

# Reproductive Consequences of Population Divergence through Sexual Conflict

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## Summary

Sexual-selection research increasingly focuses on reproductive conflicts between the sexes [1–4]. Sexual conflict, divergent evolutionary interests of males and females [5], can cause rapid antagonistic coevolution of reproductive traits [6] and is a potentially powerful speciation engine [7–11]. This idea has theoretical and comparative support [10–12] but remains controversial [13–14]. Recent experimental evidence from *Sepsis cynipsea* indicates that populations with greater sexual conflict diverged more quickly; females were less likely to mate with males from other populations when flies had evolved under high levels of sexual conflict [15]. The consequences of this divergence have not been addressed, so here we assess two female fitness surrogates after 44 generations of evolving (and diverging) under three different levels of sexual conflict. Longevity after copulation was negatively associated with the degree of sexual conflict under which flies evolved, and housing females with males also reduced female longevity. Female lifetime reproductive success (LRS) also tended to decrease with increasing conflict. However, there was evidence of either sexual-selection fitness benefits at intermediate levels of sexual selection and conflict or inbreeding depression in the smallest populations (those with the lowest levels of conflict). Nevertheless, the results indicate that there can be a fitness load associated with sexual selection [2] and support claims that sexual conflict can lead to reproductive isolation [7–11, 15].

## Results and Discussion

After exposing *Sepsis cynipsea* to 44 generations of experimental evolution under varying levels of sexual conflict, we assessed the fitness consequences of mating within or across populations (but within treatments: high conflict, low conflict, and relaxed conflict; see Experimental Procedures below) for female *Sepsis cynipsea*. We have previously shown that sexual conflict varies as expected with our experimental manipulations (i.e., increased population size/density leads to an increased number of sexual interactions, including female reluctance behavior, and hence more intense sexual conflict. See [15]). Additionally, under these varying levels of conflict, one measure of population divergence,

the proportion of pairs that did not mate in a 30 min observation period, was greater in high-conflict populations in spite of their larger population size [15]. This contrasts with classical population genetics theory, which predicts faster divergence in smaller populations [16], but is an explicit prediction of divergence through sexual conflict [10] (also see [17]).

Here we used multi- and univariate general linear models (GLM) to investigate the fitness consequences of this apparent divergence. We initially used a multivariate model to look at the effects of treatment (evolution under high conflict, low conflict, and relaxed conflict), mating type (within or between populations exposed to the same treatment), and housing (with or without males) and their interactions on female longevity and lifetime reproductive success (LRS = number of offspring produced). We also included female size as a covariate in this analysis because body size may influence both of these dependent variables (LRS and longevity). All three factors had significant multivariate effects (Wilks' Lambda:  $F > 4.71$ ;  $p < 0.02$ ), but none of the interactions was significant (Wilks' Lambda: all  $F < 2.354$ ; all  $p > 0.069$ ). There was also no significant effect of body size on the multivariate combination of dependent variables (Wilks' Lambda:  $F_{2,22} = 1.20$ ;  $p = 0.319$ ).

Univariate GLM revealed that treatment ( $F_{2,23} = 10.83$ ;  $p < 0.001$ ) and housing ( $F_{1,23} = 78.69$ ;  $p = 0.001$ ) significantly influenced longevity after copulation (Figure 1), but no other predictor term was close to significant (all  $F < 2.00$ ; all  $p > 0.17$ ). Females from the high-conflict populations died sooner, females from the low-conflict treatment were intermediate, and females from the relaxed conflict (monogamous) treatment lived the longest. Post-hoc tests (Fisher's PLSD) indicated that the two conflict populations did not differ significantly (difference = 0.013; critical difference = 0.018;  $p = 0.14$ ), but both were significantly different from the relaxed-conflict treatment (difference  $> 0.25$ ; critical difference = 0.018;  $p < 0.006$ ). This indicates either that the base-line mortality of flies from the conflict treatments had evolved to differ from the monogamous populations or that copulation costs have evolved differently during experimental evolution.

It has previously been shown that males harm females during copulation [18], and hence these results could be generated because females from conflict populations became less resistant to male harm, males became more harmful, or a combination of the two. Conversely, females from the relaxed-conflict populations may have also become more resistant to harm, males may have become less harmful, or a combination of the two. If either scenario were correct, one may expect housing with males to further accentuate this effect. Females housed with males died more rapidly than those housed alone (Figure 1). Additionally, when females were housed with males from conflict populations, this cost was slightly, but not significantly, accentuated (the decrease in longevity due to housing with males was 11.9% for monogamous females and 15.9% and 13.5% for low-

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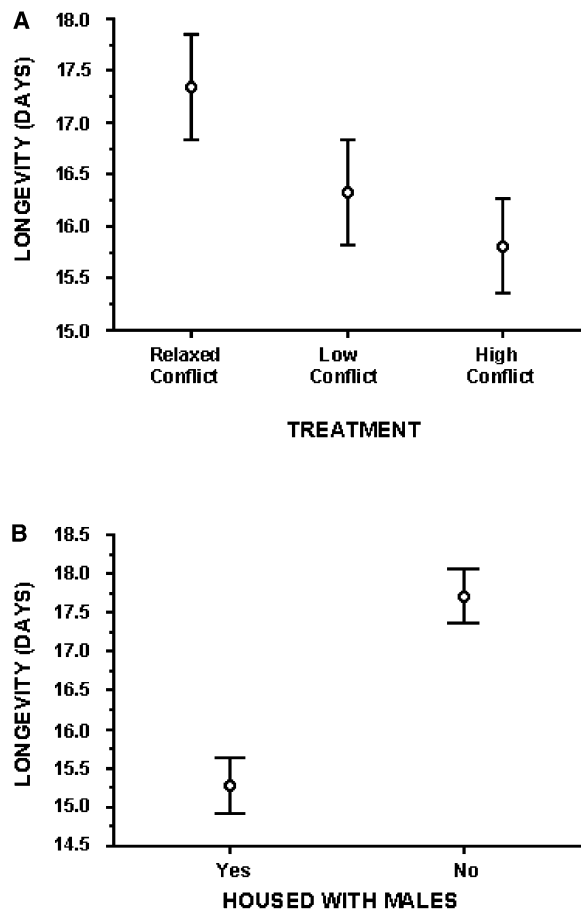


Figure 1. Female Longevity

Female longevity (mean  $\pm$  SE) after copulation as a function of (A) treatment (high, low, or relaxed conflict) and (B) housing (with or without males). Note that copulations were always within the same treatment (i.e., between males and females from relaxed-conflict, low-conflict, or high-conflict populations).

and high-conflict females). However, we have no way of knowing how frequently females copulated when housed with males from each treatment. As a result, the housing-with-males treatment does little to clarify exactly whether base-line mortality or harm evolved during the experiment. Nevertheless, the mortality differences noted are largely as predicted under antagonistic evolution regardless of whether base-line mortality or harm/resistance evolved. We also note that housing females with males has been shown to decrease female fitness components previously (e.g., longevity, LRS [15, 19]),

Identical univariate analysis of LRS revealed a significant treatment-mating type interaction that influenced offspring production ( $F_{2,23} = 5.40$ ;  $p = 0.012$ ; Figure 2). The three factors were also all individually significant in this analysis (treatment:  $F_{2,23} = 6.14$ ,  $p = 0.007$ ; mating type:  $F_{1,23} = 8.99$ ,  $p = 0.006$ ; housing:  $F_{1,23} = 20.38$ ,  $p = 0.0002$ ), but female size was not ( $F_{1,23} = 2.52$ ,  $p = 0.126$ ), nor were any other interaction terms (all  $F < 3.38$ , all  $p > 0.079$ ). LRS was lower when females were housed with males (mean offspring number  $\pm$  SE: with males,

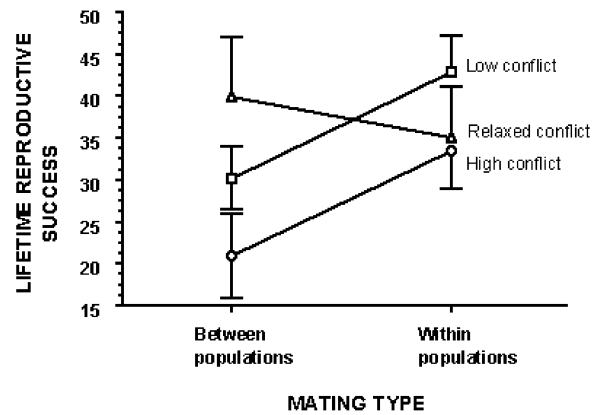


Figure 2. Female Reproductive Success

Female lifetime reproductive success (number of offspring produced, mean  $\pm$  SE) as a function of treatment (high [circles], low [squares], or relaxed [triangles] conflict) and mating type (within or between populations). Note that copulations were always within the same treatment (i.e., between males and females from relaxed-conflict, low-conflict, or high-conflict populations).

$27.2 \pm 2.5$ ; without males,  $40.2 \pm 3.0$ ), but when longevity is included as a covariate in this analysis, housing is no longer significant (see Experimental Procedures below). Although both treatment and mating type were individually significant, it is the significant interaction term that we must interpret [20]. Again, it appears that evolving under high levels of sexual conflict is costly; it reduces fitness in this experimental situation and does so even more when females copulate with males from other conflict populations. However, LRS in the relaxed conflict treatment was intermediate (between the conflict populations) in within-population pairings.

The reduction that we found here in LRS of the between-population conflict pairings may be linked to the increase in copulation duration that we detected in between-population matings (mean duration [min]  $\pm$  SE: within =  $23.4 \pm 0.6$ ; between =  $25.7 \pm 0.8$ ;  $F_{1,16} = 5.38$ ;  $p = 0.034$ ). Previous work indicates that longer copulations reduce female fitness [22] and that copula duration is likely to be largely male controlled [23], but why it is that copulation lasts longer in across-population pairings is unclear. Perhaps males that succeed in copulating in between-population conflict-crosses are in relatively better condition, or perhaps females in these pairings are in worse condition. That is, males need to be relatively stronger to overcome female resistance in these pairings, and hence copulation duration is closer to the male optima. If so, this could also explain the lower LRS in such pairings. However, the reason why the magnitude of this difference is not greater in the high-conflict lines is unclear, but the longevity costs of the two conflict treatments were also similar. In any case, all of this would only explain the result from the two conflict treatments; the opposite effect was found in relaxed-conflict pairs (Figure 2). One possible explanation for the opposite situation in the monogamous (relaxed-conflict) populations is that they had become slightly inbred and that mating with benign males from other relaxed-conflict populations reverses this effect.

However, it is unclear if effective population size was smaller in the monogamous populations than in the low-conflict groups because in the latter treatment the scope for some males to contribute more genes to subsequent generations is probably increased. Furthermore, there was no indication that these two treatments differed in inbreeding at generation 27 [21]. An additional factor that may contribute to the higher fitness of low-conflict flies in the within-population comparison (Figure 2) is that under these conditions perhaps the benefits of sexual selection outweigh any costs of sexually antagonistic coevolution. Overall, the significant effects in the multivariate analysis are driven by effects on LRS alone (mating type and the interaction between mating type and treatment) or by effects on both dependent variables (treatment and housing).

Our results parallel those reported in other experimental evolution studies [1, 2, 24–27]. For example, work on *Drosophila melanogaster* shows that males become more successful at securing mates and stopping female remating when females are artificially prevented from evolving with them, and this comes at a cost to female survival [1]. Similarly, using artificial constructs that forced more than 90% of the genome to segregate like a giant Y chromosome led to an increase in measures of male fitness compared to that of control males, and the mortality of tester females increased when they mated with the experimental males [24]. Both results are consistent with sexually antagonistic coevolution [24]. More recently, it has also been shown that evolving under sexual selection does not increase nonsexual fitness in *D. melanogaster* [26] (also see [28]) and that males preferred by female *D. melanogaster* are more harmful [29, 30].

Studies such as these have been questioned partly because they may not reflect what occurs in nature [31]. Although this criticism is in some ways justified, laboratory evolution nevertheless shows what is possible. If results are not representative of what occurs in wild populations, the question is why not, and in at least some taxa, strong evidence of sexually antagonistic evolution through sexual conflict has been documented in nature [3, 32, 33]. The question of female fitness benefits via sexy sons has also frequently been raised as a concern in these and similar studies [31, 34, 35]. However, a number of quantitative genetic models suggest these benefits have little effect on evolutionary equilibria, especially when there is direct selection on female preference [36–39], and cast at least some doubt on this concern. Additionally, indirect benefits of any sort are unlikely to overcome the substantial direct fitness costs of mating [40, 41]. Finally, we emphasize that the results we documented here are a consequence of whatever divergence occurred during our experimental evolution and that we are in no way implying that any reinforcement occurred because populations were always in allopatry. Nevertheless, exactly what has diverged to cause the fitness outcomes documented here remains unknown.

In conclusion, we show that foreign males from conflict populations reduced female fitness more than familiar males. Our results also support the suggestion of a load associated with sexual selection, as previously

reported for *Drosophila melanogaster* [2], because fitness was frequently lower in populations with (more) sexual selection. However, our results also suggest that fitness benefits may at times outweigh costs with intermediate levels of sexual selection and sexual conflict. Nevertheless, it seems that sexual conflict has probably driven population divergence in these populations, further emphasizing that male-female coevolution was antagonistic and that sexual conflict can lead to reproductive isolation.

## Experimental Procedures

### Fly Populations

We investigated the reproductive consequences of population divergence through sexual conflict in replicate laboratory populations of *Sepsis cynipsea*. In *S. cynipsea* sexual conflict over mating is obvious, with long and violent precopulatory struggles [18, 19, 42–44]. Conflict over mating seems to be partly due to injuries that males inflict on females during copulation, and these greatly increase female mortality [18]. Copulation duration appears to be largely controlled by males [23], and longer copulations also reduce female fitness [22]. Additionally, previous work indicates that increased population size/density leads to increased sexual conflict and that increasing the number of males with which a female interacts reduces her fitness, consistent with increased sexual conflict [15].

To investigate the effects of sexual conflict intensity on reproduction, we used artificial evolution with three treatments, each with three replicate populations. These consisted of two conflict treatments, high-conflict (high-density populations HD1, 2, and 3, each with 250 males and 250 females per container), low-conflict (low-density populations LD1, 2, and 3, each with 25 males and 25 females per container), and relaxed-conflict populations (monogamy M1, M2, M3, each consisting of 20 pairs in individual vials). For more detailed information concerning these selection lines and laboratory protocols, see [15]. After 42 generations of selection (different levels of conflict), flies were housed under relaxed selection for two generations. This was done to eliminate differential maternal effects and potential phenotypic effects generated by population density variation (all populations from all treatments were in containers with 50 males and 50 females and kept for 12 days per generation, as in the selection populations). The offspring of these flies were used for the experiments.

### Fitness Assay

To investigate reproductive consequences of population divergence, we assessed lifetime reproductive success (i.e., number of offspring produced) and female longevity. Experimental females were placed separately with single males from the same treatment (relaxed, low, or high conflict) but from their own (within) or different (between) populations and allowed to copulate. There were significant effects of both these factors on the time that expired before copulation occurred (treatment:  $F_{2,12} = 23.5$ ;  $p = 0.0001$ ; Fisher's PLSD indicated that all three treatments differed,  $p < 0.036$ . Mating type:  $F_{1,12} = 6.24$ ;  $p = 0.028$ ). High-conflict population crosses took the longest time to occur, relaxed-conflict (monogamous) crosses took the least time, and low-conflict lines fell between these extremes; between-population crosses took longer than within-population crosses. All of this is consistent with previous work [15]. We also found that the proportion of pairs that finally copulated was also influenced by treatment and mating type (treatment:  $F_{2,12} = 36.2$ ;  $p = 0.0001$ ; Fisher's PLSD indicated all three treatments differed,  $p < 0.0016$ . Mating type:  $F_{1,12} = 8.63$ ;  $p = 0.012$ ). Fewer pairs from high-conflict pairings copulated than did those from the low-conflict treatment, and monogamous pairs copulated the most. Within populations, there were proportionally more copulations than in between-population crosses. Again, this is consistent with previous work [15]. If copulation occurred, its duration was recorded.

After 20 females per pairing type had copulated, females were transferred to 100 ml vials. Ten of these females were housed alone,

and the other ten were individually housed with two males from the corresponding population (these two males were replaced if they died before the female during the experiments). All vials were provided with dung (oviposition substrate), water, pollen, and sugar. Housing with or without males allowed us to investigate potential effects of male presence on female longevity (harassment, additional copulations) and reproductive success (possibility to remate and replenish sperm stores). Dung portions were replaced regularly with fresh dung, and the old portions were placed in a climate chamber and left until offspring emerged. Offspring were then counted, and the longevity (in days) of all females was recorded (NB all of a females's offspring were raised until all the females had died and the last offspring had emerged). In subsequent analyses, cross type (relaxed conflict by relaxed conflict [monogamous  $\times$  monogamous], low conflict by low conflict [low density  $\times$  low density], or high conflict by high conflict [high density  $\times$  high density]) was the unit of replication (and not individual females) and consisted of pairings between populations or within populations. Mean values for female longevity, offspring numbers (i.e., lifetime reproductive success, LRS), and copulation duration were calculated per population combination (e.g., combination of M1 and M2 and according to whether females were housed alone or with males, i.e., one mean value for crosses of populations M1 and M2 with females housed alone and one value for those females housed with males). These were used instead of values for individual females in the analysis (i.e., individuals were not treated as the unit of replication). Thus, data were organized in the analyses according to three factors: selection (either relaxed [monogamous], low conflict, or high conflict), mating type (between different or within the same population), and presence of males (yes or no). Data were checked against the assumptions of our tests and were transformed to meet them when appropriate. Because each dependent variable (longevity or LRS) may have influenced the other, we also carried out two additional univariate analyses, one with longevity as a covariate and LRS as the dependent and one with LRS as a covariate and longevity as the dependent. In neither case were these covariates significant, and our interpretation of other factors did not change, except that the housing effect on LRS became nonsignificant ( $p = 0.094$ ). For this reason, we do not think the housing effect on LRS is very strong in our experiments, and hence our discussion of it is limited.

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