

1 **Title: Consequences of population structure for sex allocation and sexual conflict**

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14

15 **Abstract**

16 Both sex allocation and sexual conflict can be modulated by spatial structure. However, how the  
17 interplay between the type of dispersal and the scale of competition simultaneously affects these  
18 traits in sub-divided populations is rarely considered.

19 We investigated sex allocation and sexual conflict evolution in meta-populations of the spider mite  
20 *Tetranychus urticae* evolving under budding (pairing females from the same patch) or random  
21 (pairing females from different patches) dispersal and either local (fixed sampling from each  
22 subpopulation) or global (sampling as a function of subpopulation productivity) competition.

23

24 Females evolving under budding dispersal produced less female-biased offspring sex ratios than  
25 those from the random dispersal selection regimes, contradicting theoretical predictions. In  
26 contrast, the scale of competition did not strongly affect sex allocation. Offspring sex ratio and  
27 female fecundity were unaffected by the number of mates, but female fecundity was highest when  
28 their mates evolved under budding dispersal, suggesting these males inflict less harm than those  
29 evolving under random dispersal.

30

31 This work highlights that population structure can impact the evolution of sex allocation and sexual  
32 conflict. Moreover, selection on either trait may reciprocally affect the evolution of the other, for  
33 example via effects on fecundity.

34

35 **Keywords:** local mate competition, hard and soft selection, experimental evolution, budding  
36 dispersal, scale of competition, *Tetranychus urticae*.

37 **Introduction**

38 Many organisms exist in structured populations, sub-divided into patches, that are linked and  
39 shaped by demographic factors such as dispersal. The frequency and type of dispersal can  
40 determine whether interactions are more likely to occur among related or unrelated individuals  
41 (Hamilton, 1964, Bulmer, 1986, Queller, 1992, Rousset, 2004, Courteau & Lessard, 2000, West,  
42 2009). For instance, if dispersal is limited, such that only some individuals disperse, the probability  
43 of interactions among genetically related individuals in a patch increases compared to populations  
44 in which all individuals disperse (Taylor, 1992, Wilson et al., 1992, Taylor & Crespi, 1994,  
45 Hamilton, 1964). However, if individuals disperse in groups from the same patch (i.e., if there is  
46 budding dispersal), interactions among genetically related individuals can be maintained, even if  
47 dispersal rates are high (Gardner et al., 2009, Gardner & West, 2006, Lehmann et al., 2006,  
48 Lehmann & Rousset, 2010, Aviles, 1993).

49         Dispersal frequency and timing also influence the scale of competition. For example, high  
50 dispersal, and dispersal occurring prior to the competitive interaction, leads to global competition,  
51 in which individuals compete with equal probability with others in the population (West et al.,  
52 2002a, Griffin et al., 2004, Taylor, 1992). In contrast, limited dispersal, and/or dispersal occurring  
53 after the competitive interaction, is associated with local competition (i.e., competition within the  
54 natal patch) (West et al., 2002a, Griffin et al., 2004, Taylor, 1992, Frank, 1998, Wilson et al.,  
55 1992). Therefore, the type, frequency and timing of dispersal can have a significant impact on the  
56 scale at which competitive interactions occur.

57         In turn, both relatedness and the scale of competition can affect sex allocation - the  
58 differential investment into male and female offspring. Indeed, in subdivided populations, sex  
59 allocation theory predicts an offspring sex-bias towards the sex for which local competition

60 between kin is less intense (Wilson et al., 1992, Hamilton, 1967, Charnov, 1982). For example,  
61 more female-biased offspring sex ratios are predicted when males compete locally on their natal  
62 patch for mates, and mated females disperse and compete globally for new patches (Hamilton,  
63 1967, Taylor, 1981, Herre, 1985). If there is budding dispersal, relatedness among the offspring of  
64 foundresses increases, exacerbating local competition between related males for mates, thus  
65 selecting for even more female-biased sex ratios (Gardner et al., 2009, Aviles, 1993). However, if  
66 the proportion of individuals dispersing is limited, and females compete locally for resources,  
67 competition becomes intense for both sexes and selection favours a more balanced offspring sex  
68 ratio (Table S1; Courteau & Lessard, 2000, Taylor & Crespi, 1994, Frank, 1985, Herre, 1985,  
69 Bulmer, 1986, Frank, 1986). A few empirical studies to date have investigated the consequences  
70 of budding dispersal (Kummerli et al., 2009), or disentangled the relative effects of the scale of  
71 competition and relatedness (Griffin et al., 2004) on the evolution of kin-selected behaviours, but  
72 none have disentangled the effect of these two factors on sex allocation.

73       Population structure is also predicted to impact the evolution of sexual conflict, i.e.,  
74 asymmetric reproductive interests between mating partners (Pizzari et al., 2015, Bourke, 2009,  
75 Faria et al., 2020). Competition for mates between males often involves strategies that are harmful  
76 to females reducing their survival and reproductive output (Arnqvist & Rowe, 2005, Parker, 2006,  
77 Wigby & Chapman, 2004, Crudgington et al., 2005). However, under global competition, when  
78 interactions occur among kin, reduced harming behaviour in males is expected to be selected for,  
79 as harm reduces patch productivity (Pizzari et al., 2015, Pizzari & Gardner, 2012, Rankin, 2011).  
80 A number of empirical studies are compatible with this prediction (Lukasiewicz et al., 2017,  
81 Carazo et al., 2014, Hollis et al., 2015, Le Page et al., 2017), but see (Chippindale et al., 2015).  
82 For instance, in the fruit fly *Drosophila melanogaster*, females repeatedly exposed to related, as

83 opposed to unrelated, males presented a higher lifetime reproductive success (Carazo et al., 2014).  
84 The evolution of sexual conflict may also depend on the frequency (Eldakar & Wilson, 2011) and  
85 type of dispersal (Faria et al., 2020). Because random dispersal reduces relatedness among  
86 competitors, it is predicted to increase the intensity of sexual conflict (Faria et al., 2015, Rankin,  
87 2011). This may be alleviated by budding dispersal, which maintains interactions among kin,  
88 reducing sexual conflict and harm inflicted to females, hence increasing their fecundity (Faria et  
89 al., 2020).

90 Although population structure is predicted to affect sex allocation and sexual conflict  
91 (Bourke, 2009), no study to date has disentangled how the type of dispersal and the scale of  
92 competition impacts the evolution of both within the same set-up. This is at odds with the fact that  
93 evolution under different population structures may simultaneously impact sex allocation and  
94 sexual conflict in a non-independent manner, highlighting the need to integrate studies on these  
95 traits (Chapman, 2009, Schärer & Janicke, 2009). For instance, changes in sex allocation may  
96 result in the production of more or fewer individuals of each sex, which impacts sexual conflict.  
97 At the same time, sexual conflict may impact the number of offspring produced (Carazo et al.,  
98 2014, Lukasiwicz et al., 2017), which may in turn influence sex allocation (Stubblefield & Seger,  
99 1990). This is supported by studies showing that multiple mating can impede optimal sex  
100 allocation in the parasitoid wasp *Nasonia vitripennis* (Boulton et al., 2019, Boulton & Shuker,  
101 2015).

102 Here, we uncover the effects of the type of dispersal and the scale of competition on the  
103 evolution of sex allocation and sexual conflict in the spider mite *Tetranychus urticae*. Previous  
104 work in this species has shown the evolution of sex allocation, and conflict between the sexes over  
105 the optimal offspring sex-ratio, in populations evolving under panmixy or local mate competition

106 (Macke et al., 2011, Macke et al., 2014). In addition, it is known that in this species only the sperm  
107 from the first copulation fertilises a female's eggs (Rodrigues et al., 2020). Still, multiple mating  
108 is common in this species, imposing costs in females by reducing the proportion of daughters as  
109 well as female fecundity (Rodrigues et al., 2020, Macke et al., 2012). This reduction in female  
110 reproductive output is thought to be a consequence of strong competition among males and, thus,  
111 evidence of male harm due to sexual conflict (Rodrigues et al., 2020).

112 In a fully crossed design, using experimental evolution, we placed replicate populations of  
113 *T. urticae* in 4 selection regimes with either local or global competition, and random *versus*  
114 budding dispersal. This design enabled us to follow evolution of both sex ratio and sexual conflict  
115 under different population structures. We expect sex allocation evolution under global competition  
116 to be towards more female biased offspring sex ratios than under local competition, due to reduced  
117 competition between related females locally in the patch. Furthermore, global competition, when  
118 coupled with budding dispersal, should produce the most female biased offspring sex ratios (see  
119 Table S1 for precise predictions for sex allocation under our different selection regimes). In  
120 contrast, as there is no mixing under local competition with budding dispersal, we do not expect  
121 selection to occur in this treatment.

122 Sexual conflict should also evolve differently in the different selection regimes. We predict  
123 that female harm inflicted by males, expressed as reduced fecundity, will be diminished when  
124 males evolve under budding dispersal, where interactions occur among kin.

125

126

## 127 **Material and Methods**

### 128 *Biological model*

129 The two-spotted spider mite, *T. urticae* Koch (Acari: Tetranychidae), is a generalist herbivore with  
130 a host range of over 1100 plant species (Migeon & Dorkeld, 2019, Helle & Sabelis, 1985). *T.*  
131 *urticae* has an arrhenotokous haplodiploid life cycle (~14 days egg – adult at 20-25°C): sons  
132 develop from unfertilised, haploid eggs and daughters from fertilised, diploid eggs. In this species,  
133 females are the dispersing sex, whereas males generally remain on their natal patch with mating  
134 occurring before female dispersal (Kennedy & Smitley, 1985, Mitchell, 1973). We report tertiary  
135 sex ratios (adult males divided by the total number of adult offspring) as males and females can  
136 only be distinguished as adults using microscopy: males are smaller than females and possess a  
137 pointed abdomen.

### 138 *Population origins*

139 In 2013, 10 different *T. urticae* populations were collected and separately maintained on bean  
140 plants at  $25 \pm 2^\circ\text{C}$ , with a 16h light: 8h dark cycle at the University of Lisbon. These populations  
141 comprised seven populations from Portugal (Lou, DC, AMP, DF, CH, COL and RF), two from  
142 Spain (Albe and Alro) and one from France (FR) (Zélé et al., 2018). All populations were treated  
143 with antibiotics to ensure that they were free of bacterial endosymbionts, known to be sex ratio  
144 distorters (Breeuwer, 1997). The sex ratio of each individual population ranges from 0.22 to 0.40  
145 (Zélé et al., 2020). In November 2015, more than 50 females from each of the 10 populations were  
146 transferred to the University of Montpellier and mixed to form a genetically diverse population to  
147 seed the experiment (hereafter called the ‘ancestral population’). This newly mixed population was  
148 maintained on 12 whole bean plants (variety: Pongo) in a plastic box (395 mm length x 335 mm

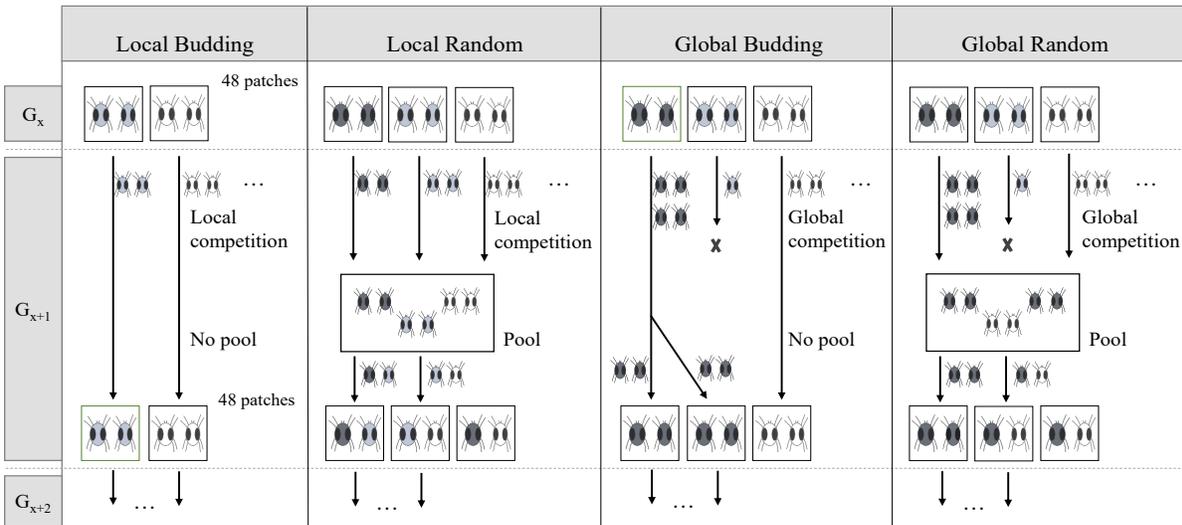
149 width x 323 mm height) at 25°C with a 16h light: 8h dark cycle. Each week, old plants were  
150 removed and replaced with young, un-infested plants. All bean plants used to maintain mite  
151 populations and for all experiments described below were grown from seeds in an isolated,  
152 herbivore-free room at 23 ± 1°C with a photoperiod of 12h light: 12h dark at the University of  
153 Montpellier.

154 Fourteen days before starting the experiment, 10 independent groups of 40 females were  
155 haphazardly sampled from the ancestral population and put on a patch (10-15 bean leaves placed  
156 together) on water-saturated cotton wool to lay eggs. This allowed maternal effects to be equalised  
157 and ensured that females seeding the experiment were of the same age. Two weeks later, when  
158 mites of the following generation had reached adulthood, all 10 groups were mixed, and the newly  
159 emerged mated adult females were haphazardly assigned to the different selection regimes.

#### 160 *Establishment and maintenance of selection regimes*

161 The impact of different types of dispersal (budding *versus* random) and scales of competition (local  
162 *versus* global) on the evolution of sex allocation and sexual conflict in *T. urticae* was investigated  
163 using a fully crossed experimental design (Figure 1): 1) global competition, budding dispersal  
164 ('Global Budding', GB), 2) global competition, random dispersal ('Global Random', GR), 3) local  
165 competition, budding dispersal ('Local Budding', LB) and 4) local competition, random dispersal  
166 ('Local Random', LR). Each regime was replicated three times (GB-1, GB-2, GB-3, GR-1, GR-2,  
167 GR-3, LB-1, LB-2, LB-3, LR-1, LR-2 and LR-3).

168 For each replicate population, each generation comprised a total of 96 mated adult females, being  
169 assigned in pairs to 48 bean leaf patches (4 cm<sup>2</sup> each, corresponding to a very mild infestation  
170 level, thus low competition) placed on water-saturated cotton wool in a plastic box (255 mm length



171

172 **Figure 1. Description of the selection regimes.** Four selection regimes were established and  
 173 maintained for 33 generations, each with three experimental replicates. In ‘Local Budding’, 2  
 174 females from each of the 48 patches (squares) were transferred in pairs to a new patch for the next  
 175 generation ( $G_{x+1}$ ). In ‘Local Random’, an equal number of females (2 – 4; the number was adjusted  
 176 each generation to account for mortality) from each patch were pooled together on a large common  
 177 leaf patch (‘mixing patch’, rectangle), from which females were subsequently haphazardly  
 178 transferred in pairs to 48 new patches. In ‘Global Budding’, the number of adult females per patch  
 179 was counted before each transfer to calculate fecundity relative to that of the other 47 patches in  
 180 the replicate. Each patch contributed with a number of female pairs, to the following generation,  
 181 proportional to its relative fecundity. In ‘Global Random’ all 48 patches were placed on a ‘mixing  
 182 patch’ onto which females could disperse for ~4 hours, after which adult females were haphazardly  
 183 transferred in pairs to 48 new patches for the next generation. Related females within a treatment  
 184 are denoted as the same shade of grey.

185

186 x 183 mm width x 77 mm height). Mating occurred on the natal patch among the offspring of the  
 187 2 females, before mated daughters dispersed. A single male is capable of fertilising up to 15  
 188 females in a single day (Krainacker & Carey, 1989), hence the number of males available to

189 fertilise all females on a patch was not limiting. All replicates from all regimes were maintained  
190 in the same conditions, the only difference being how populations were mixed and transferred to  
191 new patches at each generation (Figure 1). Note that such variation in population structure  
192 inherently affects the effective population size and thus the degree of inbreeding in populations.

193         In the budding dispersal regimes, females were always transferred with another female  
194 from the same patch to form the next generation. In contrast, in the random dispersal regimes,  
195 females from different patches were placed together on a ‘mixing patch’ (10 bean leaves placed  
196 together) before being transferred, in haphazardly chosen pairs, to a new patch. Local competition  
197 was imposed by letting an equal number of adult females per patch seed the next generation (2 –  
198 4 females per patch in ‘Local Random’; adjusted each generation to accommodate mortality).  
199 Under global competition, relative patch productivity (the total number of daughters produced  
200 compared to that of the other patches within the replicate) determined the number of female adult  
201 offspring transferred to the next generation: in the ‘Global Random’ regime, all 48 patches were  
202 placed on a ‘mixing patch’ onto which adult females dispersed (patches with more female  
203 offspring having a higher representation on the ‘mixing patch’) before being transferred in pairs;  
204 in the ‘Global Budding’ regime, the number of adult females on each patch was counted to  
205 calculate relative fecundity (i.e. dividing the number of females per patch by the total number of  
206 females across the 48 patches), so that patches with the most offspring contributed more pairs of  
207 females to the next generation.

208         Due to the time taken for each transfer, transfers from one generation to the next were done  
209 over 1, 2 or 3 days. When done over more than one day, at least one replicate population from each  
210 regime was transferred on the same day. All replicates were maintained in a climate chamber at  
211  $25 \pm 2^\circ\text{C}$ , with a photoperiod of 16h light: 8h dark. During the selection experiment, all replicates

212 in the ‘Local Budding’ regime were lost after generation 14, and 1 replicate in the ‘Global  
213 Budding’ regime was lost at generation 22 (GB-3). These lines were lost because females on the  
214 different patches did not produce any offspring, or only male offspring, leading to extinction of  
215 lines. In total, 33 generations of selection were performed.

## 216 *Responses to selection*

### 217 *1. Sex allocation during experimental evolution*

218 The sex allocation of females was measured directly in the replicate populations of each selection  
219 regime at generations 12, 17, 20 and 31. This was done by counting the number of males and  
220 females per patch within each experimental replicate prior to the following transfer. Thus, sex ratio  
221 comprised the combined output of the two females per patch.

### 222 *2. Sex allocation in a common environment*

223 In this assay, all regimes were each exposed to a common environment for 1 generation to  
224 equilibrate maternal effects before measuring the offspring sex-ratios of females that mated  
225 randomly with males from their selection regime (Figure S1). For this, at generation 31, 96 mated  
226 daughters were haphazardly chosen from the 48 patches within each selection regime and placed  
227 on a large leaf patch (~200cm<sup>2</sup>) where they laid eggs together. Fourteen days later, the offspring  
228 on these patches emerged as adults and mated amongst themselves (Generation 31 + 1). Ninety-  
229 six mated female offspring from each mixing patch were then haphazardly chosen to measure their  
230 offspring sex-ratio; 48 were placed individually on 2cm<sup>2</sup> patches, and another 48 placed in pairs  
231 on 4 cm<sup>2</sup> patches. Females were allowed to lay eggs for 7 days on these new patches, before being  
232 killed. After 2 weeks, once offspring had emerged as adults, the number of daughters and sons on

233 each patch was counted. This experiment was set up over three days, with one replicate per regime  
234 being treated each day.

### 235 *3. Sex allocation in response to patch fecundity*

236 Measures of offspring sex ratio on patches concern the sex allocation of two females on that patch.  
237 While this is informative, it may obscure responses to selection, especially if offspring sex ratio  
238 differs between females, for instance, if a focal female's sex allocation changes in response to her  
239 own fecundity only, or also to that of her patch mate (Stubblefield & Seger, 1990). To test this  
240 hypothesis, we measured the fecundity and sex allocation of single females from our selection  
241 regimes in response to the presence of eggs laid by sterile females on the same patch (Osouli et  
242 al., 2014). Because the eggs of the sterilised females do not hatch, we can distinguish the offspring  
243 of the focal female (adult individuals) from that of the sterilised one (unhatched eggs) within a  
244 single patch.

245         This experiment was implemented after 33 generations of selection. As for the preceding  
246 experiment, individuals within each replicate population were subject to a common environment.  
247 However, in this experiment it was over two generations (generation 33 + 2; Figure S1). At the  
248 same time, females from the ancestral population were placed in a common environment for 2  
249 generations, as done with females from the selection regimes (Figure S1) to generate sterile  
250 females. To sterilise these females, they were exposed to 100 Gy, at a dose of 2.7 Gy minute<sup>-1</sup>,  
251 using a Xstrahl XenX pre-clinical irradiator at the Institute of Cancer Research, Montpellier  
252 (IRCM). Preliminary studies revealed that this dose of X-ray irradiation is sufficient to sterilise  
253 females, that lay eggs that do not hatch (see Table S2).

254         Single adult females from the different selection regimes were placed on individual leaf

255 patches with one sterile female and allowed to lay eggs for 5 days. Both females were then killed  
256 and patch fecundity, the total number of eggs per patch (laid by the sterile and the fertile female,  
257 coming from the ancestral population and from one of the selection regimes, respectively) was  
258 measured. Nine days later the adult offspring of fertile females were counted, and the offspring  
259 sex ratio measured. A total of 48 leaf patches (4 cm<sup>2</sup>) were set up per replicate population.

#### 260 *4. Sexual conflict*

261 The impact of mating with males evolved under the ‘Global Budding’ and ‘Global Random’  
262 selection regimes on the fecundity of females from the ancestral population was compared in a  
263 separate assay. Females were collected from the different selection regimes at generation 33 and  
264 spent two further generations in a common environment before the experiment (G33 + 2, as above;  
265 Figure S1). The females from the ancestral population experienced one generation in a common  
266 environment, being placed in 2 boxes, each containing 100 females on a large ‘mixing patch’.  
267 Thirteen days later, 240 immature, virgin females (i.e., daughters) were isolated on 4 cm<sup>2</sup>  
268 individual leaf patches later used to measure the degree of sexual conflict.

269 To obtain males from each selection regime, on days 10 and 11 of the second generation in  
270 the common environment (G33 + 2), 30 immature, juvenile females were isolated from each  
271 replicate population and each placed on a 4cm<sup>2</sup> leaf patch. These virgin females emerged as adults  
272 and laid eggs for six days. Because spider mites are haplodiploid, only male progeny emerged  
273 from these eggs. Due to female mortality or failure to lay eggs, the total number of patches  
274 containing virgin males from each line varied from 17 to 28 (GB-1 = 28, GB-2 = 17, GR-1 = 21,  
275 GR-2 = 21 and GR-3 = 21). On day 1 of the experiment, males from the different patches within  
276 each replicate population were mixed on a large leaf patch so they could be haphazardly distributed

277 across treatments (see below).

278           The 240 immature, virgin females (i.e. daughters) were taken from the ancestral population  
279 and kept isolated for 2 days on their individual patches. Subsequently, the eggs laid by these  
280 females were removed and patches were assigned to males from either the ‘Global Random’ or  
281 ‘Global Budding’ selection regime, and to a ‘single’ or ‘double’ mate treatment (N=30 per  
282 treatment). In all treatments, males from the selection regimes were placed with the virgin females  
283 for 5 hours on day 1 of the experiment. Twenty-four hours later (day 2), in patches assigned to the  
284 double mating treatment, a second male was placed on the patch and left for 5 hours. This mating  
285 period was chosen to take into account the time females were together with their mates each  
286 generation prior to their transfer to new patches. In both treatments, females were left to lay eggs  
287 and on day 6 of the experiment, female mortality was checked and alive females were removed  
288 from the patches. The total number of eggs per patch was counted and, 8 days later, offspring sex-  
289 ratio was measured.

### 290 *Statistical analysis*

291 All analyses were carried out using the R statistical package (v. 3.0.3) and JMP13. We used  
292 Generalised Linear Mixed Models (GLMMs, package glmmTMB; Brooks et al., 2017) with a beta-  
293 binomial error structure and logit link function, and quasi-poisson or negative binomial error  
294 structures and log link function, to analyse the effect of selection regime on sex ratio and mean  
295 offspring production, respectively. Maximal models were simplified by sequentially eliminating  
296 non-significant terms ( $p < 0.05$ ) from the highest- to the simplest-order interaction, with the highest  
297 p-value to establish a minimal model (Crawley, 2007). The significance of the explanatory  
298 variables in the minimal models was established using chi-squared tests (Bolker et al., 2009). *A*

299 *posteriori* contrasts with Bonferroni corrections were done to interpret the effect of selection  
300 regime when significant (glht, multcomp package; Hothorn et al., 2008). Details of all models are  
301 given in Table S3.

### 302 **1. Sex allocation during experimental evolution**

303 To analyse the impact of the selection regime on offspring sex ratio, generation (12, 17, 20 and  
304 31) and selection regime (GB, GR and LR) were included in the model as fixed factors, as well as  
305 their interaction. Generation was analysed as a covariate and was log transformed to improve the  
306 fit of the model. Experimental replicate (GB-1, GB-2, GR-1, GR-2, GR-3, LR-2 and LR-3,) was  
307 included as a random factor nested within selection regime, and the day measurements were taken  
308 as a random factor nested within generation.

### 309 **2. Sex allocation in a common environment**

310 To investigate the effect of selection regime on offspring sex ratio in a common environment, we  
311 used a model with selection regime (GB, GR and LR) and the number of females per patch (1 or  
312 2) as fixed factors, as well as their interaction, and replicate population (GR-3, GB-1, GB-2, GR-  
313 1, GR-2, LR-2 and LR-3), nested within selection regime as a random factor. This analysis  
314 excluded replicate LR-1 due to fewer than 8 patches with more than 3 offspring. For this variable,  
315 the best fit model included a parameter to account for zero inflation (ziformula~1; package  
316 glmmTMB; Brooks et al., 2017).

### 317 **3. Sex allocation in response to patch fecundity**

318 In a second analysis, using data from the '*Sex allocation in response to patch fecundity*'  
319 experiment, we investigated whether the sex allocation of the focal female depended on her relative

320 fecundity ('relative patch fecundity': the number of eggs laid by the focal female divided by the  
321 total number of eggs laid on the patch) and on the total number of eggs present in the patch ('total  
322 patch fecundity'). In this analysis, the selection regime of the focal female (GB, GR and LR),  
323 relative (or total) patch fecundity were included in models as fixed factors, as well as their  
324 interaction, and experimental replicate (GB-1, GB-2, GR-2, GR-3, LR-2 and LR-3) nested within  
325 selection regime was added as a random factor. These analyses only included females alive on day  
326 4 of the experiment and excluded replicates GR-1 and LR-1, due to fewer than 10 patches with  
327 more than 3 offspring.

328         We used data from this experiment to compare observed offspring sex ratios with predicted  
329 values from theoretical models (see Supplementary Materials Table S1 for details) using two tailed  
330 t-tests in JMP13. Observed offspring sex ratios were mean values for fertile females from each  
331 selection regime.

#### 332 ***4. Sexual conflict***

333 To test whether selection regime affected the intensity of sexual conflict and male-male  
334 competition, female fecundity and offspring sex-ratio were analysed including the number of  
335 mates (one or two mates) and the selection regime of the male ('Global Budding' *versus* 'Global  
336 Random') as discrete, fixed variables in the model, as well as their interaction. Replicate  
337 population (GB-1, GB-2, GR-1, GR-2 and GR-3) and box (the container in which several  
338 individual replicates were maintained) were included nested within dispersal type as random  
339 factors. In the analysis of female fecundity, all individual replicates in which females died before  
340 day six were excluded.

341

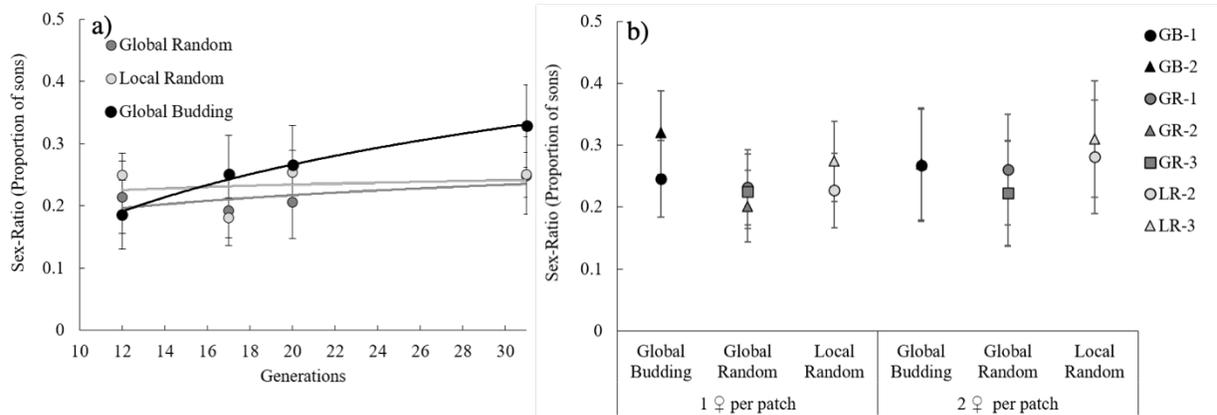
## 342 **Results**

### 343 *Sex allocation during experimental evolution and in a common environment*

344 There was a consistent significant effect of selection regime on sex allocation during the selection  
345 experiment and after a generation in a common environment (during selection experiment:  $X^2_2 =$   
346 14.046,  $p < 0.001$ ; common environment:  $X^2_2 = 11.845$ ,  $p = 0.002$ ; Figures 2a and 2b, Table S4).  
347 Females from the ‘Global Budding’ regime produced less female-biased offspring sex ratios than  
348 females from the ‘Global Random’ regime (during selection experiment:  $Z = -3.741$ ,  $p < 0.001$ ;  
349 the ‘Global Budding’ regime to produce a less female-biased offspring sex ratio than females from  
350 the ‘Local Random’ regime during the selection experiment ( $Z = -2.289$ ,  $p = 0.066$ ), but not after a  
351 generation in a common environment ( $Z = -1.53$ ,  $p = 0.331$  Figures 2a and 2b; Table S5). There was  
352 no difference in sex allocation between females from the ‘Global Random’ and ‘Local Random’  
353 regimes (during selection experiment:  $Z = 1.554$ ,  $p = 0.361$ ; common environment:  $Z = -1.597$ ,  $p =$   
354 0.3776; Figures 2a and 2b; Table S5). The number of females on a patch did not affect offspring  
355 sex ratio (selection regime x number of females per patch:  $X^2_2 = 4.114$ ,  $p = 0.128$ ; number of  
356 females per patch:  $X^2_1 = 0.94$ ,  $p = 0.331$ ; Table S4).

### 357 *Comparing observed offspring sex-ratios with theoretical predictions*

358 In table S1, we present the theoretical predictions corresponding to the selection regimes in which  
359 spider mite populations evolved (Herre, 1985, Taylor & Bulmer, 1980, Gardner et al., 2009).  
360 Females from the ‘Global Random’ selection regime produced an offspring sex ratio of 0.19 ( $\pm$   
361 0.19 SE), that does not differ from the predictions of Taylor and Bulmer (1980) and of Gardner



362

363 **Figure 2. Mean offspring sex ratio ( $\pm$  standard error) of females from the ‘Global Random’**  
 364 **(GR, dark grey), ‘Global Budding’ (GB, black) and ‘Local Random’ (LR, light grey)**  
 365 **selection regimes across generations.** The proportion of male offspring was measured a) during  
 366 experimental evolution at generations 12, 17, 20 and 31 (experimental replicate means shown for  
 367 each selection regime) and b) at generation 31 + 1 after one generation in a common environment,  
 368 in patches with one or two females (means shown for each experimental replicate (different  
 369 symbols) in each selection regime).

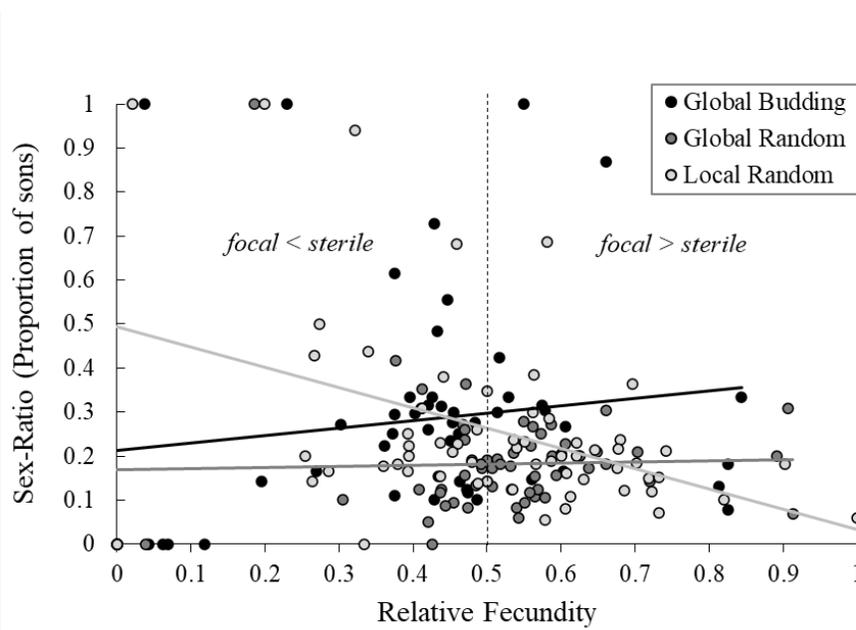
370

371 et al (2009) ( $t = 0.932$ ,  $df = 69$ ,  $p = 0.3544$ ). In contrast, the evolved offspring sex ratios in the  
 372 ‘Global Budding’ and ‘Local Random’ selection regimes differed from theoretical predictions.  
 373 Specifically, females from the ‘Global Budding’ selection regime produced a less female-biased  
 374 offspring sex ratio (mean  $0.30 \pm 0.03$  SE;  $t = 9.54$ ,  $df = 55$ ,  $p < 0.0001$ ), and females from the  
 375 ‘Local Random’ regime a more female-biased offspring sex ratio (mean  $0.24 \pm 0.02$  SE;  $t = 7.99$ ,  
 376  $df = 74$ ,  $p < 0.0001$ ), than predicted by theory.

### 377 *Sex allocation in response to patch fecundity*

378 Offspring sex ratios changed according to the selection regime of focal females and their relative  
 379 patch fecundity (selection regime:  $X^2_2 = 10.90$ ,  $p = 0.004$ ; relative patch fecundity:  $X^2_1 = 6.87$ ,  $p =$

380 0.009; Figure 3, Table S4). As before, females from the ‘Global Budding’ regime produced less  
381 female-biased offspring sex ratio than females from the ‘Global Random’ regime ( $Z=-3.298$ ,  $p =$   
382 0.003; Figure 3, Table S5). The offspring sex ratio of females from the ‘Local Random’ treatment



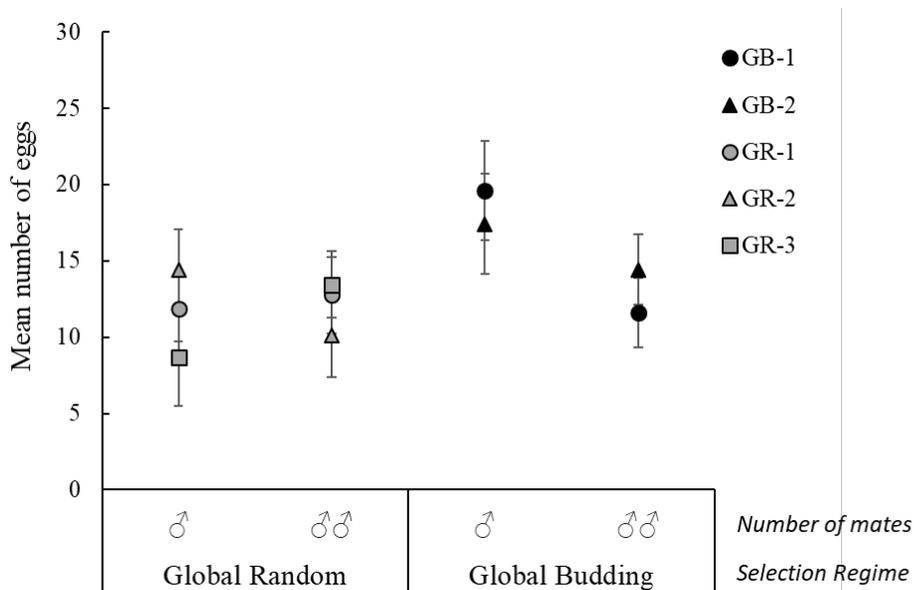
383

384 **Figure 3.** Offspring sex ratio as a function of relative patch fecundity per patch in the ‘Global  
385 Budding’ (GB, black), ‘Global Random’ (GR, dark grey) and ‘Local Random’ (LR, light  
386 grey) selection regimes. Females from the different selection regimes were placed on individual  
387 patches (one per patch) with a sterile female from the base population. For each patch, the  
388 proportion of offspring produced by the focal female (i.e. from the selection regime) was calculated  
389 as the proportion of eggs that hatched and became adult (relative patch fecundity), and her  
390 offspring sex-ratio was measured. Each dot represents an individual replicate (i.e., patch from  
391 which measurements were taken).

392

393 did not differ from that of the other two selection regimes (Table S5). Across all treatments,  
394 females with higher relative patch fecundity produced more female-biased offspring sex-ratios  
395 (selection regime x relative patch fecundity:  $X^2_2 = 2.55$ ,  $p = 0.28$ ; Figure 3). These results did not

396 change when using total patch fecundity (sum number of eggs laid by the fertile and sterile female  
 397 on each patch, Figure S2, Tables S4 and S5).



398

399 **Figure 4.** Mean fecundity ( $\pm$  standard error) of females from the ancestral population  
 400 presented with either one or two males from the ‘Global Budding’ (GB, black) or ‘Global  
 401 Random’ (GR, dark grey) selection regimes. Means shown for each experimental replicate  
 402 (different symbols) in each selection regime at generation 33, after two generations in a common  
 403 environment.

404

#### 405 *Sexual conflict*

406 Overall, there was no significant effect of mate number ( $X^2_1=0.024$ ,  $p = 0.876$ ), male selection  
 407 regime ( $X^2_1=0.028$ ,  $p = 0.867$ ), or their interaction ( $X^2_1=0.073$ ,  $p = 0.788$ ) on the offspring sex-  
 408 ratio of females from the ancestral population (Figure S3, Table S4). However, the total number  
 409 of offspring produced was higher when females mated with a male from the ‘Global Budding’, as  
 410 opposed to the ‘Global Random’, selection regime ( $X^2_1=4.336$ ,  $p = 0.036$ ; Figure 4, Table S4).

411

412 **Discussion**

413 Both sex allocation and sexual conflict responded to selection under different population  
414 structures. Sex allocation responses were mainly driven by dispersal type (budding *vs* random),  
415 which influences whether interactions occur among kin or unrelated individuals, and not by the  
416 scale of competition. Females from the ‘Global Budding’ regime consistently produced more  
417 male-biased offspring sex ratios than those from the ‘Global Random’ selection regime. We also  
418 found that higher relative patch fecundity was associated with more female-biased offspring sex-  
419 ratios across all selection regimes. Finally, when comparing the intensity of sexual conflict,  
420 females from the ancestral population mated to males from the ‘Global Budding’ regime had  
421 higher fecundity than those mated to males from the ‘Global Random’ regime.

422 ***Sex Allocation***

423 Females from the ‘Global Random’ selection regime produced offspring sex ratios consistent with  
424 theory (Taylor & Bulmer, 1980). This corroborates previous findings in mites (Macke et al., 2011)  
425 and is consistent with sex ratio observations in other haplodiploid and diploid systems (e.g. Reece  
426 et al., 2004, Reece et al., 2008, Herre, 1985). However, the ‘Local Random’ and the ‘Global  
427 Budding’ regimes present offspring sex ratios that differ from theoretical predictions, being more  
428 and less female-biased than predicted, respectively (see Table S1). The fact that offspring sex  
429 ratios in the ‘Global Budding’ treatment were not as predicted, and that sex ratios in the ‘Local  
430 Random’ and ‘Global Random’ regimes were equivalent, suggest that other factors, besides the  
431 type of dispersal and the scale of competition, may be at play. Below, we highlight potential factors  
432 that may account for the observed patterns.

433 First, inbreeding is affected by dispersal type (with high inbreeding expected for the  
434 budding dispersal regime). Inbreeding can select for more female-biased offspring sex ratios  
435 (Herre, 1985, Chung et al., 2019, Frank, 1985). If coupled with high levels of juvenile mortality  
436 this could, in some cases, result in no males in a patch (Chung et al., 2019, West et al., 2002b),  
437 potentially explaining why all 3 replicates of the ‘Local Budding’ and 1 replicate of the ‘Global  
438 Budding’ regimes were lost. Moreover, different inbreeding levels may lead to different levels of  
439 inbreeding depression among selection regimes, being highest under budding dispersal. Thus, in  
440 our surviving selection regimes, the consequences of inbreeding depression may be more  
441 pronounced in the ‘Global Budding’ regime. In haplodiploids like spider mites, inbreeding  
442 depression is expressed mainly in female traits (Tien et al., 2015). Therefore, the accrued costs of  
443 inbreeding may negate any benefit of female-biased sex ratios in the ‘Global Budding’ regime in  
444 the replicates that survived (Greeff, 1996). As such, there might be selection to augment the  
445 production of sons in patches with low fecundity and/or high mortality due to inbreeding  
446 depression to ensure female fertilisation (Chung et al., 2019, West et al., 2002b). In line with this,  
447 females in the ‘Global Budding’ regime produced fewer offspring that became adult, which might  
448 be due to higher offspring mortality or lower fecundity (Figure S4, Table S5). These females also  
449 produced slightly more male offspring than those from the ‘Global Random’ regime (Figure S5a,  
450 Table S5).

451 Another factor that may affect sex allocation is variable clutch size. Indeed, when females  
452 with asymmetric fecundities oviposit simultaneously in the same patch, the sons of a more fecund  
453 female are subject to more intense competition for mates, as they mostly compete among brothers  
454 to mate with sisters, whereas the sons of a less fecund female mostly compete with unrelated males  
455 to access unrelated females (Stubblefield & Seger, 1990). More fecund females are thus expected

456 to produce more female-biased sex ratios, while less fecund females should produce less female-  
457 biased sex ratios. As a result, the patch sex ratio becomes skewed towards that produced by the  
458 more fecund females, i.e. a more female-biased sex ratio (Stubblefield & Seger, 1990, West, 2009).  
459 In addition, theory predicts that this adjustment by females should emerge from a differential  
460 investment in daughters, while maintaining a constant production of sons, known as the ‘constant  
461 male hypothesis’ (Yamaguchi, 1985, Frank, 1987). Here, we found that, for all selection regimes,  
462 the sex ratio declined as the relative fertility of the focal female increased (the same was observed  
463 for total patch fecundity, Figure S2), showing that female fecundity and sex-ratio are not  
464 independent traits. Furthermore, although not significantly different from the other regimes, ‘Local  
465 Random’ females generated the steepest slope. Coupled with higher overall fecundity observed in  
466 this selection regime (Figure S4, Table S5), this may explain why offspring sex ratios are more  
467 female-biased than expected. Finally, although son production is not constant across selection  
468 regimes (Figure S5a, Table S4), its variation is much lower than that for the number of daughters  
469 produced (Figure S5b). Again, this seems to be in line with an effect of clutch size on sex  
470 allocation. Note however that the constant male hypothesis is expected to break down under local  
471 competition (Rodrigues & Gardner, 2015), a result we do not recapitulate here.

472         A final possibility for why populations in the ‘Local Random’ and ‘Global Budding’  
473 selection regimes do not produce the predicted offspring sex ratios is that they have not reached  
474 their evolutionary equilibrium. Another selection experiment found an initial reduction in  
475 cooperation under budding dispersal followed by a subsequent increase in this trait value  
476 (Kummerli et al., 2009). Although our experimental evolution was done over 33 generations,  
477 which is a large number for non-microbial organisms, populations may still have been in a transient  
478 stage when traits were measured.

479 *Sexual Conflict*

480 Theory predicts that multiple mating with unrelated males causes a greater reduction in female  
481 reproductive success than multiple mating with related males (Pizzari et al., 2015). More precisely,  
482 Faria et al (2020) predict that budding dispersal leads to higher relatedness between males, thus  
483 reducing levels of sexual conflict and increasing levels of female fecundity. In accordance with  
484 this we found that females mated to males from the ‘Global Budding’ regime had higher fecundity  
485 than those mated to males from the ‘Global Random’ regime, suggesting that ‘Global Budding’  
486 males inflicted less harm. This replicates previous findings showing that evolving with kin reduced  
487 the level of male harm inflicted to females in bulb mites (Lukasiewicz et al., 2017). Other studies  
488 have shown that a reduction in male harm may be a plastic response to the presence of kin (Carazo  
489 et al., 2014, Lymbery & Simmons, 2017). However, in our experiment, since mating was with  
490 unrelated females from the ancestral population, there were no direct cues indicating the presence  
491 of kin. In addition, competitor males coming from the same selection regime experienced 2  
492 generations of common garden prior to the experiment which probably reduced relatedness among  
493 them. This means that if the response were plastic, then there should be no difference between  
494 selection regimes. Thus, reduced harm was most likely an evolved response in our study.

495         Contrary to expectations (Arnqvist & Rowe, 2005), we did not find that multiple mating  
496 reduced fecundity in either selection regime. Possibly, the differences in harm inflicted by one or  
497 two mates over a single, or two successive five-hour periods respectively, might have been  
498 insufficient to detect differences in fecundity between the two treatments. Previous studies with  
499 spider mites found fecundity costs when females were simultaneously exposed to multiple mates  
500 for two consecutive periods of 24 hours with an interval of 24h hours between them (Rodrigues et  
501 al., 2020), or exposed to two mates on multiple occasions during their lifetime (Macke et al., 2012).

502 Here we only tested the effect of the type of dispersal on sexual conflict. However, the  
503 outcome of sexual conflict may also change according to the type of population regulation. Indeed,  
504 under local competition, increased competition among relatives is predicted to cancel out the  
505 benefits of cooperation (Queller, 1992, Taylor, 1992, Wilson et al., 1992). This means that sexual  
506 conflict might be maintained among related individuals when competition is local (Pizzari et al.,  
507 2015, Wild et al., 2011). Yet, despite its general interest, we are not aware of any studies that  
508 explicitly test this.

### 509 *The interplay between sex allocation and sexual conflict*

510 Evolution under different population structures may simultaneously impact sex allocation and  
511 sexual conflict in a non-independent manner (Chapman, 2009, Schärer & Janicke, 2009). One  
512 possibility is that sexual conflict might impact sex allocation if a reduction in female fecundity  
513 prevents the production of optimal offspring sex ratios. Our sexual conflict experiment showed  
514 that females from the ancestral population mated to males from the ‘Global Random’ regime had  
515 the lowest fecundity, suggesting that these males inflict more harm. Yet females from the ‘Global  
516 Random’ regime produced offspring sex ratios closest to those predicted by theory (Table S1). In  
517 addition, ‘Global Random’ females, when mated to ‘Global Random’ males in the sex allocation  
518 experiment had higher fecundity (Figure S4, Table S4). This suggests that ‘Global Random’  
519 females may have evolved mechanisms to overcome male harassment or induced harm, as shown  
520 in this (Macke et al., 2014) and other (Wigby & Chapman, 2004, Michalczyk et al., 2011) systems.  
521 Female resistance to harassment may thus be one trait involved simultaneously in the outcome of  
522 sexual conflict and sex allocation.

523 Conversely, sex allocation may also impact sexual conflict through changes in levels of

524 male-male competition; as the sex ratio becomes more male biased so will the intensity of  
525 competition. Indeed, evolving with kin may reduce male harm and be associated with more female-  
526 biased offspring sex ratios (Lukasiewicz et al., 2017); although the latter was not significantly  
527 different from the non-kin evolution treatment). In our sex allocation experiment, sex ratio was the  
528 least female-biased in the ‘Global Budding’ selection regime. However, males from this regime  
529 inflicted the least harm to females from the ancestral population (sexual conflict experiment),  
530 suggesting sex allocation evolution did not result in stronger sexual conflict.

### 531 **Conclusions**

532 To date, we are only aware of one study that considers the evolution of both sex allocation and  
533 sexual conflict under different population structures (Lukasiewicz et al., 2017). Although the latter  
534 study is a very important step toward integrating responses of both traits, relatedness was the only  
535 variable that differed between treatments. Therefore, much remains to be investigated concerning  
536 the consequences of populations structure for these traits, which have been found to be non-  
537 independent in a number of systems (Macke et al., 2014, Boulton et al., 2019, Boulton & Shuker,  
538 2015). Here, we show that responses to selection on these traits under different population  
539 structures mostly depended on the dispersal regime. Clearly, more studies simultaneously  
540 investigating the evolution of sex allocation and sexual conflict are needed, as interactions among  
541 these traits may account for data not matching theoretical predictions.

542

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550

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552

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