

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

daughters.

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

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

Here, we use the spider mite *Tetranychus urticae* to clarify which cues, are used by females to adjust the sex-ratio of their offspring. Specifically, we investigate whether females alter their sex allocation in response to the number of females 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.

MATERIAL AND METHODS

Biological model

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

Study population

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

maintained on 4 - 6 cucumber plants contained in a single plastic box (505mm length x 310mm width x 380mm height) with a hole in the lid covered in a fine mesh to avoid condensation. Plants were watered every week and rotten plants removed and replaced with young, mite-free plants; plants were placed beside infested ones to facilitate mite dispersal.

To start the experiment (generation 0), twelve independent samples from the base population consisting of 40 adult females each were created; this ensures that females seeding the experiment were of the same age. Each sample was placed on a cucumber leaf resting on water-saturated cotton wool in a plastic box (255mm x 185mm x 77mm) to prevent both leaf dehydration and mite dispersal. Females were allowed to lay eggs and, 14 days later, their mature adult daughters were sampled to start the 'sex allocation in response to LMC across generations' experiment. In the 'sib mating' experiment, slightly older daughters were used as the experiment was started 20 and 21 days after females started laying eggs in generation 0. In all experiments, mites were maintained in the same plastic boxes on leaf fragments placed on saturated cotton wool in climate chambers at $25 \pm 2^\circ\text{C}$ with a 16L:8D cycle. Prior to starting, and during the experiments we confirmed that our *T. urticae* populations were *Wolbachia*-free (see Supplementary Materials for details).

Sex allocation in response to LMC across generations

This experiment investigated the sex allocation of females over two generations. In generation 1 (the 'maternal generation'), we investigated the offspring sex ratios in response to high (1 foundress) versus low (15 foundresses) LMC experienced by laying females (these are the daughters of females from Generation 0). Each patch

from Generation 0 contributed equal numbers of females, haphazardly sampled, to the high and low LMC treatments. In generation 2 (the 'daughter generation'), we investigated the sex allocation under high LMC of daughters from generation 1, which had experienced either high or low LMC. Thus, in generation 2 we tested whether LMC experienced by mothers influenced the offspring sex ratios of their daughters. In other words, we tested if the sex ratio of the offspring is influenced by the LMC conditions experienced by their grand-mothers; the LMC conditions of all mothers was the same.

Generation 1 (maternal generation):

In the high LMC treatment, 240 females were individually placed on a 4-cm² cucumber leaf patch. We had 5 boxes, each containing 48 of these high LMC patches. In the low LMC treatment, a total of 360 females, in groups of 15 females, were placed on 60-cm² cucumber leaf patches. We had 6 boxes each containing 4 low LMC patches. Each box was divided into 4 quarters, each quarter containing 12 patches in high LMC boxes and 1 patch in low LMC boxes. In both treatments, females laid eggs and fourteen days later, we counted the number of emerging adults on each patch, and determined their sex, using a stereo light microscope in a subset of 3 high LMC boxes and 2 low LMC boxes. Note however that females in Generation 2 could come from patches that were not used in the analysis of sex ratio variation in Generation 1.

Generation 2 (daughter generation):

Independently of LMC levels, 12 females were randomly collected from each quarter of each box from Generation 1 and individually placed under high LMC on a 4 cm²

leaf patch. Due to differential fecundity, some females from Generation 1 may thus have contributed more offspring to Generation 2 than did others. All patches were randomly spread across 6 boxes (48 patches per box). Females were allowed to lay eggs and their offspring sex ratio was measured 14 days after transfer. Some of the females transferred to the generation 2 patches died before laying eggs. In total, we measured the offspring sex ratio on 114 patches, from 20 quarters, in low LMC boxes (5 - 6 females per quarter) and 116 patches, from 14 quarters, in high LMC boxes (3 - 12 females per quarter).

Sex allocation adjustment in response to sib mating

This experiment investigated whether females mated with their brothers produced a more female biased offspring sex ratio than did females mated with unrelated males. In total, 96 females sampled from Generation 0 were individually placed on 4 cm² patches spread across 2 boxes (48 patches per box). Females were allowed to lay eggs and 9 days later quiescent daughters (2 from each patch, later allocated to each of the two treatments), were individually placed on 4 cm² patches to ensure all mites assigned to our mating treatments were virgin. The same was done for emerged or quiescent sons. Mating pairs were formed three days later, when all quiescent females were adult. Females were then placed individually on a 4 cm² patch and assigned to one of two mating treatments: (1) the sib-mating treatment, in which one haphazardly sampled brother was added to their patch or (2) the unrelated mating treatment, in which a male from another patch was added to their patch. Males were removed after 3 days, females were allowed to lay eggs and 14 days later the sex ratio and number of emerging adults was measured. In total, we formed 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

distribution and a Poisson distribution, respectively. We accounted for the LMC treatment of the mothers as a fixed effect, and quarter from which each female originated as a random factor nested within LMC treatment.

Sex allocation adjustment in response to sib mating

In the sib-mating experiment, to test whether mate relatedness affected the offspring sex ratio and fecundity, variation in the number of males and the total number of offspring were analysed with generalised linear mixed models, assuming a binomial distribution and a Poisson distribution, respectively. The model included the mating treatments of the mothers as a fixed effect and the patch of each mother, as well as the temporal block as random factors.

RESULTS

In all experiments, fecundity was not affected by the intensity of LMC in current or past generations, or by the relatedness between mates (p-values > 0.1; Table 1). The absence of an effect on fecundity ensures that the estimates of sex-ratio have the same precision between LMC and sib-mating treatments.

Sex allocation in response to LMC across two generations

At generation 1, females did not modify their offspring sex ratio according to the number of other females laying in the same patch (only 1.6% of the bootstraps were significant; Figure 1A). In contrast, at generation 2, the maternal environment affected the sex allocation of their daughters, as females produced a more female biased offspring sex ratio if their mothers had experienced high, compared to low, 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

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

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

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

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

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

Egg size is thought to be one way by which female *T. urticae* can plastically adjust their sex allocation, as fertilisation probability increases with egg size, thus bigger eggs will be more likely to develop into females (Macke *et al.*, 2011b). Furthermore, *T. urticae* evolving under high LMC produce larger eggs and a more female biased sex ratio (Macke *et al.*, 2012). Thus, a maternal influence on a 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 \pm 1 confidence interval.

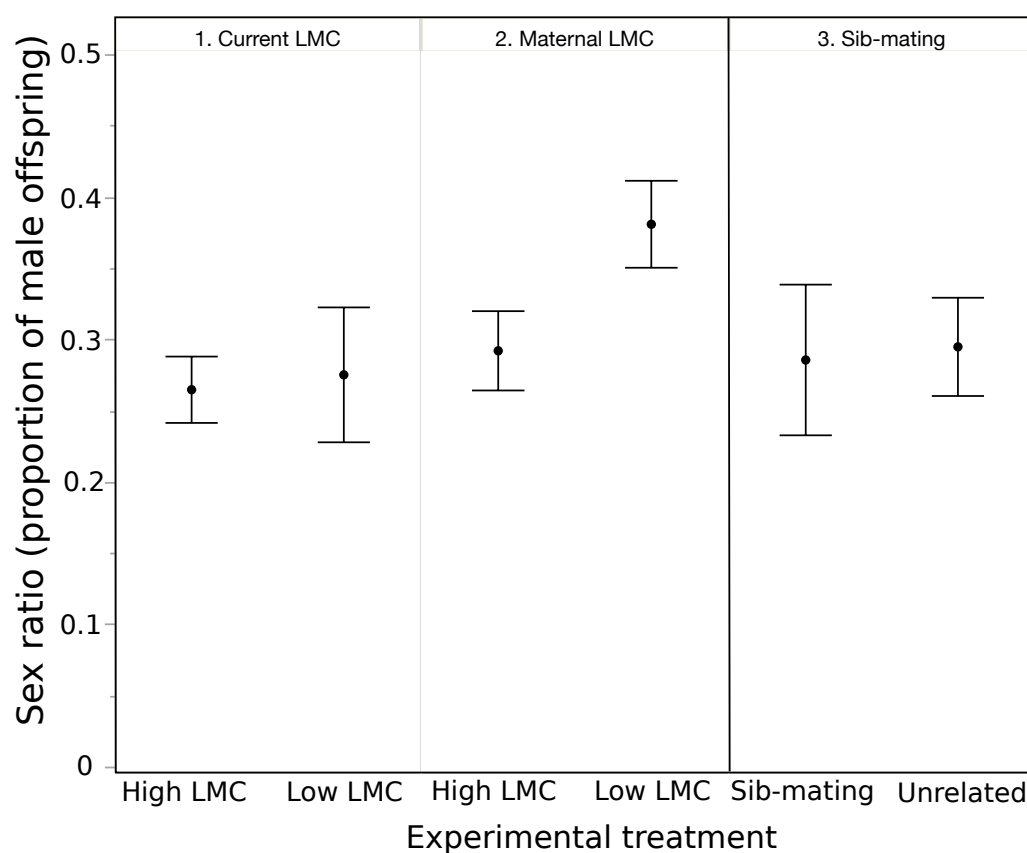


Table 1. Female fecundity A) measured as the mean number of offspring per female in each quarter for generations 1, when females experience high or low LMC, and generation 2 when mothers are exposed to high or low LMC, and B) per patch when females mate with their brother or an unrelated male. The table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

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

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