Transgenerational cues about local mate competition affect offspring sex ratios in the spider mite *Tetranychus urticae*

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Abstract

In structured populations, competition between closely related males for mates, termed Local Mate Competition (LMC), is expected to select for female-biased offspring sex ratios. However, the cues underlying sex allocation decisions remain poorly studied. Here, we test for several cues in the spider mite *Tetranychus urticae*, a species that was previously found to adjust the sex ratio of its offspring in response to indicators of LMC. In particular, we investigate whether the offspring sex ratio of *T. urticae* females changes in response to 1) the current number of females laying eggs in the same patch, 2) the number of females in the patches of their mothers and 3) their relatedness to their mate. Females on patches with 1 (high LMC) or 15 (low LMC) other females produced similar sex ratios. Similarly, the offspring sex ratios of females mated with their brother did not differ with those of females mated with an unrelated male. In contrast, females produced a more female-biased offspring sex ratio if their mother laid eggs on a patch with 1 other female compared to 15 other females. Thus, the maternal environment is used as a cue affecting the sex allocation of daughters. We discuss the conditions under which the maternal environment can be a reliable predictor of inclusive fitness.

INTRODUCTION

In structured populations, competition between closely related males for mates, termed Local Mate Competition (LMC), is expected to select for female-biased offspring sex ratios (Hamilton, 1967; Maynard Smith, 1978; Charnov, 1982; Frank, 1985; Herre, 1985). LMC reduces the value of producing sons relative to daughters, resulting in females allocating more resources to the production of daughters (Hamilton, 1967; Taylor, 1981). Furthermore, in haplodiploid species, mothers that mate with a brother are more related to their daughters than they are to their sons, which should also select for more female-biased offspring sex ratios (Greeff, 1996; Reece *et al.*, 2004).

Population structure often varies in time, and consequently so does the intensity of LMC and sib-mating. In such a situation, selection should favour females that facultatively (plastically) adjust their sex allocation in response to immediate cues in the environment that reflect the intensity of LMC that sons will experience, and sib-mating (Frank, 1985; Herre, 1985; Nagelkerke & Sabelis, 1996; Macke *et al.*, 2011a). For example, females should adjust their offspring sex ratios in response to the number of females laying eggs (foundresses) in the same patch or to the level of relatedness with their mate. LMC and inbreeding may also change in a predictable fashion over generations. This scenario is predicted by haystack models of LMC in which sub-divided populations may persist over multiple generations, and relatedness between females sharing a patch may increase across generations (Nagelkerke & Sabelis, 1996). This increased relatedness can select for even more female biased offspring sex ratios (Frank, 1985; Gardner *et al.*, 2009). If change in the sub-divided population structure is predictable through time, it may be the case that information enabling optimal sex allocation is transferred from mothers to

61 daughters.

62 Consistent with expectations that females respond to current cues in the
63 environment, more female biased offspring sex ratios are observed under high LMC,
64 e.g. in patches formed by a single foundress as compared to patches founded by
65 multiple foundresses, across a whole range of species (Shuker *et al.*, 2005; West *et*
66 *al.*, 2005; Sato & Saito, 2007; Wang *et al.*, 2015). Several studies, however, found
67 that females do not adjust their offspring sex ratio in response to different numbers of
68 foundresses on a patch (Innocent *et al.*, 2007; Abe *et al.*, 2014; Lievens *et al.*, 2016).
69 Furthermore, there is no empirical support that sib-mating results in more female
70 biased offspring sex ratios than random matings (Reece *et al.*, 2004; Burton-Chellew
71 *et al.*, 2008).

72 Variation in the occurrence of adaptive sex allocation adjustment may be
73 related to the fact that the underlying cues remain under-studied. In principle, these
74 cues could stem from both the current or past environment, that is factors
75 experienced in the immediate whilst a female is laying her eggs, or those
76 encountered as a juvenile, or even in previous generations. For example, *Nasonia*
77 *vitripennis* females change their sex allocation in response to the current number of
78 foundresses, female odours and the presence of eggs in the environment (Shuker *et*
79 *al.*, 2005, 2007). In contrast, house wrens produce a more female biased offspring
80 sex ratio when raised in a smaller brood (Bowers *et al.*, 2017) and female mice
81 exposed to stress in utero produce more daughters (Edwards et al., 2016).

82 Here, we use the spider mite *Tetranychus urticae* to clarify which cues, are
83 used by females to adjust the sex-ratio of their offspring. Specifically, we investigate
84 whether females alter their sex allocation in response to the number of females
85 laying in the same patch (i.e. current conditions of LMC), the number of females

laying in the patch of their mothers (i.e. past LMC conditions) and sib-mating. An earlier study showed that spider mites evolving under constant, high LMC lost the ability to facultatively adjust their offspring sex ratio, whilst those evolving under weaker LMC retained the ability for facultative sex allocation in response the number of foundresses (Macke *et al.*, 2011a). In this study, sex ratio was measured after mites had spent two generations on patches containing the same number of foundresses. Thus, both mothers and their daughters experienced cues indicating the same intensity of LMC for their sons. Therefore, the set-up did not allow disentangling the exact cue(s) to which females responded. Other studies have shown that spider mites can respond to both maternal cues and those in the immediate environment. Indeed, *T. urticae* females produce more female-biased offspring sex ratios when there are fewer females sharing a patch (Wrensch & Young, 1975; Roeder, 1992). They are also able to recognise kin (Tien *et al.*, 2011; Bitume *et al.*, 2014) and dispersal distance, fecundity, diapause and offspring sex ratio were shown to be affected by maternal effects (Oku *et al.*, 2003; Magalhães *et al.*, 2011; Bitume *et al.*, 2014; Marinosci *et al.*, 2015). Uncovering the interplay between immediate cues in the environment and those experienced in the previous generation will contribute to better understanding about the evolution of plasticity in sex allocation, as well as the genetic and environmental determinism of female biased sex ratios.

108 MATERIAL AND METHODS

109

110 **Biological model**

111 The two-spotted spider mite, *T. urticae* Koch (Acari: Tetranychidae), is a generalist
112 herbivore (Migeon & Dorkeld, n.d.; Helle & Sabelis, 1985) with a short generation
113 time (less than 15 days at 20°C on a suitable host; (Helle & Sabelis, 1985; Riahi *et*
114 *al.*, 2013). It has an arrhenotokous haplodiploid life cycle in which females produce
115 haploid sons parthenogenetically and diploid daughters from fertilised eggs. After the
116 egg stage, all individuals pass through a larval and two nymphal (protonymph and
117 deutonymph) stages, with short quiescence stages between each, before reaching
118 maturity. Mature females are approximately twice as big as, and rounder than,
119 mature males. The sex ratios measured in this study are tertiary sex ratios defined as
120 the proportion of adult males on a patch.

121

122 **Study population**

123 This study uses the same base population as Macke et al. 2011. This population was
124 collected from a cucumber greenhouse in Pijnacker, The Netherlands, in May 1994.
125 Mites were transferred to a climate chamber at the University of Amsterdam where
126 they were maintained on the same host plant. In September 2007, after ~240 months
127 (~480 generations), approximately 5000 mites were sampled from the Amsterdam
128 population to create two populations on cucumber plants at the University of
129 Montpellier. Mite populations were maintained under the same conditions in a single
130 climate chamber at 25°C±2°C, with a photoperiod of 14L:10D. A new base population
131 was created in February 2011 by mixing and transferring approximately 200 adult
132 females from the two previous populations. This new base population was

133 maintained on 4 - 6 cucumber plants contained in a single plastic box (505mm length
134 x 310mm width x 380mm height) with a hole in the lid covered in a fine mesh to avoid
135 condensation. Plants were watered every week and rotten plants removed and
136 replaced with young, mite-free plants; plants were placed beside infested ones to
137 facilitate mite dispersal.
138
139 To start the experiment (generation 0), twelve independent samples from the base
140 population consisting of 40 adult females each were created; this ensures that
141 females seeding the experiment were of the same age. Each sample was placed on
142 a cucumber leaf resting on water-saturated cotton wool in a plastic box (255mm x
143 185mm x 77mm) to prevent both leaf dehydration and mite dispersal. Females were
144 allowed to lay eggs and, 14 days later, their mature adult daughters were sampled to
145 start the 'sex allocation in response to LMC across generations' experiment. In the
146 'sib mating' experiment, slightly older daughters were used as the experiment was
147 started 20 and 21 days after females started laying eggs in generation 0. In all
148 experiments, mites were maintained in the same plastic boxes on leaf fragments
149 placed on saturated cotton wool in climate chambers at 25 ± 2ºC with a 16L:8D cycle.
150 Prior to starting, and during the experiments we confirmed that our *T. urticae*
151 populations were *Wolbachia*-free (see Supplementary Materials for details).
152
153 **Sex allocation in response to LMC across generations**
154 This experiment investigated the sex allocation of females over two generations. In
155 generation 1 (the 'maternal generation'), we investigated the offspring sex ratios in
156 response to high (1 foundress) versus low (15 foundresses) LMC experienced by
157 laying females (these are the daughters of females from Generation 0). Each patch

158 from Generation 0 contributed equal numbers of females, haphazardly sampled, to
159 the high and low LMC treatments. In generation 2 (the 'daughter generation'), we
160 investigated the sex allocation under high LMC of daughters from generation 1,
161 which had experienced either high or low LMC. Thus, in generation 2 we tested
162 whether LMC experienced by mothers influenced the offspring sex ratios of their
163 daughters. In other words, we tested if the sex ratio of the offspring is influenced by
164 the LMC conditions experienced by their grand-mothers; the LMC conditions of all
165 mothers was the same.
166
167 *Generation 1 (maternal generation):*
168 In the high LMC treatment, 240 females were individually placed on a 4-cm^2
169 cucumber leaf patch. We had 5 boxes, each containing 48 of these high LMC
170 patches. In the low LMC treatment, a total of 360 females, in groups of 15 females,
171 were placed on 60-cm^2 cucumber leaf patches. We had 6 boxes each containing 4
172 low LMC patches. Each box was divided into 4 quarters, each quarter containing 12
173 patches in high LMC boxes and 1 patch in low LMC boxes. In both treatments,
174 females laid eggs and fourteen days later, we counted the number of emerging
175 adults on each patch, and determined their sex, using a stereo light microscope in a
176 subset of 3 high LMC boxes and 2 low LMC boxes. Note however that females in
177 Generation 2 could come from patches that were not used in the analysis of sex ratio
178 variation in Generation 1.
179
180 *Generation 2 (daughter generation):*
181 Independently of LMC levels, 12 females were randomly collected from each quarter
182 of each box from Generation 1 and individually placed under high LMC on a 4 cm^2

183 leaf patch. Due to differential fecundity, some females from Generation 1 may thus
184 have contributed more offspring to Generation 2 than did others. All patches were
185 randomly spread across 6 boxes (48 patches per box). Females were allowed to lay
186 eggs and their offspring sex ratio was measured 14 days after transfer. Some of the
187 females transferred to the generation 2 patches died before laying eggs. In total, we
188 measured the offspring sex ratio on 114 patches, from 20 quarters, in low LMC boxes
189 (5 - 6 females per quarter) and 116 patches, from 14 quarters, in high LMC boxes (3
190 - 12 females per quarter).
191
192 **Sex allocation adjustment in response to sib mating**
193 This experiment investigated whether females mated with their brothers produced a
194 more female biased offspring sex ratio than did females mated with unrelated males.
195 In total, 96 females sampled from Generation 0 were individually placed on 4 cm^2
196 patches spread across 2 boxes (48 patches per box). Females were allowed to lay
197 eggs and 9 days later quiescent daughters (2 from each patch, later allocated to
198 each of the two treatments), were individually placed on 4 cm^2 patches to ensure all
199 mites assigned to our mating treatments were virgin. The same was done for
200 emerged or quiescent sons. Mating pairs were formed three days later, when all
201 quiescent females were adult. Females were then placed individually on a 4 cm^2
202 patch and assigned to one of two mating treatments: (1) the sib-mating treatment, in
203 which one haphazardly sampled brother was added to their patch or (2) the unrelated
204 mating treatment, in which a male from another patch was added to their patch.
205 Males were removed after 3 days, females were allowed to lay eggs and 14 days
206 later the sex ratio and number of emerging adults was measured. In total, we formed
207 24 patches with unrelated mates and 28 patches with sib-mating.

208

209 **Statistical analyses**

210 All models were performed in SAS using the GLIMMIX model procedure.

211 *Sex allocation in response to LMC across two generations*

212 In generation 1, we analysed variation in the number of sons in response to the
213 number of other females laying in the same patch using a generalised linear model
214 assuming a binomial distribution. The sex ratio of individual females could only be
215 obtained under high LMC while the sex ratio of groups of 15 females was measured
216 under low LMC. To analyse the number of sons across groups of females of the
217 same size, we randomly formed 9 groups of 15 females under high LMC, and
218 retrieved the total number of sons they produced. This was repeated for 10000
219 different randomly drawn groups from the high LMC treatments, which were
220 compared with the number of sons from the low LMC treatments. This bootstrap
221 analysis provided an empirical distribution of p-values for the effect of LMC; we
222 concluded that LMC affected sex ratio if more than 5% of runs had a p-value below
223 5%.

224 We also analysed variation in mean fecundity per female in each quarter (total
225 number of eggs divided by the number of laying females) to test whether this trait
226 held constant in the different treatments. We analysed variation in brood size using a
227 generalised linear mixed model assuming a Poisson distribution and accounting for
228 LMC treatment as a fixed effect, and the box containing each quarter as a random
229 effect nested within LMC treatment.

230 At generation 2, we tested the effect of the LMC conditions experienced by the
231 mother at generation 1 on the number of sons produced by, and the fecundity of,
232 their daughters using generalised linear mixed models, assuming a binomial

233 distribution and a Poisson distribution, respectively. We accounted for the LMC
234 treatment of the mothers as a fixed effect, and quarter from which each female
235 originated as a random factor nested within LMC treatment.
236
237 *Sex allocation adjustment in response to sib mating*
238 In the sib-mating experiment, to test whether mate relatedness affected the offspring
239 sex ratio and fecundity, variation in the number of males and the total number of
240 offspring were analysed with generalised linear mixed models, assuming a binomial
241 distribution and a Poisson distribution, respectively. The model included the mating
242 treatments of the mothers as a fixed effect and the patch of each mother, as well as
243 the temporal block as random factors.
244
245 RESULTS
246 In all experiments, fecundity was not affected by the intensity of LMC in current or
247 past generations, or by the relatedness between mates (p-values > 0.1; Table 1). The
248 absence of an effect on fecundity ensures that the estimates of sex-ratio have the
249 same precision between LMC and sib-mating treatments.
250
251 *Sex allocation in response to LMC across two generations*
252 At generation 1, females did not modify their offspring sex ratio according to the
253 number of other females laying in the same patch (only 1.6% of the bootstraps were
254 significant; Figure 1A). In contrast, at generation 2, the maternal environment
255 affected the sex allocation of their daughters, as females produced a more female
256 biased offspring sex ratio if their mothers had experienced high, compared to low,
257 LMC ($F_{1,32}$ = 10.98, p-value = 0.002, Figure 1B).

Sex allocation adjustment in response to sib-mating

Mating with a brother or an unrelated male did not change sex allocation ($F_{1,21}$ = 0.06, p-value = 0.82; Fig .1C). This means the effect of high LMC over two generations, on sex allocation, are not due to sib-mating.

DISCUSSION

We found that sex allocation in the spider mite *T. urticae* does not depend on the number of females laying together on the same patch or on the relatedness between mates. Instead, we found that females alter their offspring sex ratio according to the number of females laying on the patch of their mothers. Thus, we show that maternal effects may be important for female sex allocation. None of the treatments -current or maternal levels of LMC, or mating with a sibling- impacted female fecundity.

Female *T. urticae* did not produce a more female-biased sex ratio when they mate with their brother compared to an unrelated male. As spider mites do recognise kin (Tien *et al.*, 2011; Bitume *et al.*, 2013), this calls for an explanation. One possibility may be that there is sexual conflict over the optimal offspring sex ratio (Shuker *et al.*, 2009; Macke *et al.*, 2014). Indeed, in haplodiploids, males only pass their genes to the next generation via daughters thus it is always beneficial to produce a more female-biased offspring sex ratio, whereas for females the optimal offspring sex ratio depends on levels of LMC (Shuker *et al.*, 2009; Macke *et al.*, 2014; Shuker & Cook, 2014). Since male *T. urticae* can manipulate females to produce more female-biased offspring sex ratios (Macke *et al.*, 2014) females may be reluctant to alter their sex allocation in response to any cue provided by their mate, whatever the level of LMC. Another possible explanation is that females do not alter

283 their sex allocation in response to sib mating due to selection to reduce inbreeding
284 (Greeff, 1996). Indeed, *T. urticae* suffers from inbreeding depression (Tien *et al.*,
285 2014; Yoshioka & Yano, 2014). Furthermore, *T. urticae* actively avoids mating with
286 kin as females prefer to mate with unrelated males over brothers (Tien *et al.*, 2011)
287 and males prefer to mate with unfamiliar females (Yoshioka & Yano, 2014).

288 As for a number of other species (Reece *et al.*, 2005; Innocent *et al.*, 2007;
289 Abe *et al.*, 2014) we did not find that female *T. urticae* alter their sex allocation in
290 response to the number of other females laying in the same patch (but see (Wrensch
291 & Young, 1975; Roeder, 1992). This suggests that, for this population, the number of
292 females in the current patch does not provide reliable information about the LMC
293 level that the offspring will experience, as found in other studies. For example, in
294 *Nasonia vitripennis* egg presence and the relative number of eggs a female
295 contributes to a patch had a stronger influence on sex allocation than the number of
296 foundresses (Shuker & West, 2004; Burton-Chellew *et al.*, 2008). This may be
297 because (a) females often move away from their laying patches or (b) females have
298 high mortality during the egg laying period. In spider mites, the first hypothesis is
299 unlikely, as patches are relatively-stable entities (Janssen & Sabelis, 1992). The
300 second hypothesis is likely if females are on low-quality plants (Magalhaes *et al.*,
301 2007; Marinosci *et al.*, 2015).

302 Instead, we found that females produce a more female biased offspring sex
303 ratio when their mothers experience high LMC. Maternal effects have been shown to
304 have a role determining many offspring traits including offspring size and juvenile
305 survival (Pick *et al.*, 2016), resistance to parasites (Little *et al.*, 2003) and competitive
306 ability (Bentz *et al.*, 2016). In *T. urticae* maternal effects have been shown to partly
307 determine offspring phenotypes for dispersal distance (Bitume *et al.*, 2014), diapause

308 incidence (Oku *et al.*, 2003) and offspring life-history traits (Magalhães *et al.*, 2011;
309 Marinosci *et al.*, 2015). To the best of our knowledge, maternal effects have not been
310 previously shown to play a role in determining offspring sex allocation. One exception
311 may be a study in mice, which found that females produce a more female bias
312 offspring sex ratio if they experience stress *in utero* (Edwards *et al.*, 2016). Whether
313 this can be considered as a maternal effect is debatable, as the developing individual
314 is itself experiencing the stress. This maternal effect may be responsible for the
315 results observed in the selection experiment by Macke *et al.* (2011). Indeed, in this
316 experiment, mothers and daughters experienced the same LMC level, so the sex
317 ratio adjustment may be explained by the maternal environment. Under predictable,
318 high LMC, the maternal transfer of information determining a females' sex allocation
319 may have been lost.

320 We cannot exclude the juvenile environment as a possible proximal cause of
321 female biased sex allocation depending on maternal LMC. When mothers experience
322 high LMC, and are alone on a patch, females will develop with their siblings whereas
323 under low LMC juveniles will share their patch with both related and unrelated
324 individuals. Furthermore, although we controlled for density and fecundity variation
325 across treatments, under low LMC there is a higher absolute number of juveniles
326 than under high LMC.

327 Egg size is thought to be one way by which female *T. urticae* can plastically
328 adjust their sex allocation, as fertilisation probability increases with egg size, thus
329 bigger eggs will be more likely to develop into females (Macke *et al.*, 2011b).
330 Furthermore, *T. urticae* evolving under high LMC produce larger eggs and a more
331 female biased sex ratio (Macke *et al.*, 2012). Thus, a maternal influence on a
332 daughter's sex allocation might come about via manipulation of a daughter's egg

complement. Female *T. urticae* possess a single ovary within which eggs develop and are fertilised (Feiertag-Koppen & Pijnacker, 1982). Female larvae that have recently emerged from their eggs possess cells in their ovaries undergoing meiosis (Feiertag-Koppen & Pijnacker, 1982; Mothes-Wagner, 1984), thus a mother could manipulate resources by influencing egg development such that her daughters will produce eggs of different sizes.

Why then, should individuals use maternal cues over those present in the immediate environment to alter the sex ratio of their offspring? If levels of LMC change in a predictable fashion across generations (for example, increasing or decreasing LMC) then it may be beneficial for a female to integrate information provided by mothers (Mousseau & Fox, 1998). Indeed, *T. urticae* life-history is such that predictable population groupings may occur across multiple generations on the same host plant, as described by haystack models of LMC (Nagelkerke & Sabelis, 1996). Hence, maternal cues may provide reliable information about the current state of a population. Moreover, patches may be subdivided into smaller ones (Nagelkerke & Sabelis, 1996), making it difficult to properly assess the spatial scale at which competition for mates will take place. It may be that maternal cues provide an integrative measure of population structure, whereas the number of females a female interacts with will convey a more local information, which may be less relevant for sex allocation decisions. Clearly, more studies are needed to assess the relative value of cues informing on population structure and on the precise scale at which competition for mates actually occurs.

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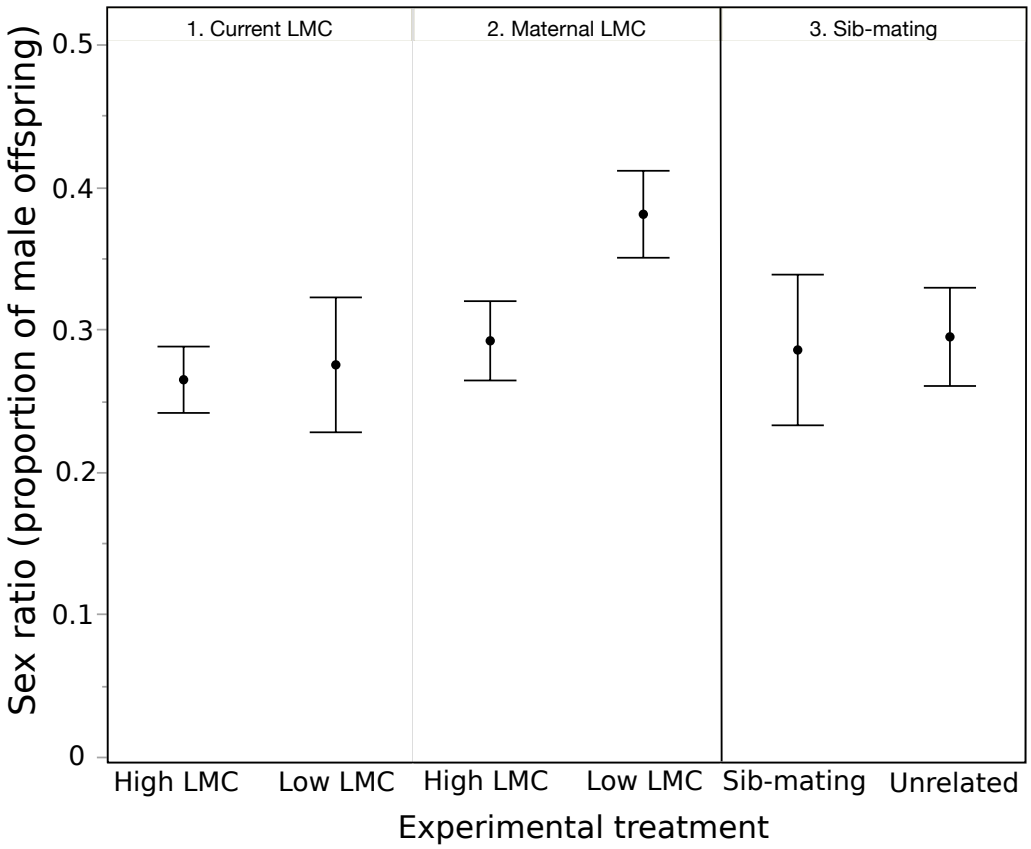
Figure 1. Offspring sex ratio measured as the proportion of male offspring for (A) females experiencing high (1 female) or low (15 females) LMC, (B) females whose mothers experienced high or low LMC and (C) females mated with their brother or an unrelated male. Figures show empirical means ± 1 confidence interval.



368 **Table 1.** Female fecundity A) measured as the mean number of offspring per female
369 in each quarter for generations 1, when females experience high or low LMC, and
370 generation 2 when mothers are exposed to high or low LMC, and B) per patch when
371 females mate with their brother or an unrelated male. The table shows empirical
372 means ± 1 standard error.
373

A. Sex allocation across two generations		
	High LMC	Low LMC
Generation 1	19.01 ± 1.630	20.16 ± 0.920
Generation 2	13.05 ± 0.860	14.79 ± 0.744
B. Sex allocation adjustment in response to sib mating		
	Sib-mating	Unrelated mate
	14.54 ± 1.088	16.42 ± 0.934

374

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