Plant metabolites modulate animal social networks and lifespan

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Author Contributions

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

Abstract

Social interactions are prevalent in animals, with some individuals participating in more interactions than others. However, the mechanisms underlying this heterogeneity in social interaction frequency and its potential fitness consequences remain poorly characterized. Here, we examine the effects of non-nutritive plant metabolites ('clerodanoids') on social networks and lifespan in the turnip sawfly. The adult sawflies facultatively acquire clerodanoids from non-host plant species. We found high variation in clerodanoid amounts in both wild-caught and lab-reared individuals. Furthermore, sawflies could acquire clerodanoids by nibbling on conspecifics that had prior access to these metabolites, with asymmetric clerodanoid access leading to increased agonistic interactions. Network analysis revealed more social interactions in sawfly groups where some or all individuals had prior access to clerodanoids, while groups with no prior access had fewer interactions. The number of social interactions depended on the clerodanoid status of the focal individual and also of other conspecifics. Finally, we observed a shorter lifespan in adults with prior clerodanoid access when paired with individuals without prior access, suggesting that social interactions to obtain clerodanoids have fitness costs. Our findings demonstrate that individual variation in non-nutritional sequestered plant metabolites can skew social interactions, with potential fitness implications that impact individual social niches.

Keywords

Athalia rosae, mating behaviour, aggressive behaviour, pharmacophagy, specialized plant metabolites, non-nutritional plant-insect interactions

Introduction

Social interactions are widespread in animals (Frank 2007) and lead to the emergence of diverse social networks in groups, from nominally to highly interactive. Such social interactions can range from mating to agonistic. A common aspect of animal social networks is skewness in the number of interactions individuals have, with some individuals having many interactions while others have few (Fisher *et al.* 2019; Krause *et al.* 2015). 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 (Guo & Dukas 2020). These 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 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 own defence (Nishida 2014; Opitz & Müller 2009). This phenomenon is called pharmacophagy (Boppré 1984). Such uptake of non-nutritive plant metabolites can have direct effects on the behaviour of the individuals (Amano *et al.* 1999; Conner *et al.* 2000; Gonzalez *et al.* 1999). Remarkably, such plant metabolites, or further metabolised compounds, can also be acquired from other animals, either conspecifics (Paul *et al.* 2021; Singh *et al.* 2022) or heterospecifics (Tea *et al.* 2021). This can impact social interactions, which in turn can influence the fitness of individuals (Formica *et al.* 2012; Royle *et al.* 2012; Silk *et al.* 2003) and thus the individualised social niche (Kaiser et al. under revision).

Moreover, taking up plant metabolites may come with some costs, e.g. if the metabolites are toxic (Kortbeek *et al.* 2019).

Interestingly, many species show intraspecific variation in the presence and quantity of sequestered plant metabolites (Liu *et al.* 2023; Mattila *et al.* 2021; Opitz & Müller 2009; Speed *et al.* 2012). Such variation can result from variation in the plant metabolite concentration per se, potential costs related to metabolite acquisition by the animal or factors such as age, sex or immunological status of the individuals (Dimarco & Fordyce 2017; Massad *et al.* 2011; Moore *et al.* 2014; Smilanich *et al.* 2009; Zvereva & Kozlov 2015). This leads us to the question of whether such intraspecific variation in sequestered plant metabolites can shape social interactions in animals and whether the sequestered plant metabolites affect fitness proxies such as lifespan of individuals, either directly or via altered social interactions. In cases where plant metabolites are passed between individuals, we can expect them to alter the social interactions of both the focal and the recipient individual. However, despite the documented effect of sequestered plant metabolites on individual behaviour (Boppré 1984; Nishida 2014), we still know relatively little about how these effects scale up to impact social interactions.

To target these open questions, we examined the effect of intraspecific variation in sequestered plant metabolites on social interactions and survivorship in the turnip sawfly, *Athalia rosae* (Hymenoptera: Tenthredinidae). The adults of *A. rosae* feed on floral nectar, usually from Apiaceae plants. In addition, they visit non-host plant species such as *Ajuga reptans* (Lamiaceae), on which they exhibit pharmacophagy by taking up specialised metabolites, neo-clerodane diterpenoids (hereafter called 'clerodanoids'), which they further metabolize slightly (Brueggemann *et al.* 2023). These clerodanoids can serve as a resource for *A. rosae* adults in multiple ways (Paul *et al.* 2022). Access to clerodanoids increases their mating success (Amano *et al.* 1999) and also provides chemical defence against predators

93 (Nishida & Fukami 1990; Singh *et al.* 2022). Moreover, individuals can obtain clerodanoids
94 via agonistic interactions like nibbling on conspecifics that have had access to plant
95 clerodanoids (Paul & Müller 2021; Singh *et al.* 2022). Such agonistic interactions can
96 potentially be costly, as suggested by the transcriptional upregulation of metabolic pathways
97 in sawflies that nibbled on conspecifics (Paul *et al.* 2021). Moreover, the uptake of
98 clerodanoids may also directly pose fitness costs, such as a reduced lifespan (Zanchi *et al.*
99 2021). However, laboratory cultures of *A. rosae* are usually maintained without access to
100 clerodanoids, indicating that clerodanoids are not essential for individual survival.

101 In the present study, we first examined the clerodanoid contents of wild-caught sawflies to
102 investigate whether there is intraspecific variation in amounts of these metabolites in natural
103 *A. rosae* populations. Next, we tested the effect of no access, indirect access to clerodanoids
104 via conspecifics and direct access to clerodanoids via *A. reptans* leaves on behavioural
105 interactions in laboratory-reared individuals and analysed their clerodanoid contents. As we
106 observed intraspecific variation in clerodanoid content in both wild-caught and laboratory-
107 reared individuals, we, next, tested how such variation may impact social interactions.
108 Therefore, we set up a gradient of treatment groups with intraspecific variation in
109 clerodanoids, such that in a group either none, half or all individuals had access to
110 clerodanoids. We used network analysis to construct social interaction networks and calculate
111 individual and group measures of the network (Wey *et al.* 2008), allowing us to estimate the
112 effects of intraspecific variation in clerodanoids. Finally, we examined potential fitness costs
113 of clerodanoid acquisition and the resulting social interactions on lifespan, by setting up pairs
114 of individuals, in which either none, one or both individuals had prior access to clerodanoids.

115 We expected intraspecific variation in clerodanoids in wild-caught individuals. Furthermore,
116 we hypothesised that groups with no clerodanoid access should have the least social
117 interactions, while asymmetric clerodanoid acquisition should lead to increased social

interactions. We expected individuals without clerodanoid access to show differences in their social interaction frequency depending on the clerodanoid acquisition status of other conspecifics. Finally, we expected agonistic interactions to obtain clerodanoids from conspecifics to be costly and to lead to a reduced lifespan.

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, lyophilized, homogenized 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 millipore 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.

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

167 We investigated the effects of no clerodanoid access (C-), indirect clerodanoid access from a
168 conspecific (C+ from conspecific), or direct clerodanoid access from a plant (C+ from plant)
169 by females on social interactions with C- males. Note that 'C+ from conspecific' has been
170 previously referred to as AC+ (Paul *et al.* 2021; Singh *et al.* 2022). For the 'C+ from plant'
171 treatment, freshly eclosed females were provided individually an *A. reptans* leaf disc (~1 cm^2)
172 for 48 h, while 'C+ from conspecific' treatment females were kept with a C+ conspecific (2-4
173 days old female that had previously contact to *A. reptans*) for 48 h. Males (6- 10 days post-
174 eclosion) were kept individually in Petri dishes for 48 h prior to the assays. The males were
175 mated to a non-focal C- female (~3 days old) 24 h prior to the experiment to avoid any
176 differences arising due to first mating (Torres-Vila & Jennions 2005). For the behavioural
177 assays, one female was set up with one male in a clean Petri dish (5.5 cm diameter) and
178 recorded (Sony HDR-CX410VE camcorder, AVCHD – 1920 × 1080 − 25 fps) for 25 minutes
179 (16 replicates per treatment combination). One to two replicates of each treatment were setup
180 simultaneously in a trial. An observer scored the number of occurrences of agonistic
181 behaviour, including frontlimb battling, fighting and nibbling events (see Paul & Müller, 2021
182 for detailed behavioural descriptions), and mating behaviour (number of matings, latency
183 until first mating) in each movie replicate using the software BORIS v7.13.9 (Friard &
184 Gamba 2016), being blind to the treatment. After the behavioural assays, five females from
185 each treatment were chosen randomly, frozen and analysed for their clerodanoid content as in
186 experiment 1.

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

188 We found intraspecific variation in clerodanoid content in both wild-caught and lab-reared
189 sawflies (see Results). To test the effect of clerodanoid acquisition status on social networks,
190 we set up three group types of two males and two females each, in which either no individual

191 had prior access to clerodanoids (C- group), only one female and one male had prior access to
192 clerodanoids (Mixed group) or all individuals had prior access to clerodanoids from
193 *A. reptans* leaves (C+ group). We set up 15 replicates per group type. Two hours before the
194 behavioural assay, each individual was colour-marked (green, red, white, or yellow; Uni
195 POSCA marker pens, Japan) on its thorax and wings to enable identification during behaviour
196 scoring. The colours were alternated between sexes and treatments across replicates to avoid
197 any colour effects. In each trial, one to two replicates per group type were set up
198 simultaneously in Petri dishes and recorded for 25 minutes. Agonistic and mating interactions
199 of each individual in a movie replicate were scored by an observer as in experiment 2.

200 For the social network analysis, we analysed group as well as individual measures of the
201 network. Therefore, we constructed an undirected weighted network for the social interactions
202 (sum of agonistic and mating interactions) in each replicate. We calculated the network
203 weights as the total number of interactions between each pair of individuals (dyad). We
204 normalised the weights at the replicate level and at the dyadic level in each replicate, by
205 dividing by the maximum number of interactions across all replicates and all dyads,
206 respectively. At the group level, we calculated the density of social interactions in each
207 replicate, as following:

208 $$Density = \frac{sum\ of\ weights}{[number\ of\ vertices * (number\ of\ vertices - 1) * 0.5},$$

209 where vertices is the number of individuals in a replicate. Moreover, at the group level, we
210 counted the number of distinct dyads that exhibited social interactions in each replicate. At
211 the individual level, we calculated the strength (Barrat *et al.* 2004) and harmonic centrality
212 (Marchiori & Latora 2000). The strength is a measure of how strongly an individual is
213 directly connected to other individuals in the network. Harmonic centrality calculates the

214 'average' distance of an individual to the other individuals in the network. We used harmonic
215 centrality as we had disconnected networks in some replicates.

216 ***Experiment 4. Costs of direct and indirect clerodanoid acquisition on lifespan***

217 To examine potential fitness costs of direct and indirect clerodanoid acquisition, we measured
218 the lifespan of differently treated females. We collected freshly eclosed *A. rosae* females and
219 assigned them to either C- or C+, offering the latter *A. reptans* leaves for 48 hours. Next, we
220 put together either two C- individuals (C-C- pair), a C- and a C+ individual (C-C+ pair) or
221 two C+ individuals (C+C+ pair). The C- individual from the C-C+ pair could acquire
222 clerodanoids from the C+ conspecific. Individuals in a replicate were marked with green or
223 white colour paint to allow for differentiation. After 48 hours, we randomly chose one
224 individual from each replicate of the C-C- pair and the C+C+ pair, and both individuals from
225 the C-C+ pair. Each individual was placed in a Petri dish and checked daily for survivorship.

226 ***Statistical analyses***

227 All analyses were done in R version 4.2.1 (R Core Team 2022). To examine the effect of
228 female treatment on the number of agonistic and mating interaction occurrences in experiment
229 2, we used a poisson-distributed generalised linear mixed model (GLMM) with trial as
230 random effect (package 'lme4' version 1.1-30, package 'lmerTest' version 3.1-3; Bates *et al.*
231 2015; Kuznetsova *et al.* 2017). We examined the effect of female treatment on mating latency
232 using a linear mixed model (LMM) with trial as random effect. We tested for differences
233 between C-, 'C+ from conspecific' and 'C+ from plant' in clerodanoid compounds 1 and 2
234 using a Kruskal-Wallis test followed by Dunn test with Holm correction for post-hoc tests
235 ('FSA' package version 0.9.4, Ogle *et al.* 2023). In experiment 3, we ran LMM for the
236 density and binomial GLMM for number of dyads with social interactions, with group type as
237 fixed effect and trial as random effect. Next, we analysed whether the group type had an

238 effect on the individual-level network measures of strength and harmonic centrality
239 (calculated using 'igraph' package version 1.5.1, Csárdi *et al.* 2023) for C- and C+ sawflies
240 separately. We performed poisson-distributed GLMM for strength and LMM for harmonic
241 centrality. The models included group type, sex and their interaction as fixed effects and
242 replicate ID and trial as random effect. We conducted post hoc analyses with Holm correction
243 using the package 'multcomp' version 1.4-20 (Hothorn *et al.* 2008) for LMM and GLMM. If
244 the interaction terms were non-significant, they were dropped to test the significance of the
245 lower-order terms. The distribution of model residuals was checked. In experiment 4, to
246 analyse differences in lifespan of C- (from C-C- pair), C- and C+ individual (from C-C+ pair)
247 and C+ (from C+C+ pair), we produced Kaplan-Meier plots and compared the curves by the
248 log-rank test using 'survival' package version 3.5-0 (Therneau 2023) and 'survminer' package
249 version 0.4.9 (Kassambara *et al.* 2021). Similarly, we tested for differences in lifespan
250 between C+ individuals from C+C+ pairs and C+ individuals from C-C+ pairs.

251

252 *Results*

253 *Clerodanoid amounts vary in wild-caught female and male sawflies*

254 Both females and males showed intraspecific variation in the amount of putative clerodanoid
255 1 and clerodanoid 2 across the three locations from which they were collected (Figure 1).
256 Some individuals had no clerodanoids, while others had relatively high quantities, suggesting
257 that natural populations show variation in clerodanoid acquisition.

258 ***Clerodanoids from plants impact behaviour and clerodanoid amounts varies in lab-reared***
259 ***sawflies***

260 There was a significant effect of female treatment on number of agonistic interaction
261 occurrences (χ_2^2 = 48.56, P < 0.001, Figure 2a), but not on mating occurrences (χ_2^2 = 1.76, P
262 = 0.41, Figure 2b). C- and 'C+ from conspecific' treatment females had significantly fewer
263 agonistic interactions than 'C+ from plant' treatment females (SI.2a). There was a significant
264 effect of female treatment on mating latency (χ_2^2= 9.90, P = 0.007, Figure 2c) with C- females
265 having a significantly longer latency until mating than 'C+ from plant' females, while 'C+
266 from conspecific' females showed an intermediate mating latency (SI.2b).

267 There was a significant difference between C-, 'C+ from conspecific' and 'C+ from plant'
268 females in the amount of clerodanoid 1 (χ_2^2 = 8.11, P = 0.017, Figure 2d) and clerodanoid 2
269 (χ_2^2 = 8.10, P = 0.017, Figure 2e). No clerodanoids were detected in C- females, while there
270 was inter-individual variation in clerodanoids in both 'C+ from conspecific' and 'C+ from
271 plant' females, which did not differ significantly from each other (SI.2c, SI.2d).

272 ***Intraspecific variation in clerodanoids modulates social networks***

273 The group types varied in their social networks, with the C- group having fewer interactions
274 than the Mixed and the C+ group (Figure 3a). Our results showed that there was a significant
275 effect of group type on density (χ_2^2 = 27.98, P < 0.001, Figure 3b) and number of dyads with
276 social interactions (χ_2^2 = 41.29, P < 0.001, Figure 3c). Mixed groups had a significantly
277 higher density and more dyads that had interacted than C- and C+ groups (SI.3a, SI.3b).
278 Together this suggests that access to clerodanoids increases social interactions with more
279 individuals networking, particularly in the Mixed group.

280 For C- sawflies, there was a significant effect of group type (χ_1^2 = 63.55, P < 0.001) on
281 strength (Figure 4a) such that C- sawflies in Mixed groups had a higher strength, i.e., had

more social interactions, than in C- groups (SI.4a). In contrast, there was a significant effect of sex ($\chi_1^2 = 25.14$, $P < 0.001$) on strength of interactions (Figure 4b) for C+ sawflies, with males having a lower strength than females across Mixed and C+ groups (SI.4a). For harmonic centrality, there was a significant effect of group type ($\chi_1^2 = 6.88$, $P = 0.008$) in C- sawflies (Figure 4c), such that those in Mixed groups had higher harmonic centrality values. There was no significant effect of any predictor variable on harmonic centrality in C+ sawflies (Figure 4d, SI.4c).

C+ individuals from C-C+ pairs have a shorter lifespan

There was no significant difference between the lifespan of C- females from C-C- pairs, C- females from C-C+ pairs and C+ females from C+C+ pairs ($\chi_2^2 = 2.3$, $P = 0.3$, Figure 5). However, C+ adults from C-C+ pairs had a significantly shorter lifespan than C+ adults from C+C+ pairs ($\chi_1^2 = 5.2$, $P = 0.02$), indicating that agonistic interactions to obtain clerodanoids may have fitness costs.

Discussion

We observed variation in clerodanoid amounts in wild-caught *A. rosae* adults of both sexes and from all locations. A capture release study had shown that most of the *A. rosae* adults could be recaptured from within 100 m of their release, suggesting limited dispersal (Nagasaka 1992). Thus, our field populations were likely separated, indicating that intraspecific variation in sequestered compounds may be common in natural populations of sequestering herbivorous insects. Moreover, we observed intraspecific variation in clerodanoid amounts of lab-reared sawflies with both indirect (via nibbling on conspecifics) and direct (via plant leaves) access to clerodanoids, similar to previous studies (Singh *et al.* 2022). Such intraspecific variation in sequestered plant chemicals in animals is widely

306 observed (Opitz & Müller 2009; Speed *et al.* 2012). While this variation may result from
307 environmental stochasticity, e.g., variation in metabolite concentrations across plants, it could
308 also have an evolutionarily basis, e.g., some individuals of an animal species may be
309 chemically defended while others are not (Brower *et al.* 1970; Speed *et al.* 2012). This can be
310 seen as automimicry in which undefended individuals (mimics) profit from the unpalatability
311 of defended individuals (models). Studies have shown that clerodanoids can serve as a
312 chemical defence against predators, like mantids (Singh *et al.* 2022) and tree sparrows
313 (Nishida & Fukami 1990), indicating that intraspecific variation in chemical defence within
314 wild populations can lead to automimicry.

315 The presence and quantity of clerodanoids can be considered as important parts of the
316 chemical phenotype of individuals, which could play a key role in shaping the individualised
317 niche, as has been argued for plants (Müller & Junker 2022). While effects of host plants on
318 an individual's phenotype and behaviour are widely documented (Geiselhardt *et al.* 2012;
319 Jarrett & Miller 2024; Müller & Müller 2017; Singh *et al.* 2020), we demonstrate here that
320 plant chemicals sequestered from non-host plants can also modulate social interactions. Our
321 results demonstrated the highest density and number of interacting dyads in social networks
322 of Mixed groups, followed by C+ groups. The asymmetric access to clerodanoids in Mixed
323 groups may lead to an increase in social interactions, potentially due to C- trying to obtain
324 clerodanoids from C+ individuals. Moreover, C- individuals in Mixed groups could acquire
325 clerodanoids from C+ individuals, which may then increase their social interactions, as it will
326 change their clerodanoid acquisition status. Individuals in C- groups had the least density and
327 number of interacting dyads, suggesting that when all individuals lack clerodanoids, they
328 have only few intra-and inter-sex social interactions.

329 Remarkably, the social interactions of the individuals depended not only on their own
330 clerodanoid acquisition status but also on that of other conspecifics in the group. For example,

331 C- individuals had a higher number of social interactions in Mixed than in C- groups (see
332 Figure 4a,4c), suggesting that even for the same clerodanoid-holding status, an individual
333 may have low or high frequencies of social interactions, depending on the group composition.
334 Similar effects of conspecifics on an individual's behaviour have been shown in the aquatic
335 insect, *Notonecta irrorata*, where an individual's propensity to disperse depended on the
336 behavioural type of conspecifics present (Kitchen & Chalcraft 2020). Given the intraspecific
337 variation in clerodanoid content that we observed in wild-caught individuals of *A. rosae*, we
338 expect social interactions in natural populations to range from low, for example, if no *A.*
339 *reptans* plants are around from which clerodanoids can be sequestered, to high, when some
340 insects could sequester clerodanoids, leading to a skewed distribution of social interaction
341 frequency.

342 Social interactions may accrue over time, and influence fitness of individuals through
343 different ways, such as injury/mortality from agonistic interactions, frequency of mating and
344 disease progression or spread (Alwash & Levine 2019; Dawson *et al.* 2018; Guo & Dukas
345 2020; Stroeymeyt *et al.* 2018). We observed a reduced lifespan in C+ females from C-C+
346 pairs compared to C+ females from C+C+ pairs (experiment 4). In experiment 2, we saw that
347 C+ females paired with C- males had the most agonistic interactions. Similarly, in Mixed
348 groups, we found more frequent interactions than in C- or C+ groups. Thus, we can expect
349 that C+ females in C-C+ pairs had more social, especially agonistic, interactions with their C-
350 conspecific, which may have nibbled on the C- individual to get access to clerodanoids. These
351 increased agonistic interactions may have led to a decreased lifespan for C+ females in C-C+
352 pairs. Such costs of aggression have also been shown in the fruit fly, *Drosophila*
353 *melanogaster*, where flies that were engaged in aggressive behaviour had a reduced survival
354 despite not showing any differences in wing damage from no-aggression treatment flies (Guo
355 & Dukas 2020). The authors hypothesised that such costs could arise from physiological and

metabolic changes triggered by stress and aggressive conflicts. Indeed, a previous study has shown transcriptional upregulation of genes involved in metabolic pathways in *A. rosae* sawflies that nibbled on conspecifics (Paul *et al.* 2021), which may indicate potential costs of the aggressive interactions.

Along with chemical defence against predation and increased mating success, sequestered plant metabolites could 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 like defence against predation (Singh *et al.* 2022) and mating success (Amano *et al.* 1999; Paul & Müller 2021), there may be trade-off with costs in terms of 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.

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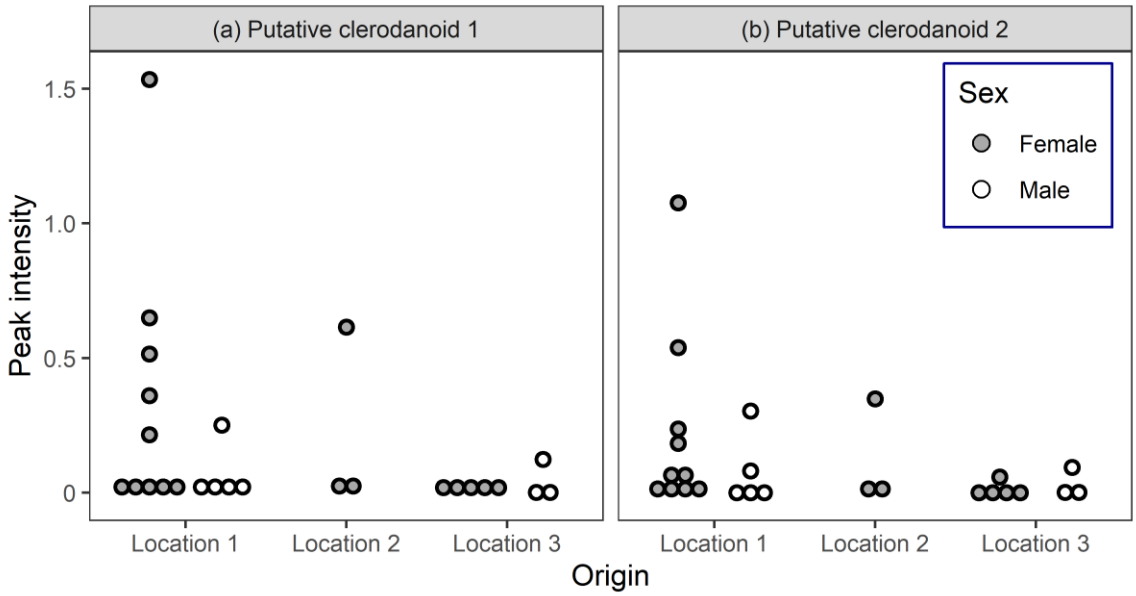
511 ***Figures***



512

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

514 clerodanoid 2 per individual in wild-caught *A. rosae* female (n=18) and male (n=8) sawflies

515 from three locations.

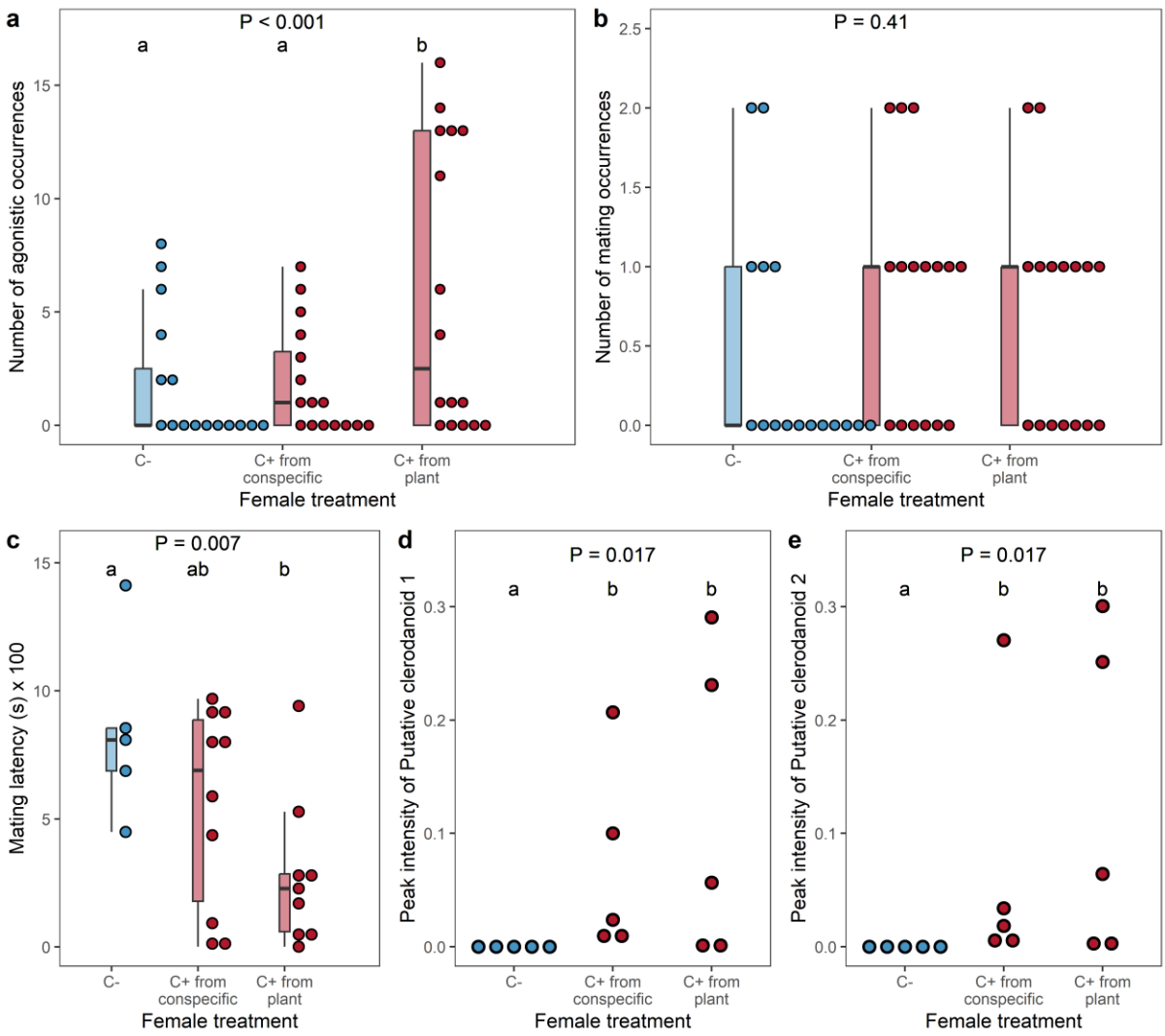


Figure 2. Effect of female treatment, no (C-), indirect (C+ from conspecific: prior contact to other female that had access to *Ajuga reptans* leaf) or direct (C+ from plant: prior access to *A. reptans* leaf) access to clerodanoids, on number of occurrences of (a) agonistic and (b) mating interactions, and on (c) mating latency with C- males of *Athalia rosae*. Effect of female treatment on peak intensity of putative (d) clerodanoid 1 and (e) clerodanoid 2 in lab-reared female sawflies. The boxplots represent the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range. The coloured circles represent individual data-points. The given *P*-values are the outcome of a GLMM (a,b), LMM (c) or Kruskal-Wallis (d,e) test. Different lower-case letters in the plots represent significant differences from post-hoc tests ($P < 0.05$).

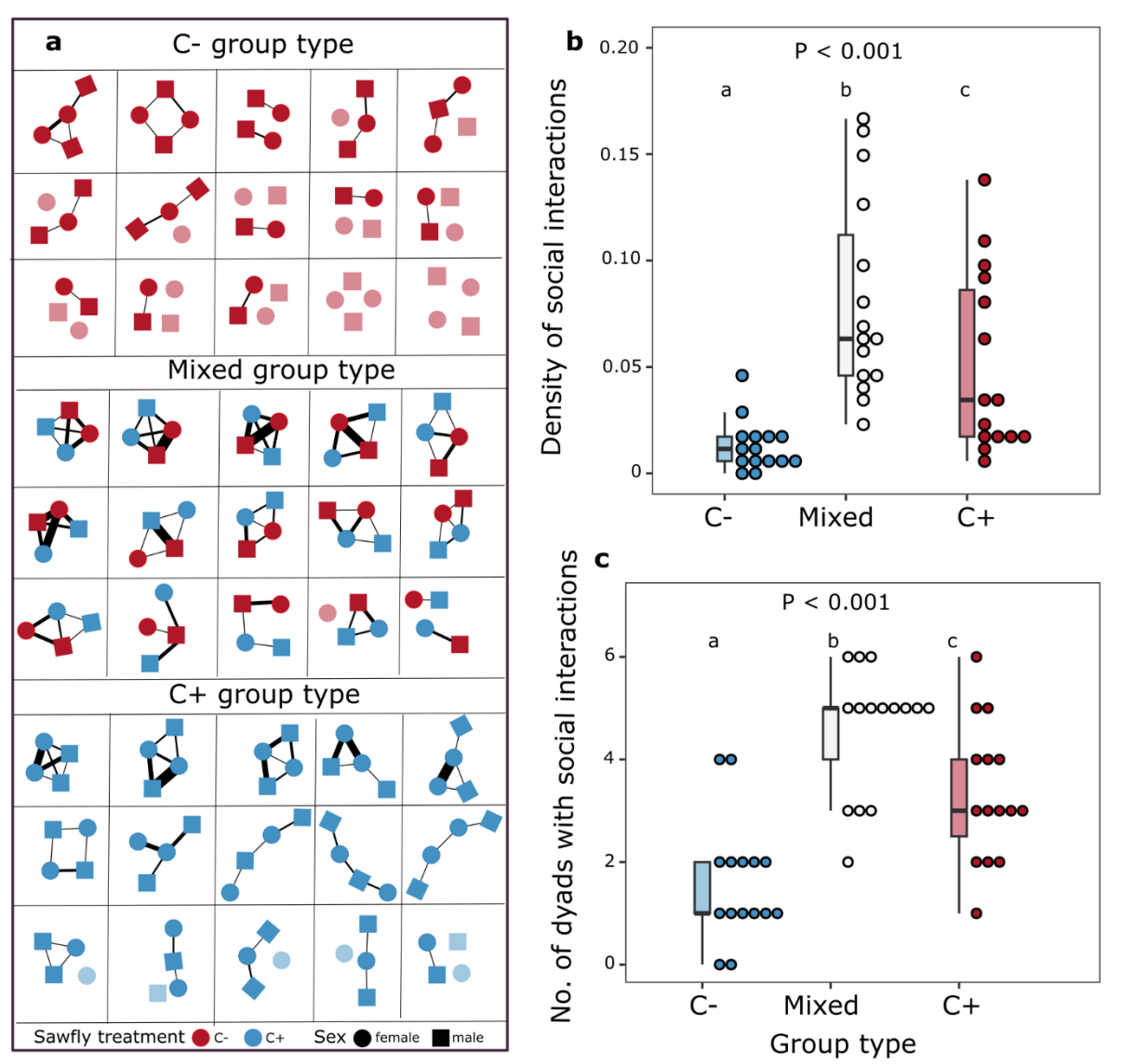


Figure 3. (a) Networks representing the C-, Mixed and C+ groups (n=15 per group type). Each cell in the grid corresponds to one replicate and consisted of two female (circular nodes) and two male (square nodes) adults of *Athalia rosae*. The sawflies had either no access (C-, red nodes) or prior access to *Ajuga reptans* (C+, blue nodes). Social interactions between a dyad are represented by lines between the individuals and the frequency of their social interactions is represented by the thickness of the line. The lighter colour-shade of nodes represents those individuals that had 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. The boxplots represent the median and 25th and 75th percentiles; the whiskers

537 indicate the values within 1.5 times the interquartile range. The coloured circles represent
538 individual data-points. The given *P*-values are the outcome of a LMM (b) or GLMM (c)
539 model. Different lower-case letters in the plots represent significant differences from post-hoc
540 tests ($P < 0.05$).

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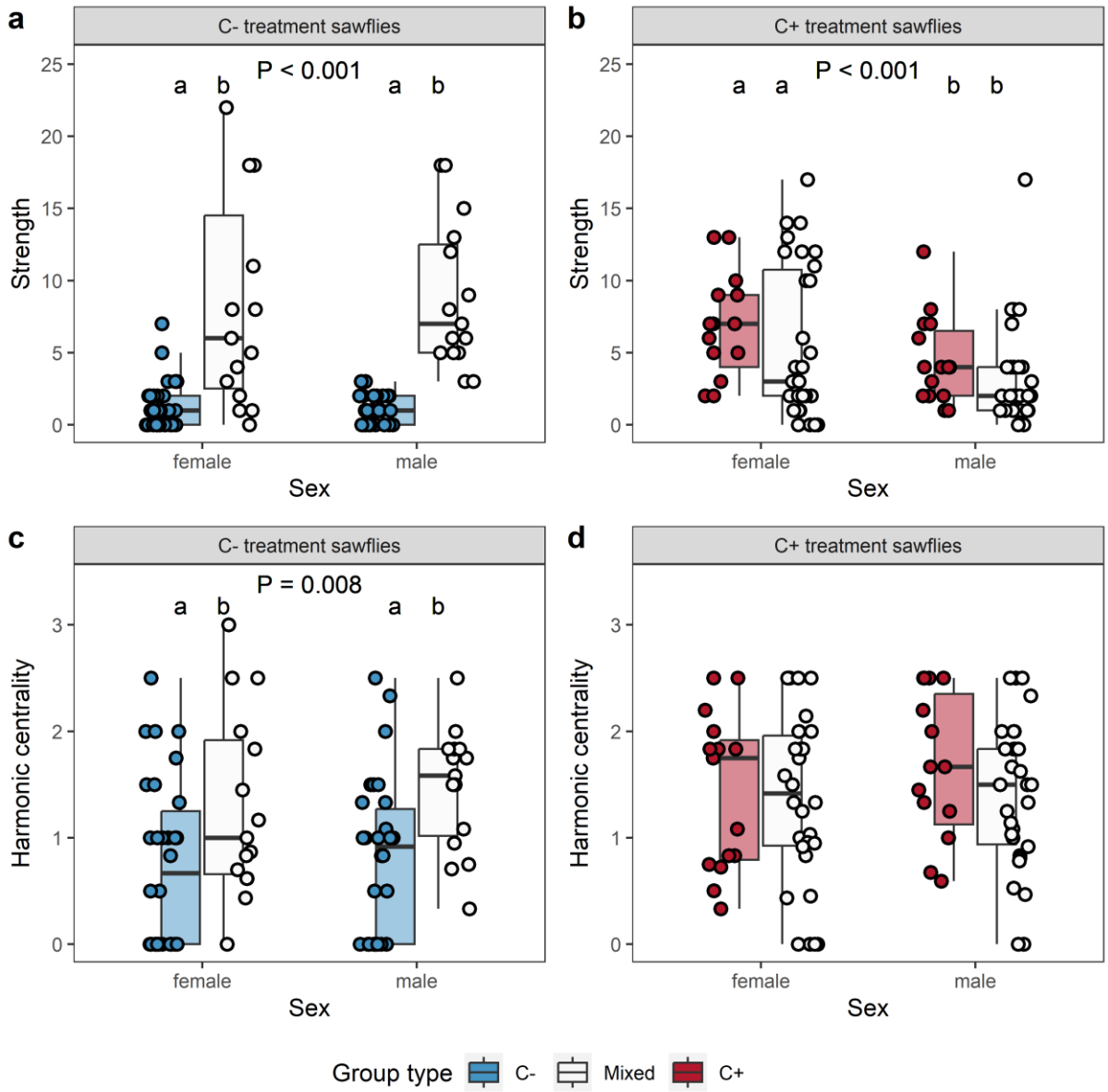

542

543 Figure 4. Effect of group type (C-, Mixed or C+ groups: represented by different colours) on
544 strength (top row) and harmonic centrality (bottom row) in C- treatment sawflies (a,c) and C+
545 treatment sawflies (b,d) for both sexes. The boxplots represent the median and 25th and 75th
546 percentiles; the whiskers indicate the values within 1.5 times the interquartile range. The
547 coloured circles represent individual data-points. The given *P*-values are the outcome of a
548 GLMM (a,b) or LMM (c,d) model. Different lower-case letters in the plots represent
549 significant differences from post-hoc tests ($P < 0.05$).

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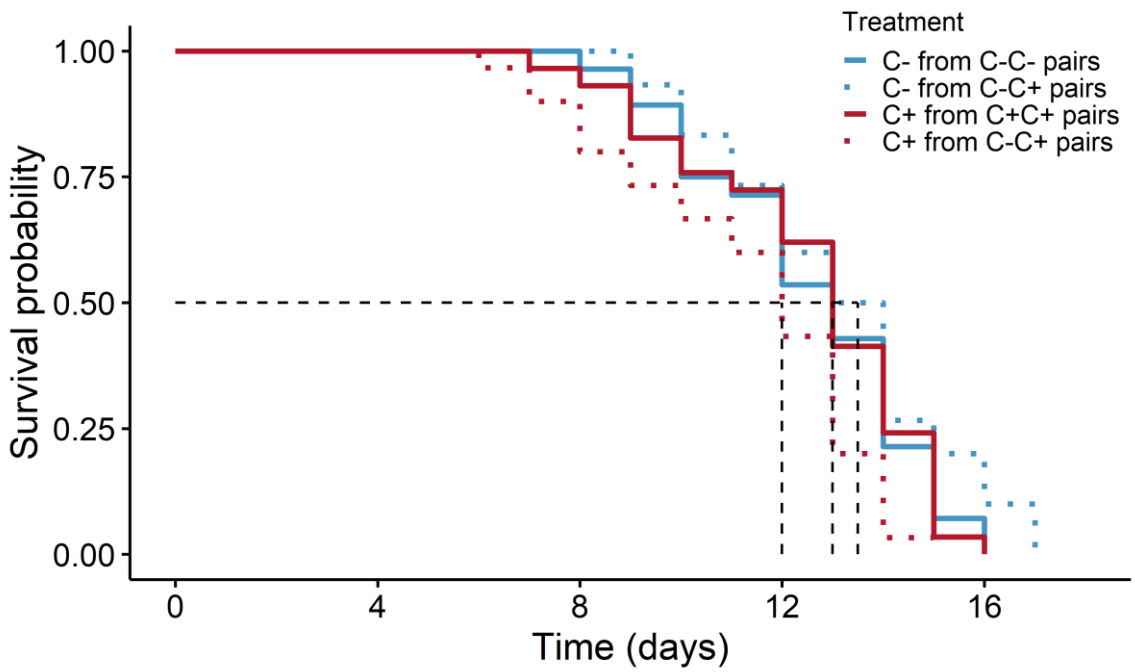

Figure 5. Survival of *Athalia rosae* females kept for 48 h with an individual of the same or a different treatment (C-: no access, C+: prior access to *Ajuga reptans* leaf), represented by Kaplan-Meier curves. The black dashed line represents the median survival of each treatment given by the point where it intersects the treatment curve. Note that the C- individuals from C-C+ pairs and C+ individuals from C-C+ pairs originate from the same treatment (see text for details).