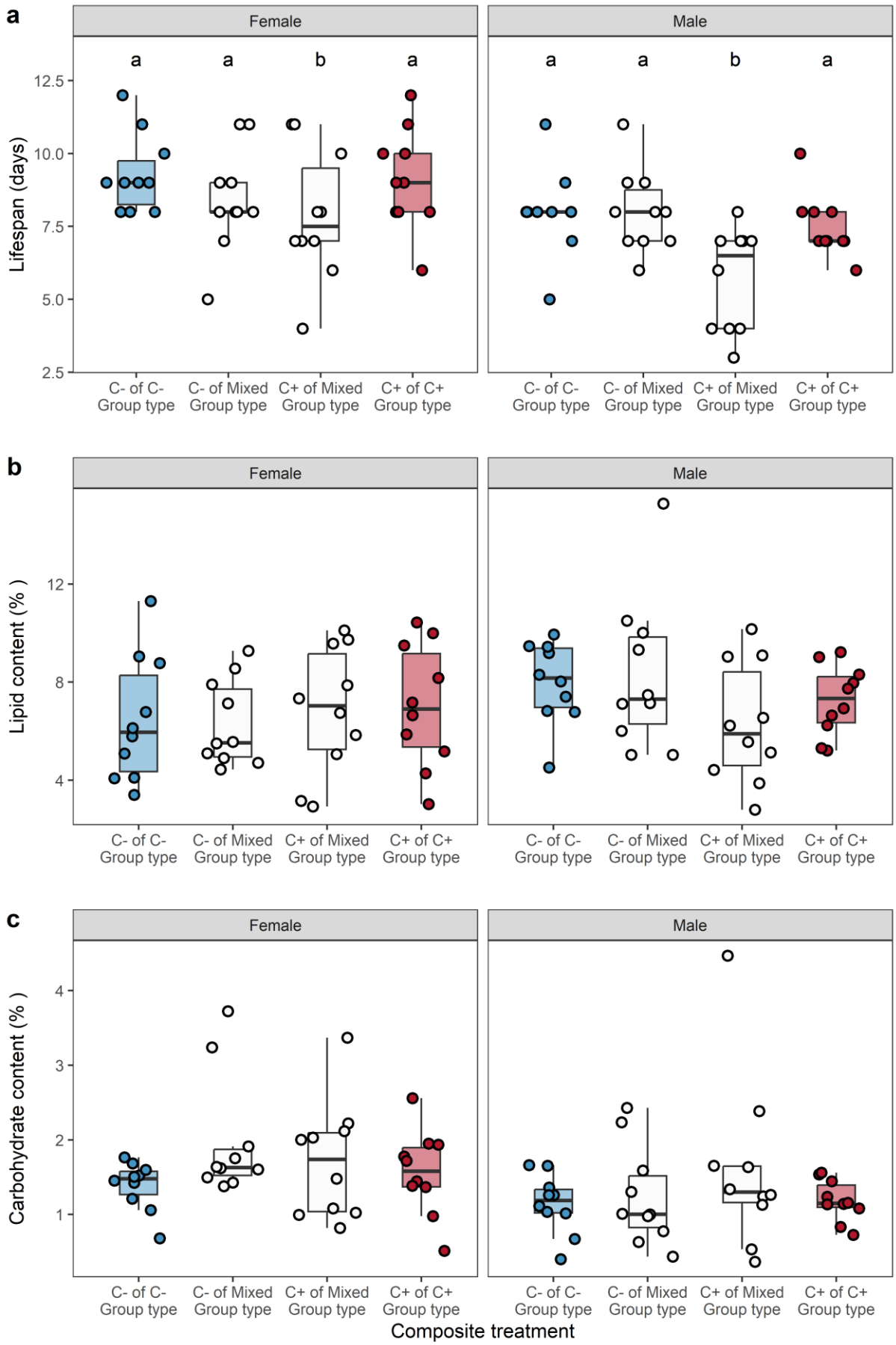
621



622

623 Figure 4. Effect of group type (C-, Mixed, or C+ groups, distinguished by different colours)
624 on strength (top row) and harmonic centrality (bottom row) in C- treatment (a, c) and C+
625 treatment (b, d) *Athalia rosae* sawflies of both sexes. Boxplots depict the median, 25th, and
626 75th percentiles, with whiskers indicating values within 1.5 times the interquartile range.
627 Coloured circles denote individual data points. Different lowercase letters indicate significant
628 differences from post-hoc tests ($P < 0.05$).

629


a
Female
Male
a
a
b
a
a
a
b
a
Lifespan (days)
12.5
10.0
7.5
5.0
2.5
C- of C- Group type
C- of Mixed Group type
C+ of Mixed Group type
C+ of C+ Group type
b
Lipid content (%)
12
8
4
c
Carbohydrate content (%)
4
3
2
1
Composite treatment

630

631 Figure 5. Effect of composite treatment on (a) lifespan, (b) carbohydrate content (%), and (c)
632 lipid content (%) in female and male *Athalia rosae* sawflies. Sawflies were assigned to either
633 C- or C+ treatment groups (C-: no access, C+: prior access to *Ajuga reptans* leaf) and grouped
634 as C-, Mixed, or C+ groups. Boxplots display the median, 25th and 75th percentiles, with
635 whiskers indicating values within 1.5 times the interquartile range. Coloured circles denote
636 individual data points. Different lowercase letters represent significant differences from post-
637 hoc tests ($P < 0.05$).