

Local Mate Competition Mediates Sexual Conflict over Sex Ratio in a Haplodiploid Spider Mite

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Summary

In haplodiploids, females pass their genes on to all their offspring, whereas a male's genes are only passed on to his daughters [1]. Hence, males always benefit from female-biased sex ratios, whereas for females the optimal offspring sex ratio depends on the level of local mate competition (LMC), ranging from highly female-biased under strict LMC to unbiased in Panmixia [2]. This generates a sexual conflict over sex ratio, the intensity of which depends on the LMC level, with most intense conflict in Panmixia [3–6]. Such conflict might lead to an evolutionary arms race, with persistence traits evolving in males and resistance traits in females. Although this prediction is theoretically straightforward, it remains untested empirically. We addressed this by crossing spider mites that evolved under varying intensities of LMC (hence of sexual conflict), to mates from inbred lines. Under high levels of sexual conflict, both sexes evolved manipulative traits to shift the sex ratio to their own advantage. In females, this was partly achieved through changes in egg size. We thus show that (1) LMC levels modulate sexual conflict over sex ratio in haplodiploids, driving the evolution of manipulative traits, and (2) fathers can affect sex ratio, challenging conventional assumptions.

Results and Discussion

The evolutionary interests of fathers and mothers over reproductive decisions rarely coincide completely, generating sexual conflicts [7, 8]. Such conflicts may drive evolutionary changes in both males and females, with adaptation in one sex followed by counteradaptation in the other sex [7, 9]. A classic example is the conflict over current versus total fecundity in *Drosophila melanogaster*, in which male seminal fluid increases female egg-laying rate, thus increasing male fitness, while having toxic side effects on females (reviewed in [10–12], see also [13], [14]). Experimental evolution studies showed that, in populations evolving under conditions implying reduced sexual conflict (e.g., monogamy), males evolved decreased female harming ability (e.g., [15–18]; but see [19]), while females evolved reduced resistance to male harm [20]. In hermaphrodites, sexual conflicts between male and female

functions in donors and receivers have been pointed out and recently tested [21–24].

Although empirical examples of sexual conflicts are abundant, the evolutionary consequences of sexual conflict over sex ratio in species with separate sexes have been empirically overlooked [25]. In haplodiploids, a female passes her genes on to all her offspring, whereas a male's genes are represented in his daughters only [1, 25]. This provides a unique opportunity to study such conflict. In subdivided populations, Local Mate Competition (LMC) theory predicts that the optimal offspring sex ratio becomes increasingly female-biased as the number of foundresses present in a patch decreases [2]. Thus, in haplodiploids, the optimal offspring sex ratio is highly female-biased for both sexes under strong LMC (i.e., few founding females). In contrast, under weak LMC (i.e., approaching Panmixia), the optimal offspring sex ratio for females tends to 50%, whereas for males it is still highly female-biased (Figure 1). Therefore, as LMC levels decrease, sexual conflict over the optimal offspring sex ratio increases [3–6]. Note that the number of foundresses present in a patch determines not only the level of LMC but also the level of inbreeding, assuming, within a population, no variation in foundress numbers among patches or in the sex ratio of their offspring [26]. LMC and inbreeding thus often go hand in hand, with strong inbreeding under high levels of LMC. The optimal sex ratio for females expected under inbreeding is the same as that predicted by LMC theory [26], because mothers become more related to their daughters as LMC increases (whereas father relatedness remains constant). However clear these theoretical predictions might be, they have never been empirically tested. This might stem from the belief that control over sex ratio in haplodiploids is exclusive to mothers. Indeed, in this system, offspring sex depends on egg fertilization, which has typically been assumed to be under maternal control (references in [25]). Hence fathers have not been expected to respond to the conflict level, on account of their supposed inability to manipulate sex allocation in their offspring [25, 27]. However, a study with *Nasonia vitripennis* has shown that males can affect their offspring's sex ratio [27], opening the door for a possible response to the above-mentioned conflict.

Here, we hypothesize that an evolutionary arms race occurs under weak LMC and low inbreeding levels, in which both sexes evolve traits to manipulate the offspring sex ratio to approach their respective optimal value. In contrast, under strong LMC and high inbreeding, both sexes should evolve to become less manipulative, assuming that manipulative traits are costly to maintain. We test this by using spider mite populations that evolved under different LMC levels.

LMC Levels Affect Sexual Conflict over Offspring Sex Ratio

We used replicate populations of the haplodiploid spider mite *Tetranychus urticae* evolving under different LMC levels: LMC+ (1 foundress per patch), LMC– (10 foundresses per patch), and Panmixia (100 foundresses per patch) [28] to test for the evolution of traits that manipulate offspring sex ratio. Under female control, the optimal sex ratio is nearly 0% of males (which has to be interpreted as the minimum proportion

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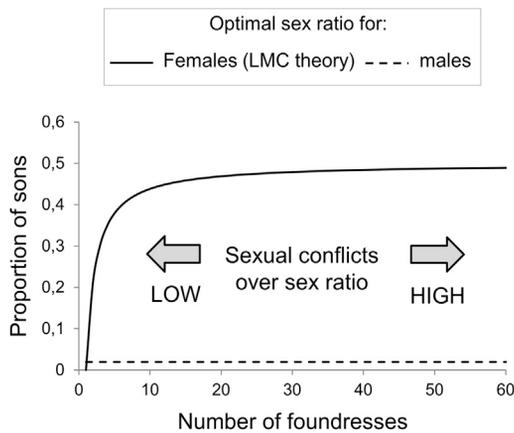


Figure 1. Effects of LMC Levels on the Intensity of Sexual Conflicts in Haplodiploids

The figure illustrates the optimal proportion of sons for females according to LMC theory (solid line) and for males (dashed lines) in a haplodiploid organism. Note that for males, or for females when $N = 1$, the optimal sex ratio is theoretically zero, which should be interpreted as the minimum proportion of sons needed to maximize the probability of insemination of their daughters [29].

of sons needed to fertilize daughters [29]) in LMC+ lines and close to 50% in the others. Males and females from these populations were crossed with mates from inbred lines, allowing us to measure manipulative traits independently in each sex. Each cross involved one male and three females. This ratio was chosen because (1) it theoretically generates sexual conflict over offspring sex ratio (the optimal offspring sex ratio is 30% and close to 0% of sons, for mothers and fathers, respectively), thus providing suitable conditions for manipulative traits to be expressed, and (2) it maximizes the probability of all females being inseminated, while maintaining a situation of conflict [30]. After mating, females laid eggs during 2 days, and offspring sex ratio was determined at adulthood. The experiment was repeated twice for each sex, but leaf desiccation hampered the use of the sex ratio data from experimental females in one block (cf. [Supplemental Experimental Procedures](#) available online).

We expect a smaller proportion of sons in the progeny of males from LMC– and Panmixia populations as compared to LMC+ populations. Conversely, females should produce a larger proportion of sons when stemming from LMC– and Panmixia populations (Table 1). Our results match these predictions. Indeed, males produced a lower proportion of sons when evolving under LMC– and Panmixia than under LMC+ (generalized linear model: $F_{2,6} = 8.16$, $p = 0.019$; contrasts: LMC– and Panmixia versus LMC+: $t_6 = 4.01$, $p = 0.007$, LMC– versus Panmixia: $t_6 = 0.5$, $p = 0.63$; Figure 2A). The opposite pattern was observed in females, which produced a larger proportion of sons when evolving under LMC– and Panmixia (generalized linear model: $F_{2,6} = 6.41$, $p = 0.032$; contrasts: LMC– and Panmixia versus LMC+: $t_6 = 3.55$, $p = 0.012$, LMC– versus Panmixia: $t_6 = 0.43$, $p = 0.69$; Figure 2A).

Together, these results strongly suggest that sexual conflict over the offspring sex ratio under low LMC levels drove an evolutionary arms race, where males evolved manipulative traits to decrease the number of sons and females counteradapted to restore an unbiased sex ratio.

Females from LMC– and Panmixia populations crossed with males from inbred lines produced more sons than the

Table 1. Expected Sex Ratio when Individuals from Either LMC+, LMC–, or Panmixia Populations Are Crossed with a Range of Different Inbred Lines

Manipulative Traits	Cross	Percentage of Sons
In males	♀ inbred x ♂ LMC+	Low
	♀ inbred x ♂ LMC–	Very Low
	♀ inbred x ♂ Panmixia	Very Low
In females	♂ inbred x ♀ LMC+	Low
	♂ inbred x ♀ LMC–	High
	♂ inbred x ♀ Panmixia	High

predicted optimum of 30%. Females might aim at producing a higher proportion of sons to compensate for manipulation by males from their own selection regime. This compensation would be revealed when females are crossed with males that did not coevolve with them but would be masked when females are crossed with males from their own population. If this is the case, it would mean that females are ahead of the arms race, with more effective manipulative traits than males. This hypothesis is consistent with the results obtained in our earlier study [28], in which intrapopulation crosses in LMC– and Panmixia selection regimes produced an offspring sex ratio that was close to optimal for females, but not for males.

Proximate Mechanisms Underlying Sex Ratio Manipulation

An important finding of our study is that both males and females can affect the sex ratio of their offspring, which is a prerequisite for the theoretical conflict to become realized, potentially fostering an evolutionary arms race [25]. While control of females over sex ratio is commonly accepted in haplodiploids, male manipulation of offspring sex ratio has been underestimated [25]. Our study strongly suggests that males can also influence the relative proportion of males and females in the progeny. Another suggestive evidence of a male effect on sex ratio comes from *Nasonia vitripennis*, in which the genotype of the inseminating male affects the fertilization rate [27]. These results raise the question of how such sex ratio control is achieved.

In spider mites, larger eggs are more likely to be fertilized, hence to develop into female offspring, than smaller eggs [31], maybe because they are more likely than smaller eggs to meet sperm, like in sea urchins [32]. Moreover, differences in sex ratio among LMC populations are correlated with differences in egg size [33]. Thus, differences in egg size might correlate with changes in sex ratio in this experiment as well. We tested this by measuring the volume of eggs from the above mentioned crosses ($n = 5$ eggs per cross). When crossed with males from inbred lines, females from LMC+ populations produced significantly larger eggs than females from LMC– and Panmixia populations (generalized linear model: $F_{2,6} = 7.33$, $p = 0.025$; contrasts: LMC– and Panmixia versus LMC+: $t_6 = 3.57$, $p = 0.012$, LMC– versus Panmixia: $t_6 = 1.4$, $p = 0.21$; Figure 2B). The interaction selection Regime * Block was significant ($F_{2,181} = 4.79$, $p = 0.009$), although the tendency was similar between blocks (cf. Figure S1). Females from LMC+ populations when remaining virgins also produced larger eggs than females from LMC– and Panmixia populations (generalized linear model: $F_{2,6} = 8.01$, $p = 0.02$; contrasts: LMC– and Panmixia versus LMC+: $t_6 = 4.00$, $p = 0.007$, LMC– versus Panmixia: $t_6 = 0.07$, $p = 0.94$; Figure 2B). These results suggest that offspring sex ratio manipulation by females is at least partly mediated by egg size, although other traits are probably also involved in sex determination, see [31]. In contrast, we found no difference in the size of eggs of females

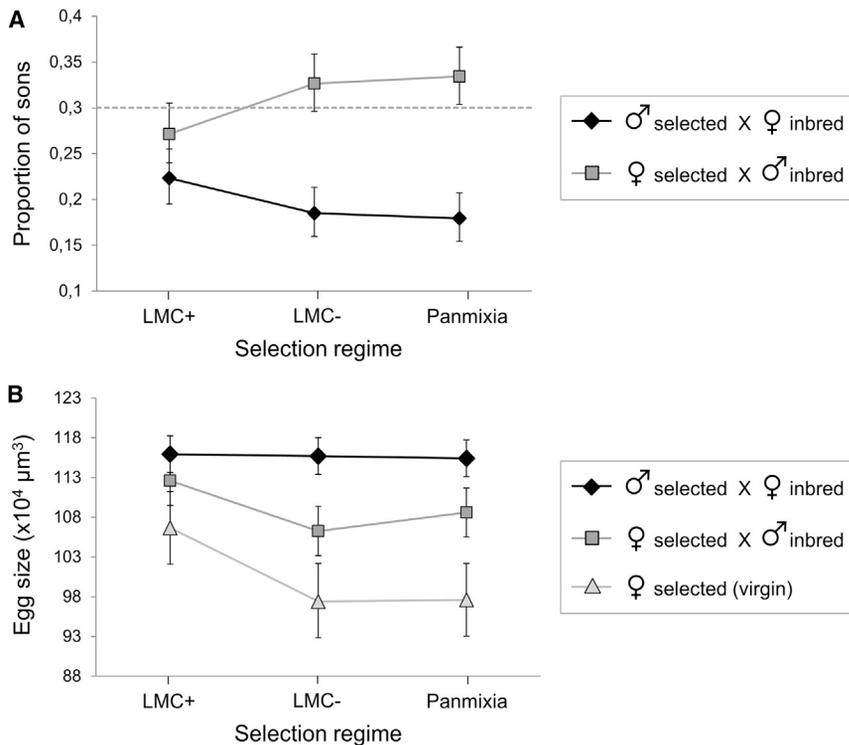


Figure 2. Proportion of Sons and Egg Size in the Offspring of Males and Females from the Different Selection Regimes

(A) Proportion of sons in the progeny of males (black lines, diamonds) and females (gray lines, squares) from populations evolving under two different LMC levels when crossed with mates from inbred lines. Symbols correspond to least-square mean values and vertical bars to 95% confidence limits. The dashed line corresponds to the optimal proportion of sons for $N = 3$ for females, as predicted by LMC theory [21].

(B) Egg size in the offspring of the crosses outlined above and in that of virgin females from the same populations (light gray line, triangles).

from inbred lines when crossed with males from the different selection regimes ($F_{2,6} = 0.14$, $p = 0.88$; Figures 2B), suggesting that males cannot manipulate the size of eggs of the females they mated with.

Males might manipulate offspring sex ratio by increasing the leakiness of the spermatheca of females, for example by transferring seminal proteins [25]. Alternatively, they might clump their sperm together or increase sperm density or fertilization ability (e.g., [34–37]). As a response, females might have developed ova more resistant to fertilization. Such arms race at the gametic level has recently been observed in house mice evolving under varying levels of sperm competition [38]. These hypotheses remain speculative, and much scope for future research remains.

A sexual conflict over offspring sex ratio in haplodiploids is theoretically straightforward. Surprisingly, however, the evolutionary consequences of such conflict have not been investigated experimentally to date, probably due to the strong belief that males have no control over offspring sex in haplodiploids. Our study challenges this traditional view and strongly suggests that males are not passive bystanders over their offspring sex ratio. Using an innovative experimental evolutionary approach, we show that both males and females can evolve manipulative traits over sex ratio, adding new insights to the understanding of sexual conflicts. Moreover, our study highlights the extraordinary potential of LMC theory to address sexual conflict over sex ratio in haplodiploids, paving the way to a new exciting research avenue.

Experimental Procedures

Populations Evolving under Different Levels of LMC

LMC populations were seeded from the same outbred base population, established at the University of Montpellier in 2007 [28]. Replicate populations were subjected to three types of selection regimes: LMC+ (one foundress per patch), LMC– (10 foundresses per patch), and Panmixia (100

foundresses per patch), each replicated three times. Population size and density were maintained constantly across treatments (cf [28] for further details).

Inbred Lines

Thirty inbred lines were obtained following 20 generations of sib-mating, after which the lines were allowed to grow to approximately 100 individuals each (cf. Supplemental Experimental Procedures for further details).

Experimental Protocol

After about 96 generations of evolution for LMC+ and LMC– populations and 56 generations for

Panmixia populations, we tested how each sex affected offspring sex ratio and egg size in all populations. By crossing individuals from each of these evolved populations with individuals from inbred lines, we were able to analyze the evolutionary changes in one sex independently of those in the other sex.

Male Effects on Sex Allocation

To start the experiment, for each evolved population, we collected 12 virgin quiescent females and placed them on a leaf fragment to lay eggs during 1 day. Because those eggs were unfertilized, they all developed into males. At adulthood, 12 males were haphazardly chosen and placed individually on a 4 cm² leaf fragment with 3 quiescent virgin females originating from the same inbred line. A total of 12 crosses were performed for each evolved population, each cross involving females from a different inbred line, and each inbred line used was crossed with all evolved populations. A total of 12 inbred lines were thus used, which were haphazardly chosen among those that presented the largest number of quiescent females. In this way, males from all LMC populations were crossed with related females, which mitigated potential confounding effects (cf. Table S1).

Males were left on the leaves until 1 day after female emergence, to ensure mating. Females were then allowed to lay eggs during 1 additional day, and five eggs per leaf were haphazardly chosen to be measured (cf. Supplemental Information). Following egg measurement, females were placed on new leaf fragments (under the same conditions as described above) for a second egg-laying period of 3 days. The eggs were allowed to complete their development, and the sex of the resulting offspring (mean number of offspring per cross: $n = 51.2 [\pm 10.5]$ and $n = 53.4 [\pm 8.9]$ for block 1 and block 2, respectively) was determined at adulthood to obtain the sex ratio.

The whole experiment was repeated twice; hence 24 males were tested per LMC population. In the second block, the inbred lines chosen were not necessarily the same as those used in the first block, because we chose lines that contained the largest number of quiescent females at the moment of the experiment.

Female Effect on Sex Allocation

The protocol used to assess female control over sex allocation was similar to that described above, except that, in this case, the three females originated from an evolved population (after being removed from selection during one generation, as in the experiment testing male effects), whereas the male came from an inbred line. A total of 12 crosses per population were performed, using males from 12 different inbred lines. The whole experiment was repeated twice, so that 24 females were tested per LMC population. In the first block, however, leaves containing the offspring of the crosses dried

accidentally before offspring had reached adulthood, making sex ratio determination impossible. Hence, we obtained egg size values in the two blocks, but values for sex ratio in the second block only. The sex ratio was estimated from on average 38 (± 5.4) offspring.

An additional experiment was performed to compare the size of eggs produced by females from the different populations independently of any male effect, by measuring the size of eggs produced by virgin females (still after being removed from selection during one generation). The protocol used was the same as above, except that females were left on their leaf fragment without any male. Twelve groups of three females were tested for each LMC population, with five eggs measured per group of females.

Statistical Analysis

All analyses were performed using the SAS software (SAS, 2002). For both male and female effects on sex ratio and egg size, differences among crosses were analyzed using a Generalized Linear Mixed Model (GLIMMIX Procedure in SAS), with Block, Selection Regime (i.e., LMC+, LMC-, and Panmixia), and Block*Selection Regime as fixed factors and Population (nested within Selection Regime), Inbred line (nested within Block), Regime*Inbred line (nested within Block), and Block*Population (nested within Selection Regime) as random factors. To obtain the final model, we first removed all the random interactions that were reported to have a null effect, and then we removed the fixed interaction term Selection Regime*Block when its *p* value was above 0.1. In the test of the female effect over the sex ratio, the Block effect was removed from the model, because the experiment was not repeated.

The size of eggs produced by virgin females was analyzed in a similar way, using a Generalized Linear Mixed Model, with Selection Regime as a fixed factor, and Population (nested within Selection Regime) as a random factor.

We specified a binomial distribution of the response variable for sex ratio and a normal distribution for egg size. For egg size, we calculated the mean egg size over the five eggs measured per cross and performed the analysis with these new values to avoid pseudoreplication.

Through the LSMEANS statement, we obtained the least-squares means (LS means) estimates of sex ratio and egg size, as well as their 95% confidence limits. Contrasts were performed with the LSMESTIMATE statement of the GLIMMIX procedure.

A graph representing differences between blocks is provided in [Supplemental Information](#).

Supplemental Information

Supplemental Information includes one figure, one table, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.10.040>.

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