

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

2

3 Authors: Leonor R. Rodrigues<sup>1 †</sup>, Mario Torralba Sáez<sup>2 †</sup>, João Alpedrinha<sup>1</sup>, Sophie Lefèvre<sup>2</sup>,  
4 Muriel Brengues<sup>3</sup>, Sara Magalhães<sup>1</sup> and Alison B. Duncan<sup>2\*</sup>.

5 †These authors contributed equally to the manuscript.

6

7 Author institutional affiliations:

8 1. cE3c: Centre for Ecology, Evolution, and Environmental Changes, Faculty of Sciences,  
9 University of Lisbon, Edifício C2, 38 piso, 1749-016 Lisboa, Portugal

10 3. Institut des Sciences de l'Évolution, Université de Montpellier, CNRS, IRD, EPHE, CC065,  
11 Place Eugène Bataillon, 34095 Montpellier Cedex 05, France

12 4. IRCM, INSERM, Univ. Montpellier, ICM, Montpellier, France

13 Leonor Rodrigues is currently a Post doc at the Department of Zoology, Stockholm University,  
14 SE-106 91 Stockholm, Sweden

15 Corresponding author: [alison.duncan@umontpellier.fr](mailto:alison.duncan@umontpellier.fr).

16

17

18 **Abstract**

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

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

27

28 Females evolving under budding dispersal produced less female-biased offspring sex ratios  
29 than those from the random dispersal selection regimes, contradicting theoretical predictions.  
30 In turn, the scale of competition did not have a strong effect on sex allocation. Males evolved  
31 under budding dispersal induced less female harm than those exposed to random dispersal, but  
32 there was no reduction in female fitness following exposure to multiple mates from either  
33 selection regime.

34

35 This work highlights that population structure can impact the evolution of sex allocation and  
36 sexual conflict. We also discuss how selection on either trait may reciprocally affect the  
37 evolution of the other, for example via effects on fecundity.

38

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

41

## 42 **Introduction**

43 Many organisms exist in structured populations, sub-divided into patches, that are linked and  
44 shaped by demographic factors such as dispersal. The frequency and type of dispersal can  
45 determine whether interactions are more likely to occur among related or unrelated individuals  
46 (Hamilton 1964; Bulmer 1986; Queller 1992; Courteau and Lessard 2000; Rousset 2004; West  
47 2009). For instance, if dispersal is limited, such that only some individuals disperse, the  
48 probability of interactions among genetically related individuals in a patch increases compared  
49 to populations in which all individuals disperse (Hamilton 1964; Taylor 1992; Wilson et al.  
50 1992; Taylor and Crespi 1994). However, if individuals disperse in groups from the same patch  
51 (i.e., if there is budding dispersal), interactions among genetically related individuals can be  
52 maintained, even if dispersal rates are high (Aviles 1993; Gardner and West 2006; Lehmann et  
53 al. 2006; Gardner et al. 2009; Lehmann and Rousset 2010). Dispersal frequency and timing  
54 also influence the scale of competition. For example, high dispersal, and dispersal occurring  
55 prior to the competitive interaction, favour global competition, in which individuals compete  
56 with equal probability with others in the population (Taylor 1992; West et al. 2002a; Griffin et  
57 al. 2004). In contrast, limited dispersal, and/or dispersal occurring after the competitive  
58 interaction, favour local competition (i.e., competition within the natal patch) (Taylor 1992;  
59 Wilson et al. 1992; Frank 1998; West et al. 2002a; Griffin et al. 2004). Therefore, the type,  
60 frequency and timing of dispersal can have a significant impact on relatedness within a patch  
61 and the scale at which competitive interactions occur.

62 In turn, both relatedness and the scale of competition can affect sex allocation - the  
63 differential investment into male and female offspring. Indeed, in subdivided populations, sex  
64 allocation theory predicts an offspring sex-bias towards the sex for which local competition  
65 between kin is less intense (Hamilton 1967; Charnov 1982; West 2009). For example, more  
66 female-biased offspring sex ratios are predicted when males compete locally on their natal  
67 patch for mates, and mated females disperse and compete globally for new patches (Hamilton  
68 1967; Taylor 1981; Herre 1985). If there is budding dispersal, relatedness among the offspring  
69 of foundresses increases, exacerbating local competition between related males for mates, thus  
70 selecting for even more female-biased sex ratios (Gardner et al. 2009). However, if the  
71 proportion of individuals dispersing is limited, and females compete locally for resources,  
72 competition becomes intense for both sexes and selection favours a more balanced offspring  
73 sex ratio (Table S1; Frank 1985; Herre 1985; Taylor and Crespi 1994; Courteau and Lessard  
74 2000). A few empirical studies to date have investigated the consequences of budding dispersal  
75 (Kummerli et al. 2009), or disentangled the relative effects of the scale of competition and

76 relatedness (Griffin et al. 2004) on the evolution of kin-selected behaviours, but none have  
77 disentangled the effect of these two factors on sex allocation.

78 Population structure is also predicted to impact the evolution of sexual conflict, i.e.,  
79 asymmetric reproductive interests between mating partners (Bourke 2009; Pizzari et al. 2015).  
80 Theoretical work predicts that global competition selects for reduced harming behaviour of  
81 males, when interactions occur among kin, as harm reduces patch productivity (Rankin 2011;  
82 Pizzari and Gardner 2012; Pizzari et al. 2015). A number of empirical studies are compatible  
83 with this prediction (Carazo et al. 2014; Hollis et al. 2015; Lukasiewicz et al. 2017, but see  
84 Chippindale et al. 2015). For instance, in the fruit fly *Drosophila melanogaster*, females  
85 repeatedly exposed to related, as opposed to unrelated, males presented a higher lifetime  
86 reproductive success (Carazo et al. 2014). The type of dispersal may also be crucial for the  
87 evolution of sexual conflict. As described above, random dispersal reduces relatedness among  
88 competitors, which might increase the intensity of sexual conflict (Rankin 2011; Faria et al.  
89 2015). In contrast, sexual conflict may be reduced by budding dispersal, which maintains  
90 interactions among kin.

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

103 Here, we uncover the effects of the type of dispersal and the scale of competition, on  
104 the evolution of sex allocation and sexual conflict in the spider mite *Tetranychus urticae*.  
105 Previous work has shown sex allocation and sexual conflict evolution in *T. urticae* when males  
106 compete locally for mates and females compete globally for patches (Macke et al. 2011; Macke  
107 et al. 2014), and that multiple mating can be costly for females (Macke et al. 2012; Rodrigues  
108 et al. 2020). In a fully crossed design, using experimental evolution, we placed replicate

109 populations of *T. urticae* in 4 selection regimes with either local or global competition, and  
110 random *versus* budding dispersal. This design enabled us to follow evolution of both sex ratio  
111 and sexual conflict under different population structures.

## 112 **Material and Methods**

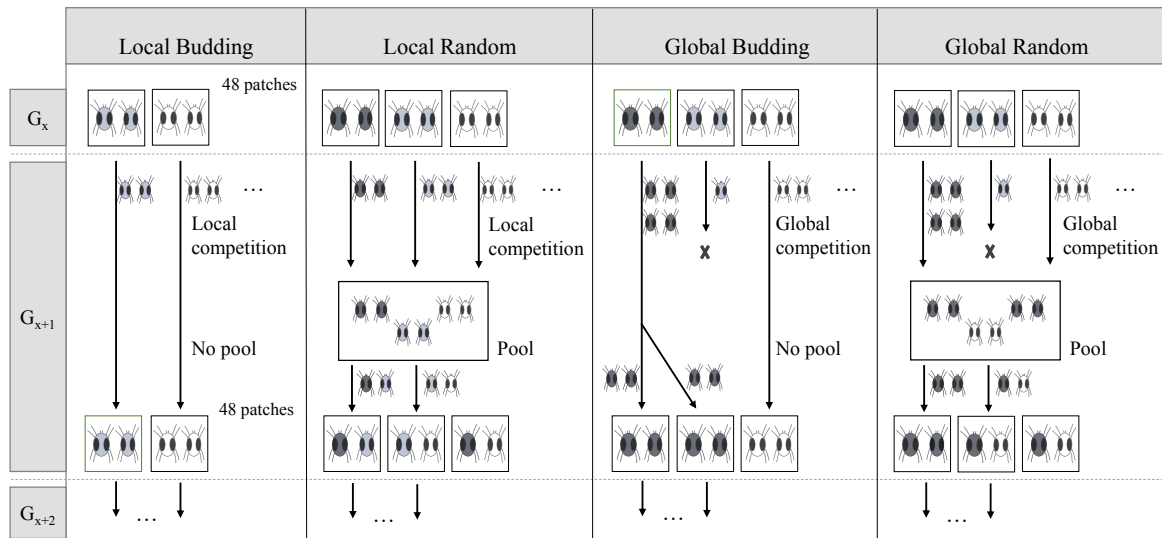
### 113 *Biological model*

114 The two-spotted spider mite, *T. urticae* Koch (Acari: Tetranychidae), is a generalist herbivore  
115 with a host range of over 1100 plant species (Helle and Sabelis 1985; Migeon and Dorkeld  
116 2019). *T. urticae* has an arrhenotokous haplodiploid life cycle (~14 days egg – adult at 20-  
117 25°C): sons develop from unfertilised, haploid eggs and daughters from fertilised, diploid eggs.  
118 We report tertiary sex ratios (adult males divided by the total number of adult offspring) as  
119 males and females can only be distinguished as adults using microscopy: males are smaller  
120 than females and possess a pointed abdomen.

### 121 *Population origins*

122 In 2013, 10 different *T. urticae* populations were collected and separately maintained on bean  
123 plants at  $25 \pm 2^\circ\text{C}$ , with a 16h light: 8h dark cycle at the University of Lisbon. These  
124 populations comprised seven populations from Portugal (Lou, DC, AMP, DF, CH, COL and  
125 RF), two from Spain (Albe and Alro) and one from France (FR) (Zélé et al. 2018). All  
126 populations were treated with antibiotics to ensure that they were free of bacterial  
127 endosymbionts, known to be sex ratio distorters (Breeuwer 1997). The sex ratio of each  
128 individual population ranges from 0.22 to 0.40 (Zélé et al. 2020). In November 2015, more  
129 than 50 females from each of the 10 populations were transferred to the University of  
130 Montpellier and mixed to form a genetically diverse population to seed the experiment  
131 (hereafter called the ‘ancestral population’). This newly mixed population was maintained on  
132 12 whole bean plants (variety: Pongo) in a plastic box (395 mm length x 335 mm width x 323  
133 mm height) at  $25^\circ\text{C}$  with a 16h light: 8h dark cycle. Each week, old plants were removed and  
134 replaced with young, un-infested plants. All bean plants used to maintain mite populations and  
135 for all experiments described below were grown from seeds in an isolated, herbivore-free room  
136 at  $23 \pm 1^\circ\text{C}$  with a photoperiod of 12h light: 12h dark at the University of Montpellier.

137 Fourteen days before starting the experiment, 10 independent groups of 40 females  
138 were haphazardly sampled from the ancestral population and put on a patch (10-15 bean leaves  
139 placed together) on water-saturated cotton wool to lay eggs. This allowed maternal effects to



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

be equalised and ensured that females seeding the experiment were of the same age. Two weeks later, when mites of the following generation had reached adulthood, all 10 groups were mixed, and the newly emerged adult females were haphazardly assigned to the different selection regimes.

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

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

157 For each replicate population, each generation comprised a total of 96 adult females, being  
158 assigned in pairs to 48 bean leaf patches (4cm<sup>2</sup> each) placed on water-saturated cotton wool in  
159 a plastic box (255 mm length x 183 mm width x 77 mm height). All replicates from all regimes  
160 were maintained in the same conditions, the only difference being how populations were mixed  
161 and transferred to new patches at each generation (Figure 1).

162 In the budding dispersal regimes, females were always transferred with another female  
163 from the same patch to form the next generation. In contrast, in the random dispersal regimes,  
164 females from different patches were placed together on a ‘mixing patch’ (10 bean leaves placed  
165 together) before being transferred, in haphazardly chosen pairs, to a new patch. Local  
166 competition was imposed by letting an equal number of adult females per patch seed the next  
167 generation (2 – 4 females per patch in ‘Local Random’; adjusted each generation to  
168 accommodate mortality). Under global competition, relative patch productivity (the total  
169 number of daughters produced compared to that of the other patches within the replicate)  
170 determined the number of female adult offspring transferred to the next generation: in the  
171 ‘Global Random’ regime, all 48 patches were placed on a ‘mixing patch’ onto which adult  
172 females dispersed (patches with more female offspring having a higher representation on the  
173 ‘mixing patch’) before being transferred in pairs; in the ‘Global Budding’ regime, the number  
174 of adult females on each patch was counted to calculate relative fecundity (i.e. dividing the  
175 number of females per patch by the total number of females across the 48 patches), so that  
176 patches with the most offspring contributed more pairs of females to the next generation.

177 Due to the time taken for each transfer, transfers from one generation to the next were  
178 done over 1, 2 or 3 days. When done over more than one day, at least one replicate population  
179 from each regime was transferred on the same day. All replicates were maintained in a climate  
180 chamber at  $25 \pm 2^\circ\text{C}$ , with a photoperiod of 16h light: 8h dark. During the selection experiment,  
181 all replicates in the ‘Local Budding’ regime were lost after generation 14, and 1 replicate in the  
182 ‘Global Budding’ regime was lost at generation 22 (GB-3). In total, 33 generations of selection  
183 were performed.

## 184 ***Responses to selection***

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

186 The sex allocation of females was measured directly in the replicate populations of each  
187 selection regime at generations 12, 17, 20 and 31. This was done by counting the number of

188 males and females per patch within each experimental replicate prior to the following transfer.  
189 Thus, sex ratio comprised the combined output of the two females per patch.

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

191 In this assay, all regimes were each exposed to a common environment for 1 generation to  
192 equilibrate maternal effects before measuring the offspring sex-ratios of females that mated  
193 randomly with males from their selection regime (Figure S1). For this, at generation 31, 96  
194 mated daughters were haphazardly chosen from the 48 patches within each selection regime  
195 and placed on a large leaf patch (~200cm<sup>2</sup>) where they laid eggs together. Fourteen days later,  
196 the offspring on these patches emerged as adults and mated amongst themselves (Generation  
197 31 + 1). Ninety-six mated female offspring from each mixing patch were then haphazardly  
198 chosen to measure their offspring sex-ratio; 48 were placed individually on 2cm<sup>2</sup> patches, and  
199 another 48 placed in pairs on 4 cm<sup>2</sup> patches. Females were allowed to lay eggs for 7 days on  
200 these new patches, before being killed. After 2 weeks, once offspring had emerged as adults,  
201 the number of daughters and sons on each patch was counted. This experiment was set up over  
202 three days, with one replicate per regime being treated each day.

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

204 Measures of offspring sex ratio on patches concern the sex allocation of two females on that  
205 patch. While this is informative, it may obscure responses to selection, especially if offspring  
206 sex ratio differs between females, for instance, if a focal female's sex allocation changes in  
207 response to her own fecundity only, or also to that of her patch mate (Stubblefield and Seger  
208 1990). To test this hypothesis, we measured the sex allocation of single females from our  
209 selection regimes in response to the presence of eggs -that do not hatch- laid by sterile females  
210 on the same patch (Osouli et al. 2014).

211 This experiment was implemented after 33 generations of selection. As for the  
212 preceding experiment, individuals within each replicate population were subject to a common  
213 environment. However, in this experiment it was over two generations (generation 33 + 2;  
214 Figure S1). At the same time, females from the ancestral population were placed in a common  
215 environment for 2 generations, as done with females from the selection regimes (Figure S1) to  
216 generate sterile females. To sterilise these females, they were exposed to 100 Gy, at a dose of  
217 2.7 Gy minute<sup>-1</sup>, using a Xstrahl XenX pre-clinical irradiator at the Institute of Cancer  
218 Research, Montpellier (IRCM). Preliminary studies revealed that this dose of X-ray irradiation



219 is sufficient to sterilise females, that lay eggs that do not hatch (see Table S2).

220 Single adult females from the different selection regimes were placed on individual leaf  
221 patches with one sterile female and allowed to lay eggs for 5 days. Both females were then  
222 killed and the total number of eggs per patch (laid by the sterile and the fertile female, coming  
223 from the ancestral population and a selection regime, respectively) was counted. Nine days  
224 later the adult offspring of the fertile female were counted, and the offspring sex ratio measured.  
225 A total of 48 leaf patches (4 cm<sup>2</sup>) were set up per replicate population.

#### 226 **4. Sexual conflict**

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

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

244 The 240 quiescent, virgin females (i.e. daughters) were taken from the ancestral  
245 population and were kept isolated for 2 days on their individual patches. Subsequently, the eggs  
246 laid by these females were removed and patches were assigned to males from either the ‘Global  
247 Random’ or ‘Global Budding’ selection regime, and to a ‘single’ or ‘double’ mate treatment  
248 (N=30 per treatment). In all treatments, males from the selection regimes were placed with the  
249 virgin females for 5 hours on day 1 of the experiment. Twenty-four hours later (day 2), in

250 patches assigned to the double mating treatment, a second male was placed on the patch and  
251 left for 5 hours. In both treatments, females were left to lay eggs and on day 6 of the experiment,  
252 female mortality was checked and females alive were removed from the patches. The total  
253 number of eggs per patch was counted and, 8 days later, offspring sex-ratio was measured.

## 254 ***Statistical analysis***

255 All analyses were carried out using the R statistical package (v. 3.0.3) and JMP13. We used  
256 Generalised Linear Mixed Models (GLMMs, package glmmTMB; Brooks et al. 2017) with a  
257 beta-binomial error structure and logit link function, and quasi-poisson or negative binomial  
258 error structures and log link function, to analyse the effect of selection regime on sex ratio and  
259 mean offspring production, respectively. Maximal models were simplified by sequentially  
260 eliminating non-significant terms ( $p < 0.05$ ) from the highest- to the simplest-order interaction,  
261 with the highest p-value to establish a minimal model (Crawley 2007). The significance of the  
262 explanatory variables in the minimal models was established using chi-squared tests (Bolker et  
263 al. 2009). *A posteriori* contrasts with Bonferroni corrections were done to interpret the effect  
264 of selection regime when significant (glht, multcomp package; Hothorn et al. 2008). Details of  
265 all models are given in Table S3.

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

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

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

274 To investigate the effect of selection regime on offspring sex ratio in a common environment,  
275 we used a model with selection regime (GB, GR and LB), the number of females per patch (1  
276 or 2) and their interaction as fixed factors, and replicate population (GR-3, GB-1, GB-2, GR-  
277 1, GR-2, LR-2 and LR-3), nested within selection regime as a random factor. This analysis  
278 excluded replicate LR-1 due to fewer than 8 patches with more than 3 offspring. For this  
279 variable, the best fit model included a parameter to account for zero inflation ( $ziformula \sim 1$ ;

280 package glmmTMB; Brooks et al. 2017).

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

282 In a second analysis, using data from the ‘*Sex allocation in response to patch fecundity*’  
283 experiment, we investigated whether the sex allocation of the focal female depended on her  
284 relative fecundity (‘relative patch fecundity’: the number of eggs laid by the focal female  
285 divided by the total number of eggs laid on the patch) and on the total number of eggs present  
286 in the patch (‘total patch fecundity’). In this analysis, the selection regime of the focal female  
287 (GB, GR and LB) and its interaction with relative (or total) patch fecundity were included in  
288 models as fixed factors, and experimental replicate (GB-1, GB-2, GR-2, GR-3, LR-2 and LR-  
289 3) nested within selection regime as a random factor. These analyses only included females  
290 alive on day 4 of the experiment and excluded replicates GR-1 and LR-1, due to fewer than 10  
291 patches with more than 3 offspring.

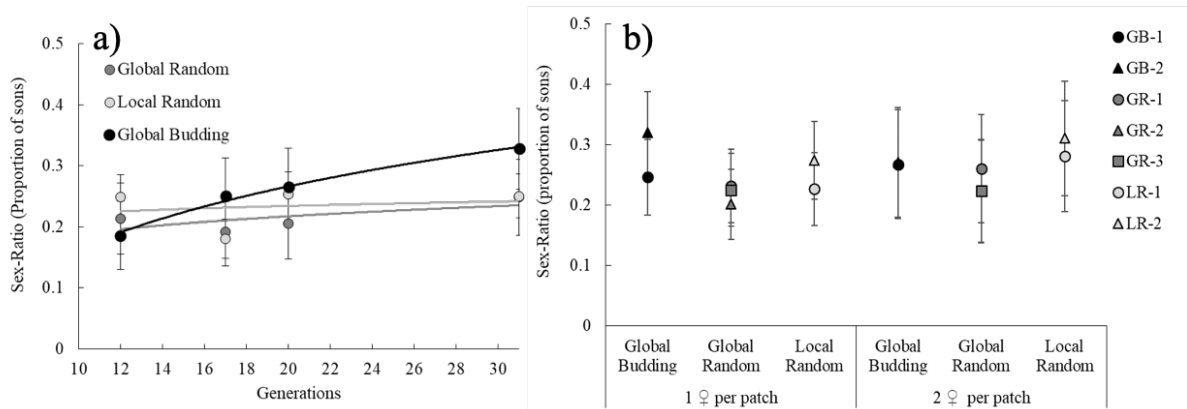
292 We used data from this experiment to compare observed offspring sex ratios with  
293 predicted values from theoretical models (see Supplementary Materials Table S1 for details)  
294 using two tailed t-tests in JMP13. Observed offspring sex ratios were mean values for fertile  
295 females from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

### 296 **4. Sexual conflict**

297 To test whether selection regime affected the intensity of sexual conflict and male-male  
298 competition, female fecundity and offspring sex-ratio were analysed including the number of  
299 mates (one or two mates), the selection regime of the male (‘Global Budding’ *versus* ‘Global  
300 Random’) and their interaction as discrete, fixed variables in the model. Replicate population  
301 (GB-1, GB-2, GR-1, GR-2 and GR-3) and box (the container in which several individual  
302 replicates were maintained) were included nested within dispersal type as random factors. In  
303 the analysis of female fecundity, all individual replicates in which females died before day six  
304 were excluded.

305

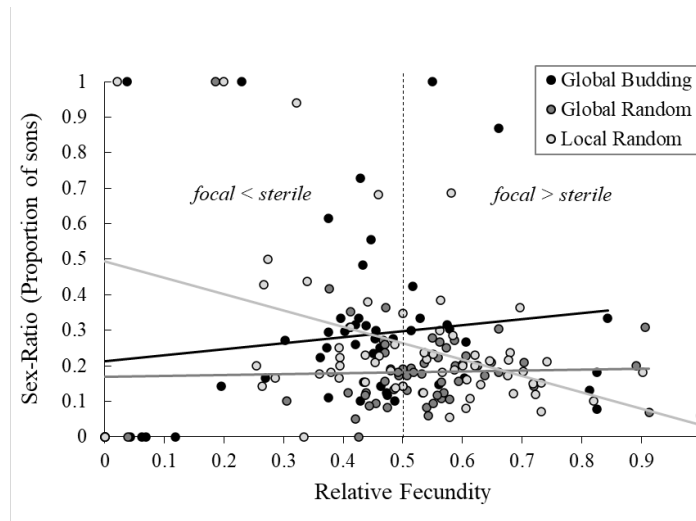
306 **Results:**



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

311 ***Sex allocation during experimental evolution and in a common environment***

312 There was a consistent significant effect of selection regime on sex allocation during the  
 313 selection experiment and after a generation in a common environment (during selection  
 314 experiment:  $X^2_2 = 14.046$ ,  $p < 0.001$ ; common environment:  $X^2_2 = 11.845$ ,  $p = 0.002$ ; Figures  
 315 2a and 2b, Table S4). Indeed, females from the ‘Global Budding’ regime produced less female-  
 316 biased offspring sex ratios than females from the ‘Global Random’ regime (during selection  
 317 experiment:  $Z = -3.741$ ,  $p < 0.001$ ; common environment:  $Z = -3.384$ ,  $p = 0.002$ ; Table S5).  
 318 There was also a trend for females from the ‘Global Budding’ regime to produce a less female-  
 319 biased offspring sex ratio than females from the ‘Local Random’ regime during the selection  
 320 experiment ( $Z = -2.289$ ,  $p = 0.066$ ), but not after a generation in a common environment ( $Z =$   
 321  $1.53$ ,  $p = 0.331$  Figures 2a and 2b; Table S5). There was no difference in sex allocation between  
 322 females from the ‘Global Random’ and ‘Local Random’ regimes (during selection experiment:  
 323  $Z = 1.554$ ,  $p = 0.361$ ; common environment:  $Z = -1.597$ ,  $p = 0.3776$ ; Figures 2a and 2b; Table  
 324 S5). The number of females on a patch had no effect on offspring sex ratio (selection regime x  
 325 number of females per patch:  $X^2_2 = 4.114$ ,  $p = 0.128$ ; number of females per patch:  $X^2_1 = 0.94$ ,  
 326  $p = 0.331$ ; Table S4).



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

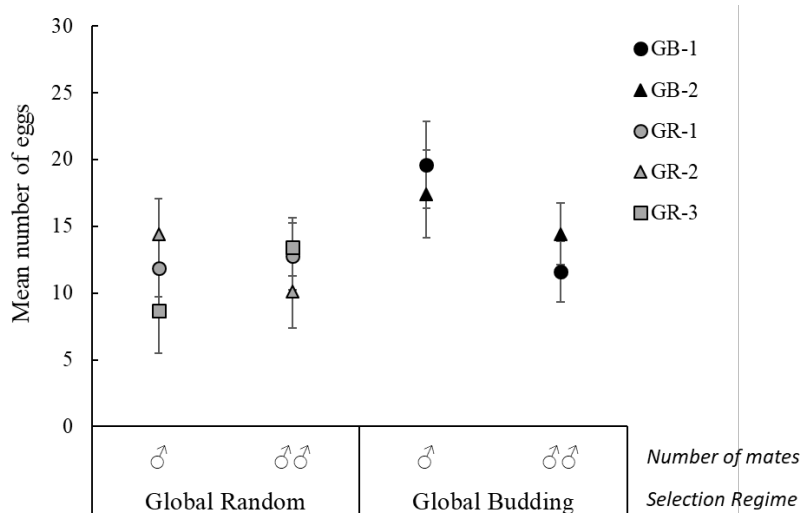
### *Comparing observed offspring sex-ratios with theoretical predictions*

We compared mean offspring sex ratios obtained in the different selection regimes with theoretical predictions (Taylor and Bulmer 1980; Herre 1985; Gardner et al. 2009) (See Table S1 for predicted offspring sex ratios).

Females from the ‘Global Random’ selection regime produced an offspring sex ratio of 0.19 ( $\pm 0.19$  SE), that does not differ from the predictions of Taylor and Bulmer (1980) and of Gardner et al (2009) ( $t = 0.932$ ,  $df = 69$ ,  $p = 0.3544$ ). In contrast, the evolved offspring sex ratios in the ‘Global Budding’ and ‘Local Random’ selection regimes differed from theoretical predictions. Specifically, females from the ‘Global Budding’ selection regime produced a less female-biased offspring sex ratio (mean  $0.30 \pm 0.03$  SE;  $t = 9.54$ ,  $df = 55$ ,  $p < 0.0001$ ), and females from the ‘Local Random’ regime a more female-biased offspring sex ratio (mean  $0.24 \pm 0.02$  SE;  $t = 7.99$ ,  $df = 74$ ,  $p < 0.0001$ ), than predicted by theory.

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

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



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

= 6.87,  $p = 0.009$ ; Figure 3, Table S4). As before, females from the ‘Global Budding’ regime produced less female-biased offspring sex ratio than females from the ‘Global Random’ regime ( $Z = -3.298$ ,  $p = 0.003$ ; Figure 3, Table S5). The offspring sex ratio of females from the ‘Local Random’ treatment did not differ from that of the other two selection regimes (Table S5). Across all treatments, females with higher relative patch fecundity produced more female-biased offspring sex-ratios (selection regime  $\times$  relative patch fecundity:  $X^2_2 = 2.55$ ,  $p = 0.28$ ; Figure 3). These results did not change when using total patch fecundity (sum number of eggs laid by the fertile and sterile female on each patch, Figure S2, Tables S4 and S5;).

### *Sexual conflict*

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

## 369 **Discussion**

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

## 380 ***Sex Allocation***

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

391 First, inbreeding is probably confounded with dispersal type (with high inbreeding  
392 expected for the budding dispersal regime) and may be a factor impacting our results.  
393 Inbreeding can in itself select for more female-biased offspring sex ratios (Frank 1985; Herre  
394 1985; Chung et al. 2019). If coupled with high levels of juvenile mortality this could, in some  
395 cases, result in no males on a patch (West et al. 2002b; Chung et al. 2019), potentially  
396 explaining why we lost all 3 replicates of the ‘Local Budding’ and 1 replicate of the ‘Global  
397 Budding’ regimes. At the same time, the accrued costs of inbreeding may negate any benefit  
398 of female-biased sex ratios in the ‘Global Budding’ regime in the replicates that survived  
399 (Greeff 1996). This might be because, in haplodiploids like spider mites, inbreeding costs are

400 expressed mainly in female traits (Tien et al. 2015). As such, there might be selection to  
401 augment the production of sons in environments, or patches, with low fecundity and/or high  
402 offspring mortality brought about by inbreeding, to ensure female fertilisation (West et al.  
403 2002b; Chung et al. 2019). Thus, it is hard to predict the exact impact of inbreeding in our  
404 selection regimes, as we do not have accurate measures of offspring mortality during  
405 experimental evolution. However, we do find that females in the ‘Global Budding’ regime  
406 produced fewer offspring that became adult, which might be due to higher offspring mortality  
407 or lower fecundity (Figure S4, Table S5). These females also produced slightly more male  
408 offspring than those from the ‘Global Random’ regime (Figure S5a, Table S5). Finally, it might  
409 be that inbreeding reduced variation in the ‘Global Budding’ regime, this way preventing it  
410 from being shaped by selection for more female-biased sex ratios. In line with this, the sex ratio  
411 in this regime was similar to that observed in natural spider mite populations, which tend to be  
412  $\sim 0.30$  (Helle and Sabelis 1985).

413         Another factor that can further affect sex allocation is variable clutch sizes in a patch.  
414 When females with asymmetric fecundities oviposit simultaneously on the same patch, the sons  
415 of a more fecund female are subject to more intense competition for mates, as they mostly  
416 compete with brothers to mate with sisters; whereas the sons of a less fecund female mostly  
417 compete with unrelated males to access unrelated females (Stubblefield and Seger 1990). More  
418 fecund females are thus expected to produce more female-biased sex ratios, while less fecund  
419 females should produce less female-biased sex ratios. As a result, the patch sex ratio becomes  
420 skewed towards that produced by the more fecund females, i.e. a more female-biased sex ratio  
421 (Stubblefield and Seger 1990; West 2009). In addition, theory predicts that this adjustment by  
422 females should emerge from a differential investment in daughters, while maintaining a  
423 constant production of sons (Yamaguchi 1985; Frank 1987). Here, we found that for all  
424 selection regimes, the sex ratio declined as the relative fertility of the focal female increased  
425 (the same was observed for total patch fecundity, Figure S2), showing that female fecundity  
426 and sex-ratio are not independent traits. Furthermore, although not significantly different from  
427 the other regimes, ‘Local Random’ females generated the steepest slope, which may suggest a  
428 greater tendency for females from this selection regime to adjust their sex allocation in response  
429 to fecundity. Coupled with higher overall fecundity observed in this selection regime (Figure  
430 S4, Table S4), this may explain why offspring sex ratios are more female-biased than expected.  
431 Finally, although son production is not constant across selection regimes (Figure S5a, Table



432 S4), its variation is much lower than that for the number of daughters produced (Figure S5b).  
433 Again, this seems to be in line with an effect of clutch size on sex allocation.

434 Overall, it may be that selection on productivity is driving our results, overriding  
435 selection for optimal sex allocation. In the ‘Global Budding’ regime an initial selective sweep  
436 for the most productive females may have reduced genetic diversity, which in turn caused  
437 higher levels of inbreeding. In the ‘Local Random’ regime (or both ‘random dispersal’  
438 regimes), competition may be selecting for more productive females, which is linked with more  
439 female-biased offspring sex ratios.

#### 440 *Sexual Conflict*

441 It was expected that multiple mating with unrelated males would cause a greater reduction in  
442 fecundity, and that they would harass females more, than related males (Pizzari et al. 2015).  
443 Accordingly, we found that females mated to males from the ‘Global Random’ regime had  
444 lower fecundity than those mated to males from the ‘Global Budding’ regime. This replicates  
445 previous findings showing that evolving with kin reduced the level of male inflicted harm (to  
446 females) in bulb mites (Lukasiewicz et al. 2017). Other studies have shown that reduced male  
447 harm may be a plastic response to the presence of kin (Carazo et al. 2014; Lymbery and  
448 Simmons 2017). However, in our experiment, since mating was with unrelated females from  
449 the ancestral population, there were no direct cues indicating the presence of kin. In addition,  
450 competitor males coming from the same selection regime experienced 2 generations of  
451 common garden prior to the experiment which probably reduced relatedness among competitor  
452 males. This means that if the response were plastic, then there should be no difference between  
453 selection regimes. Thus, reduced harm was most likely an evolved response in our study.

454 Contrary to expectations (Arnqvist and Rowe 2005), we did not find that multiple  
455 mating reduced fecundity in either selection regime. Possibly, the differences in harm inflicted  
456 by one or two mates over a single, or two successive five-hour periods respectively, might have  
457 been insufficient to detect differences in fecundity between the two treatments. Previous studies  
458 done in spider mites found fecundity costs when females were simultaneously exposed to  
459 multiple mates for periods of 24h hours (Macke et al. 2012; Rodrigues et al. 2020), or exposed  
460 to two mates on multiple occasions during their lifetime (Macke et al. 2012).

461 Here we only tested the effect of the type of dispersal on sexual conflict. However, the  
462 outcome of sexual conflict may also change according to the type of population regulation

463 occurring. Indeed, under local competition, increased competition among relatives is predicted  
464 to cancel out the benefits of cooperation (Queller 1992; Taylor 1992; Wilson et al. 1992). This  
465 means that sexual conflict might be maintained among related individuals when competition is  
466 local (Wild et al. 2011; Pizzari et al. 2015). Yet, despite its general interest, we are not aware  
467 of any studies that explicitly test this.

468

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

470 Evolution under different population structures may simultaneously impact sex allocation and  
471 sexual conflict in a non-independent manner (Chapman 2009; Scharer and Janicke 2009). One  
472 possibility is that sexual conflict might impact sex allocation if a reduction in female fecundity  
473 prevents the production of optimal offspring sex ratios. Our sexual conflict experiment showed  
474 that females from the ancestral population mated to males from the ‘Global Random’ regime  
475 had the lowest fecundity, suggesting that these males inflict more harm. Yet females from the  
476 ‘Global Random’ regime were the most accurate in their sex allocation (Table S1). In addition,  
477 ‘Global Random’ females, when mated to ‘Global Random’ males in the sex allocation  
478 experiment had higher fecundity (Figure S4, Table S4). This suggests that ‘Global Random’  
479 females may have evolved mechanisms to overcome male harassment or induced harm, as  
480 shown in this (Macke et al. 2014) and other (Wigby and Chapman 2004; Michalczyk et al.  
481 2011) systems. Female resistance to harassment may thus be one trait involved simultaneously  
482 in the outcome of sexual conflict and sex allocation.

483         Conversely, sex allocation may also impact sexual conflict through changes in levels of  
484 male-male competition; as the sex ratio becomes more male biased so will the intensity of  
485 competition. Indeed, evolving with kin has been found to reduce male harm and be associated  
486 with more female-biased offspring sex ratios (Lukasiewicz et al. 2017; although the latter was  
487 not significantly different from the non-kin evolution treatment). In our sex allocation  
488 experiment, sex ratio was the least female-biased in the ‘Global Budding’ selection regime.  
489 However, males from this regime inflicted the least harm to females from the ancestral  
490 population (sexual conflict experiment), suggesting sex allocation evolution did not result in  
491 stronger sexual conflict.

### 492 **Conclusions**

493 Our study advances upon previous work investigating the consequences of population structure  
494 on sex allocation and sexual conflict. Overall, we show that responses to selection mostly  
495 depended on dispersal regime which influences whether interactions are mostly with kin or  
496 unrelated individuals. Furthermore, we highlight the need for more studies simultaneously  
497 investigating the evolution of sex allocation and sexual conflict, to account for trade-offs  
498 between these two traits. To date, we are only aware of one study that considers the evolution  
499 of both traits under different population structures (Lukasiewicz et al. 2017), but they only  
500 considered relatedness as the variable that differed between treatments. This is despite the fact  
501 that non-independence between sex allocation and sexual conflict has been found in a number  
502 of systems (Macke et al. 2014; Boulton and Shuker 2015; Boulton et al. 2019) highlighting that  
503 associated constraints may cause observed sex ratios or fecundity to differ from predictions.

#### 504 **Acknowledgements**

505 We would like to thank François Rousset and Elsa Noël for helpful discussion about this  
506 manuscript. This work was funded by an ERC consolidating grant (COMPCON GA 725419)  
507 to SM, a joint grant from the Agence Nationale de la Recherche and the Fundação para a  
508 Ciência e a Tecnologia to Isabelle Olivieri and SM (FCT-ANR/BIA- EVF/0013/2012), a PHC-  
509 PESSOA grant (38014YC) to ABD and SM, and a SIRIC Montpellier Cancer Grant  
510 (INCa\_Inserm\_DGOS\_12553) funded the irradiator. This is ISEM contribution number 20XX-  
511 XXX.

512

#### 513 **References**

- 514 Arnqvist, G. and L. Rowe. 2005. *Sexual Conflict*. Princeton University Press, Princeton.
- 515 Aviles, L. 1993. Interdemic selection and the sex ratio: A social spider perspective. *Am. Nat.*  
516 142:320-345.
- 517 Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. Stevens, and J.  
518 S. White. 2009. Generalized linear mixed models: a practical guide for ecology and  
519 evolution. *Trends. Ecol. Evol.* 24:127-135.
- 520 Boulton, R. A., N. Cook, E. V. Greenway, G. L. Glaser, J. Green, and D. M. Shuker. 2019.  
521 Local mate competition modifies the costs of mating in a mostly monandrous parasitoid  
522 wasp. *Behav. Ecol.* 30:417-425.
- 523 Boulton, R. A. and D. M. Shuker. 2015. A sex allocation cost to polyandry in a parasitoid wasp.  
524 *Biol. Lett.* 11:20150205.

525 Bourke, A. F. 2009. The kin structure of sexual interactions. *Biol. Lett.* 5:689-692.

526 Breeuwer, J. A. 1997. *Wolbachia* and cytoplasmic incompatibility in the spider mites  
527 *Tetranychus urticae* and *T. turkestanii*. *Heredity* 79:41-47.

528 Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, A. Nielson, H. J. Skuag, M.  
529 Maechler, and B. M. Bolker. 2017. Balances Speed and Flexibility among Packages for  
530 Zero-Inated Generalized Linear Mixed Modeling. *The R Journal* 9:41-47.

531 Bulmer, M. G. 1986. Sex ratio theory in geographically structured populations. *Heredity* 56:69  
532 - 73.

533 Carazo, P., C. K. Tan, F. Allen, S. Wigby, and T. Pizzari. 2014. Within-group male relatedness  
534 reduces harm to females in *Drosophila*. *Nature* 505:672-675.

535 Chapman, T. 2009. Sexual conflict and sex allocation. *Biol. Lett.* 5:660-662.

536 Charnov, E. L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton.

537 Chippindale, A. K., M. Berggren, J. H. Alpern, and R. Montgomerie. 2015. Does kin selection  
538 moderate sexual conflict in *Drosophila*? *Proc. Roy. Soc. Lond. Ser B: Biol. Sci.*  
539 282:20151417.

540 Chung, N., J. Pienaar, and J. M. Greeff. 2019. Evolutionary stable sex ratios with non-  
541 facultative male-eggs first sex allocation in fig wasps. *Oikos* 128:859-868.

542 Courteau, J. and S. Lessard. 2000. Optimal sex ratios in structured populations. *J. Theor. Biol.*  
543 207:159 - 175.

544 Faria, G. S., S. A. Varela, and A. Gardner. 2015. Sex-biased dispersal, kin selection and the  
545 evolution of sexual conflict. *J. Evol. Biol.* 28:1901-1910.

546 Frank, S. 1985. Hierarchical selection theory and sex ratios. II. On applying the theory, and a  
547 test with fig wasps. *Evolution* 39:949-964.

548 Frank, S. 1987. Variable sex ratio among colonies of ants. *Behav. Ecol. Sociobiol.* 20:195-201.

549 Frank, S. 1998. *Foundations of social evolution*. Princeton University Press, Princeton.

550 Gardner, A., A. Arce, and J. Alpedrinha. 2009. Budding dispersal and the sex ratio. *J. Evol.*  
551 *Biol.* 22:1036-1045.

552 Gardner, A. and S. A. West. 2006. Demography, altruism, and the benefits of budding. *J. Evol.*  
553 *Biol.* 19:1707-1716.

554 Greeff, J. M. 1996. Alternative mating strategies, partial sibmating and split sex ratios in  
555 haplodiploid species. *J. Evol. Biol.* 9:855-869.

556 Griffin, A. S., S. A. West, and A. Buckling. 2004. Cooperation and competition in pathogenic  
557 bacteria. *Nature* 430:1024-1027.

558 Hamilton, W. D. 1964. The genetical evolution of social behaviour. I. *J. Theor. Biol.* 7:1 - 16.

559 Hamilton, W. D. 1967. Extraordinary sex ratios. *Science* 156:477-488.

560 Helle, W. and M. W. Sabelis. 1985. *Spider Mites: Their Biology, Natural Enemies and Control*.  
561 Elsevier, Amsterdam.

562 Herre, E. A. 1985. Sex ratio adjustment in fig wasps. *Science* 228:896-898.

563 Hollis, B., T. J. Kawecki, and L. Keller. 2015. No evidence that within-group male relatedness  
564 reduces harm to females in *Drosophila*. *Ecol. Evol.* 5:979-983.

565 Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric  
566 models. *Biometrical J.* 50:346-363.

567 Kummerli, R., A. Gardner, S. A. West, and A. S. Griffin. 2009. Limited dispersal, budding  
568 dispersal, and cooperation: an experimental study. *Evolution* 63:939-949.

569 Lehmann, L., N. Perrin, and F. Rousset. 2006. Population demography and the evolution of  
570 helping behaviors. *Evolution* 60:1137-1151.

571 Lehmann, L. and F. Rousset. 2010. How life history and demography promote or inhibit the  
572 evolution of helping behaviours. *Philos. Trans. Roy. Soc. Lond. B Biol. Sci.* 365:2599-  
573 2617.

574 Lukasiwicz, A., A. Szubert-Kruszynska, and J. W. Radwan. 2017. Kin selection promotes  
575 female productivity and cooperation between the sexes. *Sci. Adv.* 3:e1602262.

576 Lymbery, S. J. and L. W. Simmons. 2017. Males harm females less when competing with  
577 familiar relatives. *Proc. Roy. Soc. Lond. Ser B: Biol. Sci.* 284: 20171984.

578 Macke, E., S. Magalhaes, F. Bach, and I. Olivieri. 2011. Experimental evolution of reduced  
579 sex ratio adjustment under local mate competition. *Science* 334:1127-1129.

580 Macke, E., S. Magalhaes, H. Do-Thi Khanh, A. Frantz, B. Facon, and I. Olivieri. 2012. Mating  
581 modifies female life history in a haplodiploid spider mite. *Am. Nat.* 179:E147-162.

582 Macke, E., I. Olivieri, and S. Magalhaes. 2014. Local mate competition mediates sexual  
583 conflict over sex ratio in a haplodiploid spider mite. *Curr. Biol.* 24:2850-2854.

584 Michalczyk, L., A. L. Millard, O. Y. Martin, A. J. Lumley, B. C. Emerson, and M. J. Gage.  
585 2011. Experimental evolution exposes female and male responses to sexual selection  
586 and conflict in *Tribolium castaneum*. *Evolution* 65:713-724.

587 Migeon, A. and F. Dorkeld. 2019. *Spider Mites Web: A Comprehensive Database for the*  
588 *Tetranychidae*.

589 Osouli, S., K. H. I. Nejad, F. Ziaie, and M. Moghaddam. 2014. Gamma irradiation used on  
590 adult *Tetranychus urticae* Koch as a quarantine treatment. *J. Plant Prot. Res.* 54:150-  
591 155.

592 Pizzari, T., J. M. Biernaskie, and P. Carazo. 2015. Inclusive fitness and sexual conflict: how  
593 population structure can modulate the battle of the sexes. *Bioessays* 37:155-166.

594 Pizzari, T. and A. Gardner. 2012. The sociobiology of sex: inclusive fitness consequences of  
595 inter-sexual interactions. *Philos. Trans. Roy. Soc. Lond. B Biol. Sci.* 367:2314-2323.

596 Queller, D. C. 1992. Does population viscosity promote kin selection? *Trends Ecol. Evol.* 7:322  
597 - 324.

598 Rankin, D. J. 2011. Kin selection and the evolution of sexual conflict. *J. Evol. Biol.* 24:71-81.

599 Reece, S. E., D. R. Drew, and A. Gardner. 2008. Sex ratio adjustment and kin discrimination  
600 in malaria parasites. *Nature* 453:609-614.

601 Reece, S. E., D. M. Shuker, I. Pen, A. B. Duncan, A. Choudhary, C. M. Batchelor, and S. A.  
602 West. 2004. Kin discrimination and sex ratios in a parasitoid wasp. *J. Evol. Biol.*  
603 17:208-216.

604 Rodrigues, L. R., A. R. T. Figueiredo, T. Van Leeuwen, I. Olivieri, and S. Magalhaes. 2020.  
605 Costs and benefits of multiple mating in a species with first-male sperm precedence. *J.*  
606 *Anim. Ecol.* 89:1045-1054.

607 Rousset, F. 2004. Genetic structure and selection in subdivided populations. Princeton  
608 University Press, Princeton.

609 Scharer, L. and T. Janicke. 2009. Sex allocation and sexual conflict in simultaneously  
610 hermaphroditic animals. *Biol. Lett.* 5:705-708.

611 Stubblefield, J. W. and J. Seger. 1990. Local mate competition with variable fecundity:  
612 dependence of offspring sex ratios on information utilization and mode of male  
613 production. *Behav. Ecol.* 1:68-80.

614 Taylor, P. D. 1981. Intra-sex and intersex sibling interactions as sex ratio determinants. *Nature*  
615 291:64-66.

616 Taylor, P. D. 1992. Altruism in viscous populations - an inclusive fitness model. *Evol. Ecol.*  
617 6:532 - 356.

618 Taylor, P. D. and M. G. Bulmer. 1980. Local mate competition and the sex ratio. *J. Theor. Biol.*  
619 86:409-419.

620 Taylor, P. D. and B. J. Crespi. 1994. Evolutionary stable strategy sex ratios when correlates of  
621 relatedness can be assessed. *Am. Nat.* 143:297-316.

622 Tien, N. S., M. W. Sabelis, and M. Egas. 2015. Inbreeding depression and purging in a  
623 haplodiploid: gender-related effects. *Heredity* 114:327-332.

624 West, S. A. 2009. Sex Allocation. Princeton University Press, Princeton.

625 West, S. A., I. Pen, and A. S. Griffin. 2002a. Cooperation and competition between relatives.  
626 *Science* 296:72-75.

627 West, S. A., T. G. Smith, and A. F. Read. 2002b. Fertility insurance and the sex ratios of malaria  
628 and related hemosporin blood parasites. *J. Parasitol.* 88:258-263.

629 Wigby, S. and T. Chapman. 2004. Female resistance to male hamr evolves in response to  
630 manipulation of sexual conflict. *Evolution* 58:1028-1037.

631 Wild, G., T. Pizzari, and S. A. West. 2011. Sexual conflict in viscous populations: the effect of  
632 the timing of dispersal. *Theor. Popul. Biol.* 80:298-316.

633 Wilson, D. S., G. B. Pollock, and L. A. Dugatkin. 1992. Can altruism evolve in purely viscous  
634 populations? *Evol. Ecol.* 6:331-341.

635 Yamaguchi, Y. 1985. Sex ratios of an aphid subject to local mate competition with variable  
636 maternal condition. *Nature* 318:460-462.

637 Zélé, F., I. Santos, M. Matos, M. Weill, F. Vavre, and S. Magalhães. 2020. Endosymbiont  
638 diversity in natural populations of Tetranychus mites is rapidly lost under laboratory  
639 conditions. *Heredity* 124:603-617.

640 Zélé, F., I. Santos, I. Olivieri, M. Weill, O. Duron, and S. Magalhaes. 2018. Endosymbiont  
641 diversity and prevalence in herbivorous spider mite populations in South-Western  
642 Europe. *FEMS Microbiol. Ecol.* 94.

643

644