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Materials and Methods

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Experimental Evolution of Reduced Sex Ratio Adjustment Under Local Mate Competition

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Theory predicts that local mate competition (LMC) favors the evolution of female-biased sex ratios. Empirical support of this prediction is indirect and comes from comparative studies or from studies showing that individuals can adjust their offspring sex ratio in response to varying LMC intensities. Replicate lines from a population of the spider mite *Tetranychus urticae* were selected under three LMC intensities for up to 54 generations. Within each selection regime, the final sex ratio matched theoretical predictions. Furthermore, the ability of individuals to adjust their offspring sex ratio diminished in females evolving under strict LMC, but not in females evolving under relaxed LMC levels. These results provide direct experimental evidence for the evolutionary process by which LMC modifies sex-allocation strategies and suggest that evolution under strict and constant LMC may lead to a loss of phenotypic plasticity.

Hamilton's theory of local mate competition (LMC) predicts that in structured populations, when mating takes place locally among the offspring of one or few foundress mothers after which mated daughters disperse, mothers are selected to bias their offspring sex ratio toward females (*1*). As the number of foundresses contributing offspring to local mating groups increases, a more balanced sex ratio is favored and will approach an equal sex ratio, as predicted for panmictic populations (*2, 3*).

If males can inseminate several females, under LMC, brothers will compete with one another for mating partners. However, under these conditions, no such competition exists for their sisters. This reduces the value of producing sons rather than daughters, resulting in females allocating more resources to the production of

daughters. Biasing the sex ratio toward daughters allows females to maximize the fitness of her sons, by reducing her male offspring's mate competition and increasing their mating opportunities (*1, 4, 5*). Hamilton's model prediction can also be understood through a multilevel selection argument: Within groups, females maximize their number of grandchildren (hence, their fitness) by producing even (Fisherian) sex ratios, but groups that produce female-biased sex ratios are more productive because more granddaughters are produced. The relative strength of within- and among-group selective forces will depend

on the number of foundresses (*N*) per group and on the number of groups. As *N* increases or the number of groups decreases, within-group selection for an even sex ratio becomes stronger, whereas among-group selection for a female-biased sex ratio becomes weaker (*5, 6*).

Thus, theory makes three main predictions. First, populations evolving under higher LMC should exhibit a greater mean female bias. Second, individual females should adjust their sex ratios in response to different intensities of LMC through phenotypic plasticity (*7, 8*). Third, controlling the mean sex ratio and its variance is adaptive (*9*). Hence, individuals should produce precise sex ratios that exhibit lower than binomial variances, in particular under high LMC levels, where precision is expected to be under stronger selection (*9, 10*).

Support of these theoretical predictions comes predominantly from three empirical approaches. First, comparative studies have shown that in populations or species where the intensity of LMC is likely to be high, the sex ratio is more female-biased than in populations or species with lower levels of LMC [e.g., (*11*); see also (*2, 5*)]. Second, at the individual level, females of various species produce a higher proportion of sons as the number of foundresses laying eggs on a patch increases (*5, 12*). Third, the variance of the number of sons is smaller than the variance under binomial sampling, in particular for species experiencing greater LMC conditions (*10*).

However, these approaches do not directly test the evolutionary process that leads to the

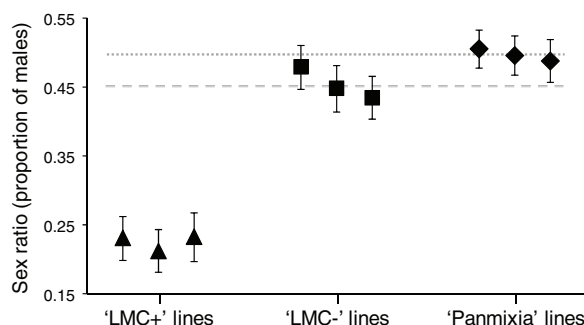


Fig. 1. The proportion of males increases with decreasing intensity of LMC imposed during the selection process. Symbols represent mean sex ratio values (triangle: LMC+ lines; squares: LMC- lines; diamonds: Panmixia lines), error bars the 95% confidence limits, and lines the predicted sex ratios (dashed: for LMC- lines; dotted: for Panmixia lines).

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extraordinary sex ratios observed as a result of LMC. Comparative studies address patterns instead of the process itself, and individual studies address within-generation phenotypic plasticity instead of genetic changes in response to selection. In contrast, experimental evolution can test how LMC affects the evolution of sex allocation and allows one to follow the evolutionary process in real time, by manipulating the number of foundresses present at each generation while leaving all other factors similar across treatments (13).

We directly tested the evolutionary process described in LMC theory, by performing experimental evolution in the haplodiploid spider mite *Tetranychus urticae* under different LMC levels, i.e., by varying the number of foundresses and letting sex ratio evolve. Spider mite ecology precisely corresponds to the typical “biofacies for LMC” set by Hamilton’s model as females can store sperm and adjust their sex ratio by laying unfertilized (male) eggs; their first egg is always unfertilized, ensuring that at least one male is produced; adult males eclose first and can mate many times; mating takes place following mate guarding, right after eclosure of adult females; and dispersal mainly concerns young mated females (1, 14). Previous studies with *T. urticae* suggest that females control their progeny sex ratio and can adjust it in response to variable LMC (15–19). Moreover, its sex ratio has been shown to be heritable and to evolve in response to artificial selection, thus fulfilling the conditions for adaptive evolution (20, 21).

Replicate lines from a single original population were allowed to evolve under three different levels of LMC intensity: “LMC+” (1 foundress per patch; high LMC intensity), “LMC–” (10 foundresses per patch; moderate LMC intensity), and “Panmixia” (>100 foundresses per patch; very low LMC intensity) (fig. S1). After 54 generations of evolution (only 14 for the Panmixia lines, which were created later), we performed a quantitative test of LMC theory by measuring sex ratio in each experimental population and comparing the observed value with the theoretical sex ratio, equal to $\frac{(N-1)(2N-1)}{N(4N-1)}$, with N the number of foundresses on each patch (5, 14, 22). To determine whether the differences observed between populations were due to a facultative adjustment or to an evolution of sex allocation in response to LMC intensity (or both), we compared the offspring sex ratio of experimental populations after one generation spent in a common environment (same number of foundresses), to mitigate eventual maternal effects (fig. S2). A persistence of differences between populations would indicate the evolution of a fixed sex-allocation strategy, whereas no difference would indicate a pure facultative adjustment. The sex ratio produced by females from the three selection regimes was compared under both “single foundress” conditions (a single female per patch, high LMC) and “several foundresses” conditions (40 females per patch, low LMC). This allowed us to test whether the ability of facultative adjustment

was maintained after multiple generations of evolution under a given LMC level.

A total of 7984 offspring were counted and sexed. As predicted by LMC theory, the proportion of males observed in the experimental populations increased with decreasing intensity of LMC under which lines had evolved, with average sex ratios of 0.23, 0.45, and 0.50 for the LMC+, LMC–, and Panmixia selection regimes, respectively (least-squares means; generalized linear model: $F_{2,6} = 183.51$, $P < 0.0001$; pairwise comparisons: LMC+ versus LMC–: $F_{1,6} = 242.99$, $P < 0.0001$; LMC+ versus Panmixia: $F_{1,6} = 355.30$, $P < 0.0001$; LMC– versus Panmixia: $F_{1,6} = 11.63$, $P = 0.0143$).

The observed sex ratios closely matched theoretical predictions (Fig. 1) (14). The sex ratios predicted for LMC– and Panmixia selection regimes (0.44 and 0.49) fit within the observed confidence intervals (0.43 to 0.48 and 0.48 to 0.52, respectively). For the LMC+ selection regime, theory predicts a sex ratio of 0% males, which should be interpreted as the minimum proportion of surviving sons needed to ensure insemination of daughters (2, 14). However, sex ratio under strict LMC should be at most equal to the sex ratio predicted for two foundresses, i.e., 0.21. This value fell within the observed confidence interval for LMC+ selection regime, 0.20 to 0.25. These results are consistent with other studies revealing that individuals could adjust their sex ratio to the level of LMC, in *T. urticae* (15–19) and in other species (5, 12), and with comparative studies showing that populations or species experiencing higher levels of LMC exhibited more female-biased sex ratios (2, 5, 11). However, our study differs in that we compared the sex ratio between populations after allowing for evolutionary changes, which fits the assumptions of LMC models. Moreover, by comparing selection lines that were initiated from the same original population and have evolved under controlled conditions, we minimized the influence of factors other than LMC intensity on the observed sex ratios and ensured that any divergence among lines when assessed in common conditions is due to evolutionary changes.

When all selection lines were tested in a common environment, we found a significant interaction between the selection regime and the

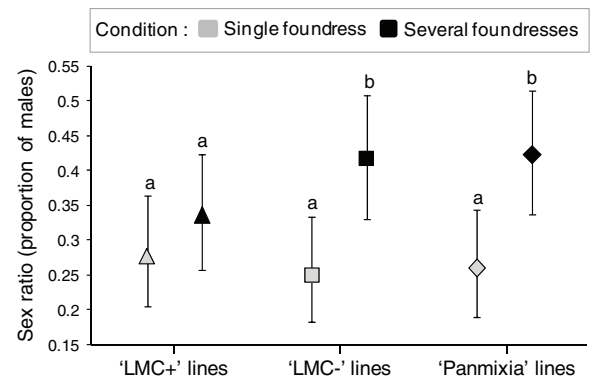
condition in which the sex ratio was measured (“single foundress” or “several foundresses”) ($F_{2,22} = 11.20$, $P = 0.0004$). This interaction indicates that females from the different selection regimes did not respond to LMC conditions in the same way, suggesting an evolution of the norm of reaction for sex ratio.

Under the single foundress condition, females from all selection regimes produced similarly low sex ratios ($F_{2,22} = 0.48$, $P = 0.63$) (Fig. 2). Under the several foundresses condition, however, females from the different selection regimes produced different sex ratios ($F_{2,22} = 8.09$, $P = 0.0023$). Indeed, lines that had evolved under LMC+ produced significantly fewer males than lines that had evolved under either Panmixia ($t_{22} = 3.62$, $P = 0.0015$) or LMC– ($t_{22} = 3.36$, $P = 0.0028$), whereas no difference was found between Panmixia and LMC– lines ($t_{22} = 0.27$, $P = 0.79$) (Fig. 2).

Moreover, females from LMC+ lines produced similar sex ratios under single foundress and several foundresses conditions ($F_{1,22} = 1.26$, $P = 0.27$), suggesting that these lines did not adjust their sex ratio in response to LMC intensity (Fig. 2). In contrast, females from both LMC– and Panmixia lines produced significantly more males under several foundresses conditions, compared to single foundress conditions ($F_{1,22} = 9.46$, $P = 0.0055$ and $F_{1,22} = 8.96$, $P = 0.0067$ for LMC– and Panmixia selection regimes, respectively) (Fig. 2) (14). Therefore, our results suggest that females evolving under strong LMC conditions had a reduced ability to adjust their sex ratio in response to the number of foundresses present on the patch, compared to females evolving under lower intensities of LMC.

Finally, we tested the hypothesis that under a single foundress condition, females should produce precise sex ratios (i.e., sex ratios with lower than binomial variance), especially for LMC+ selection regime (7–10, 14). Within each of the nine experimental populations, all but two were consistent with binomial sampling. Two lines (one from LMC– and one from Panmixia selection regimes) were slightly more precise (table S1). Overall, our results do not support the hypothesis that we would obtain a more precise sex ratio than expected under binomial sampling, in particular for LMC+ lines. Furthermore, within each line, the observed variance of sex ratio among

Fig. 2. Evolution of the reaction norm. Females from LMC– (squares) and Panmixia (diamonds) lines adjust their sex ratio in response to the number of females present on the patch, by increasing their proportion of sons under several foundresses conditions, whereas females from LMC+ lines (triangles) do not, and produce a low proportion of males in both conditions. Symbols correspond to least-squares mean values, error bars to 95% confidence limits.



females was 3 to 35 times larger than expected if the sex ratio bias of the offspring produced by each female was as precise as possible given her brood size (table S2), suggesting that females could have been more precise, even in the context of their small brood size.

We have thus tested the three main predictions of LMC theory and found that (i) populations evolving under higher LMC exhibited a greater mean female bias in their sex ratios and (ii) individual females could adjust their sex ratios to different intensities of LMC through phenotypic plasticity. However, (iii) females did not produce precise sex ratios that exhibit lower than binomial variance. We could reach these conclusions only because our setup allowed us to vary both the LMC conditions under which the populations evolved and the conditions in which they were tested. Therefore, we leveraged the advantage of both plasticity and comparative tests and added analytical power to this approach that could not be reached with correlative or observational approaches.

We found that the norm of reaction for sex ratio evolved differently in selection lines evolving under different LMC intensities. We hypothesize that LMC+ lines evolved under more constant LMC levels compared to LMC- and Panmixia lines. In LMC- and Panmixia lines, several females were present on each leaf, so that the populations may have been spatially structured, leading to more varying levels of LMC. Moreover, in some generations, one or few females may have died before laying any egg; hence, the intensity of LMC may have varied across generations. Therefore, conditions under LMC- and Panmixia lines were probably heterogeneous, which is predicted to favor adaptive phenotypic plasticity (23, 24). In contrast, in LMC+ lines, each generation of selection was initiated by a single foundress per leaf fragment; thus, the level of LMC never varied. Such homogeneous selection is predicted to favor a loss of phenotypic plasticity, particularly if phenotypic plasticity is costly to maintain (25). These results are consistent with a comparative study on fig wasps, which showed that adaptive plasticity of sex ratio was higher in species subject to more variable LMC in nature, suggesting that the selective regime shapes the evolution of sex ratio adjustment (26, 27).

It is also possible that inbreeding depression occurred in our LMC+ lines, reducing phenotypic plasticity. Inbreeding increases with the LMC level; hence, females from LMC+ lines might suffer from a reduced fitness, expressed here by a reduced ability to adaptively adjust their behavior. There is evidence for some level of inbreeding depression in female fitness components in *T. urticae* (28). Whether such inbreeding depression also affects the ability of females to precisely control their brood sex ratio remains to be studied.

Hamilton's theory of LMC has strongly influenced the field of evolutionary biology. Our results suggest that this theory is correct and that

evolutionary theories can make precise predictions, both qualitatively and quantitatively.

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Pretreatment Mitochondrial Priming Correlates with Clinical Response to Cytotoxic Chemotherapy

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Cytotoxic chemotherapy targets elements common to all nucleated human cells, such as DNA and microtubules, yet it selectively kills tumor cells. Here we show that clinical response to these drugs correlates with, and may be partially governed by, the pretreatment proximity of tumor cell mitochondria to the apoptotic threshold, a property called mitochondrial priming. We used BH3 profiling to measure priming in tumor cells from patients with multiple myeloma, acute myelogenous and lymphoblastic leukemia, and ovarian cancer. This assay measures mitochondrial response to peptides derived from proapoptotic BH3 domains of proteins critical for death signaling to mitochondria. Patients with highly primed cancers exhibited superior clinical response to chemotherapy. In contrast, chemoresistant cancers and normal tissues were poorly primed. Manipulation of mitochondrial priming might enhance the efficacy of cytotoxic agents.

Cancers that respond well to one cytotoxic agent often respond well to other cytotoxic agents even when these agents act through very different mechanisms (as in acute

lymphoblastic leukemia). Conversely, cancers that respond poorly to one type of cytotoxic agent often respond poorly to all types of chemotherapy (as in pancreatic cancer or renal cell carcinoma).