ECOLOGY LETTERS

Ecology Letters, (2014) **17**: 221–228 doi: 10.1111/ele.12222

LETTER

The ecology of sexual conflict: ecologically dependent parallel evolution of male harm and female resistance in *Drosophila melanogaster*

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Abstract

The prevalence of sexual conflict in nature, along with the potentially stochastic nature of the resulting coevolutionary trajectories, makes it an important driver of phenotypic divergence and speciation that can operate even in the absence of environmental differences. The majority of empirical work investigating sexual conflict's role in population divergence/speciation has therefore been done in uniform environments and any role of ecology has largely been ignored. However, theory suggests that natural selection can constrain phenotypes influenced by sexual conflict. We use replicate populations of *Drosophila melanogaster* adapted to alternative environments to test how ecology influences the evolution of male effects on female longevity. The extent to which males reduce female longevity, as well as female resistance to such harm, both evolved in association with adaptation to the different environments. Our results demonstrate that ecology plays a central role in shaping patterns of population divergence in traits under sexual conflict.

Keywords

Antagonistic coevolution, ecological divergence, experimental evolution, interlocus sexual conflict, sexual selection.

Ecology Letters (2014) **17**: 221–228

INTRODUCTION

Sexual conflict arises when the reproductive interests of males and females are not aligned, generating sex-specific selection on shared traits such as mating rate (Parker 1979). When the genetic basis of the trait differs between the sexes, sexually antagonistic coevolution can favour loci that enhance a male's reproductive success relative to intrasexual competitors, even if males carrying such alleles reduce female fitness. Reciprocally, selection will also favour loci that increase a female's resistance to such harm, even if these come at a cost to male fitness (Rice 1996; Holland & Rice 1998). Such interlocus sexual conflict can therefore cause repeated bouts of coevolution, driving an evolutionary 'arms race' in which any advantage gained by individuals of one sex decreases the fitness of individuals of the other, generating renewed selection that can lead to an endless evolutionary chase between the sexes (Parker 1979; Civetta & Singh 1995; Rice 1996; Chapman *et al*. 2003; Arnqvist & Rowe 2005).

The integral role sexual antagonism plays in evolution has been confirmed by experimental (Rice 1996; Martin & Hosken 2003; Stewart *et al*. 2005; Long *et al*. 2009), comparative (Arnqvist 1998; Arnqvist & Rowe 2002) and theoretical studies (Gavrilets *et al*. 2001; Gavrilets & Waxman 2002; Rowe *et al*. 2003), and there are numerous examples in nature of traits that increase male reproductive success at the expense of females, and traits that enhance female resistance to specific forms of male coercion or harm (Arnqvist & Rowe 2005). The particular phenotypes that evolve via interlocus sexual conflict are thought to be in part a product of chance, subject for example to the nature of segregating variance, new mutational input and the evolutionary history of the population in question. Consistent with this, traits enhancing male sexual fitness are extraordinarily diverse among taxa (e.g. persistent courtship and harassment, toxic ejaculates, spiny genitalia and means of traumatic insemination; Arnqvist & Rowe 2005). Together with the persistent and potentially strong selection such interlocus conflict can generate, the arbitrary nature of the resulting coevolutionary trajectories may contribute to rapid population divergence and ultimately speciation (Arnqvist 1998; Arnqvist *et al*. 2000; Gavrilets *et al*. 2001; Gavrilets & Waxman 2002; Martin & Hosken 2003) and there is therefore a great deal of interest in sexual conflict's role in diversification.

Because sexually antagonistic selection arises from an interaction of the sexes, divergence via sexual conflict has often been considered to be driven by chance events such as the order in which particular mutations occur, and is thus often cast as independent of ecology (Coyne & Orr 2004; Rundle & Nosil 2005; Schluter 2009). The majority of experimental work investigating sexually antagonistic coevolution and its consequences has therefore been done in a uniform environment (e.g. Rice 1996; Andrés & Arnqvist 2001; Long *et al*. 2006; Edward *et al*. 2011). Despite the emphasis in the speciation literature of sexual conflict as a non-ecological driver of diversification (Coyne & Orr 2004; Rundle & Nosil 2005), discussions of the evolution of traits mediating conflict have

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repeatedly noted that these do not evolve in an ecological vacuum, but rather are likely to be targets of natural selection as well (Parker 1979; Rowe *et al*. 1994; Rice & Holland 1997; Rowe & Day 2006; Fricke *et al*. 2009; Maan & Seehausen 2011). Natural selection on the traits involved in conflict could limit their exaggeration due to cost within an ecological context, thereby influencing the coevolutionary pathways followed (or not) by sexual conflict. Ecological selection could also drive trait evolution on its own, and if ecological selection is sufficiently strong, the traits underlying sexual conflict may evolve not by sexually antagonistic coevolution, or at least not by such coevolution alone. As a consequence of such effects, there is an implicit prediction of ecologically dependent parallel evolution in which populations adapting to similar ecological contexts should evolve along relatively similar evolutionary pathways with respect to sexual conflict, whereas populations in different environments should be more divergent (Rowe *et al*. 2003).

We confirmed this verbal argument concerning the potential effects of natural selection via computer simulations that extended previous models of sexual conflict (Gavrilets & Waxman 2002; Rowe *et al*. 2003; Hayashi *et al*. 2007). Our model considered multiple male and female traits under sexual conflict simultaneously and permitted natural selection to vary qualitatively among populations. When environments differed in the strength and direction of natural selection on the male traits, our simulations revealed that male stimulation/coercion and female resistance often evolved in parallel among replicate populations in correlation with the environment. The result was that the evolution of traits normally considered as phenotypic manifestations of sexual conflict (e.g. male harm, female resistance) was predictable by ecology (see Supporting Information).

Empirically, by exposing females to males from different populations (or *vice versa*), it is possible to detect evolved differences among populations in male (or female) components of sexual conflict. However, much of the experimental work employing this technique has not explicitly considered ecology. Instead, focus has been given to assessing the claim that females should be more resistant to coevolved (i.e. local), as opposed to foreign, males (Holland & Rice 1998; Andrés & Arnqvist 2001; Brown & Eady 2001; Chapman *et al*. 2003; Long *et al*. 2006; Wigby & Chapman 2006). However, this claim is not based on a robust theoretical framework and the value of population crosses for assessing sexually antagonistic coevolution has therefore been questioned (Rowe *et al*. 2003). In spite of this, such crosses do provide a powerful approach for testing ecologically dependent parallel evolution of the traits involved in conflict. In particular, if ecology is important then (1) males from populations independently adapted to the same type of habitat should have similar effects on females, and/or (2) females from the same habitat type should be similarly affected by (or resistant to) males. While ecology would promote parallel evolution of such male and female sexual conflict traits within environments in this scenario, it should simultaneously promote consistent patterns of divergence in these traits among populations occupying different environments. The ecologically dependent parallel evolution of male and/or female effects on sexual conflict would provide direct evidence of the importance of ecology in the divergence of conflict phenotypes.

A number of empirical studies have shown that the opportunity for sexual conflict, as well as its economics (i.e. costs and benefits), can depend on the ecological context under which it is assayed (Endler 1980, 1987; Magurran & Seghers 1994; Rowe *et al*. 1994; Edward & Gilburn 2007; Fricke *et al*. 2010b; Karlsson *et al*. 2010). More recently, ecology has been manipulated within the context of evolution experiments in seed beetles addressing traits involved in sexual conflict. In particular, Fricke *et al*. (2010a) showed that two food environments differed in the extent of evolutionary diversification among populations, although there was no evidence that populations from shared environments followed similar evolutionary pathways. In a separate experiment, a manipulation of the duration of the reproductive period affected the evolution of genital and mating traits (Maklakov *et al*. 2010; Cayetano *et al*. 2011).

As a test of ecology's role in sexual conflict, we used a long-term evolution experiment to assess the consequences of male–female interactions on a key life history trait among replicate populations of *Drosophila melanogaster* that had independently adapted to one of two alternative laboratory environments. We assess how much female longevity is reduced by high levels of exposure to males from different types of populations (e.g. coevolved males, males from alternative populations evolving in the same environment, or males from alternative populations evolving in a different environment). Because of the large number of interpopulation combinations, we did not measure female offspring production, and therefore do not know the impact of the observed reductions in lifespan on net fitness. Nonetheless, male-induced reductions in female longevity have classically been interpreted as an outcome of sexual conflict in fly populations (e.g. Fowler & Partridge 1989; Rice 1996) and have been shown to be associated with reductions in total fitness in other populations (Edward *et al*. 2011). Longevity is also a key life history component in populations with overlapping generations such as ours. Furthermore, males with the greatest effect on female mortality tended to have the highest fitness (Rice 1996), indicating that differences in female longevity are associated with male fitness gains and thereby provide insight into ongoing sexual conflict (Chapman 2001). Therefore, male-induced reductions in female longevity are likely manifestations of sexual conflict over fitness, though further tests would be required to confirm this. We demonstrate that male harm and female resistance to such harm both evolve in correlation with environment, providing some of the first experimental evidence that ecological adaptation may explain a substantial component of the among-population variation in traits previously thought to evolve solely via sexually antagonistic selection.

MATERIAL AND METHODS

Adaptation and sexual conflict in *Drosophila* populations

A stock population of *D. melanogaster* was founded in September 2005 from a large collection of wild flies in the Similkameen Valley, British Columbia (Yeaman *et al*. 2010). Since

its collection, this stock has been maintained at a large census size in population cages with overlapping generations. In 2007, 20 independent and isolated populations were derived from this stock, with ten of these maintained on food supplemented with 12% ethanol and the other ten on food containing 70 μg/ml cadmium chloride, while the stock continued to be maintained on their standard medium. These experimental populations (which we refer to as selection lines below) were maintained in population cages with overlapping generations like the stock, with new food added every 21/14 days in ethanol/cadmium environments respectively. (The different feeding schedules were used because the development time was initially slower in ethanol as compared to cadmium.) These environments were arbitrary, chosen only because selection was likely to differ between them and, based on past studies, adaptation was likely. All populations were kept on a 12 : 12 h light : dark cycle at 25 °C and at 50% humidity.

Our main goal was to test for the ecologically dependent parallel evolution of traits involved in sexual conflict. Prior to this, however, we first confirmed that these populations had adapted to their respective environments using a reciprocal transplant assay that measured the number of adult offspring produced by replicate male–female pairs in July 2011 (see Supporting Information for further details). We also confirmed the presence of sexual conflict in the ancestral stock population by testing for male induced harm of females. This assay was conducted in the fall of 2012 and involved measuring the longevity of stock females when exposed to their own males either continuously or for a 4 h period once per week (high vs. low exposure respectively).

Given evidence from the above assays for the adaptive divergence of these populations, as well as ongoing sexual conflict in the stock from which they were derived (see Results), we tested for ecologically dependent parallel evolution of traits involved in this conflict by measuring male harm and female resistance among a subset of eight of the selection lines, as described below. These populations were selected as the four with the highest fitness in each of the two environments.

Male harm

To test for differences among populations and environments in males' effect on female life history, in the fall of 2012 we measured the longevity of stock females when continuously exposed to males from each of the eight chosen selection lines (four ethanol and four cadmium). All flies were reared for one generation on the 'ancestral' food (i.e. no ethanol or cadmium added) prior to the assay, and individuals for use in the assay were collected as virgins using light CO_2 anaesthesia within 8 h of emergence. Thirty replicate vials were set up for each male population, with each vial containing five stock females in the ancestral medium. Each vial also contained five selection line males, and males were replaced weekly with fresh individuals that were collected on day 14 of their life cycle from the appropriate selection line. Flies were also transferred to new vials containing fresh food weekly at this time. Extra agar was added when preparing the food for all experimental vials to slow its breakdown (i.e. liquefaction) from larval feeding. Female death was recorded daily and used to estimate average female longevity for each vial. If male mortality was observed, replacement males from the appropriate population were added such that each vial always had five males present. The assay was performed in two complete blocks separated by one day, with half the replicates from each population being performed in each block.

Variation in female longevity was tested using a general linear mixed model, fit via restricted maximum likelihood (REML), using the average longevity of the five females from each replicate vial as the response variable to avoid pseudoreplication. Male environment (ethanol vs. cadmium) and experimental block were fixed effects, and population was a random effect nested within environment. Fixed effects were evaluated via standard type 3 tests, while significance of the random effect of population was evaluated via a likelihood ratio test (LRT) that compared the fit of models that included and excluded this term. Results were qualitatively similar if block was modelled as a random effect. Analyses were performed using the mixed procedure in SAS (SAS Institute, Inc., Cary, NC, USA).

Female resistance to male harm

We next tested whether adaptation to the different environments affected female resistance to male-induced harm. The assay was conducted in the fall of 2012. Females from the same eight selection lines as the male harm assay were housed under high and low male exposure treatments using common stock males. Female resistance to male-induced harm can be quantified as the extent to which female longevity is reduced in the high as compared to low exposure treatments, with smaller differences indicating greater resistance (Friberg 2005). In the high exposure treatment, females were held together with males continuously, with the males replaced weekly with fresh individuals that were collected on day 14 of their life cycle, as in the male harm assay above. In the low exposure treatment, females were held together with stock males for a 4 h period once each week, with the timing coinciding with replacement of males in the high treatment level. Short, weekly access to males provided sufficient sperm such that female offspring production was unlikely to be sperm limited while greatly reducing female exposure to male harassment and the opportunity for multiple matings.

Virgin males and females were collected as previously described. Thirty replicate vials were created for each combination of female population and exposure treatment, with each vial again containing five females. Vials in the high exposure treatment also held five males, and any males that died between transfers were replaced daily by similar aged stock males. In the low exposure treatment, the number of males added for the 4 h period was matched to the number of females alive in the vial at that time. All flies were anesthetized with light CO_2 during weekly addition/replacement of males, and high exposure vials were again anaesthetized 4 h later to coincide with the removal of males from the low exposure vials, thereby ensuring that both treatment levels were handled similarly. Flies were also transferred to new

vials containing fresh high agar food weekly at this time. The assay was performed in two complete blocks separated by one day, with half of the replicates from each combination of treatment and population being performed in each block. Female death was recorded daily.

Variation in female longevity was tested using a partially nested general linear mixed model, fit via REML, again using the average vial longevity as the response variable. Fixed effects included male exposure treatment (low vs. high), female environment (ethanol vs. cadmium), and their interaction, as well as block, while female population nested within environment, along with the interaction of this with male exposure treatment, were modelled as random effects.

Because similar average lifespans can be achieved via varied life history strategies involving different temporal patterns of mortality (Pletcher 1999), we also estimated mortality parameters for every combination of exposure treatment and female population (150 females/combination). For each combination, the best-fit mortality model from the Gompertz family was first determined using a series of nested LRTs as implemented in the program WinModest (Pletcher 1999). For the majority of population × male exposure combinations (11 of 16), female mortality was best described by a Gompertz model (Pletcher 1999), $\mu_x = \alpha e^{\beta x}$, where μ_x is the instantaneous mortality rate at age x, α is the baseline mortality and β is the senescence rate (i.e. the rate at which mortality increases with age). α and β were estimated separately for each population per treatment and analysed with the linear model above excluding both terms involving the random effect of population as there was only a single value of each mortality parameter for each population × treatment combination.

Factorial assay of selection lines

All of the above assays involved interactions between evolved individuals of one sex and stock individuals of the other. Therefore, to test the consequences of male-induced harm within and between selection lines, we also performed a full factorial experiment, measuring female longevity for all 64 pairwise combinations of males and females from all eight selection lines in the fall of 2011. Populations were raised for one generation on the ancestral food prior to conducting the assay and virgins were collected as above. Ten replicate vials were established for each male × female population combination, split evenly between two blocks separated by one day. Each vial initially contained five females and five males on the ancestral food medium. Males were replaced weekly with fresh individuals that were collected on day 14 of their life cycle, as in the male harm assay above. Flies were also transferred to new vials containing fresh high agar food weekly at this time. We checked vials daily for mortality, recording the day of death for females and replacing any dead males as in the assays above.

Variation in female longevity was tested using a general linear mixed model, fit via REML, with average female longevity of a vial as the response variable. Fixed effects included male environment (ethanol vs. cadmium), female environment (ethanol vs. cadmium) and their interaction, as well as block. Male and female population were random effects nested within male and female environment respectively. The interaction of male and female population was non-significant and was therefore excluded from further analyses.

RESULTS

Adaptation and sexual conflict in *Drosophila* populations

In a reciprocal transplant fitness assay of all populations, we found that each of the twenty selection lines had higher fitness in its selected environment than the ancestor (i.e. the stock; Fig. 1). Although adaptation to cadmium also conferred increased fitness in ethanol as a by-product (the converse did not occur), each population also produced significantly more offspring on average in its selected environment than in the other selective environment (Fig. 1; selected environment × test environment interaction: $F_{1,17} = 23.3$, $P = 0.0002$), demonstrating the adaptive divergence of these populations between the two environments. Also, consistent with past studies in this species, and indicative of ongoing sexual conflict with respect to life history in the ancestor, the longevity of stock females was decreased substantially (from 47 to 25 days on average) in the high as compared to the low male exposure treatments (*t*-test: $t_{49.08} = -12.21$, $P < 0.0001$), demonstrating substantial male-induced harm.

Male harm

We tested whether adaptation to the different environments affected male-induced harm by measuring the longevity of stock females when exposed continuously to males from each of the eight selection lines. Suggestive of population-specific effects of sexual conflict, there was observable variation in female longevity induced by exposure to males from the different populations, although this was non-significant (Fig. 2; LRT: $\chi^2 = 2.3$,

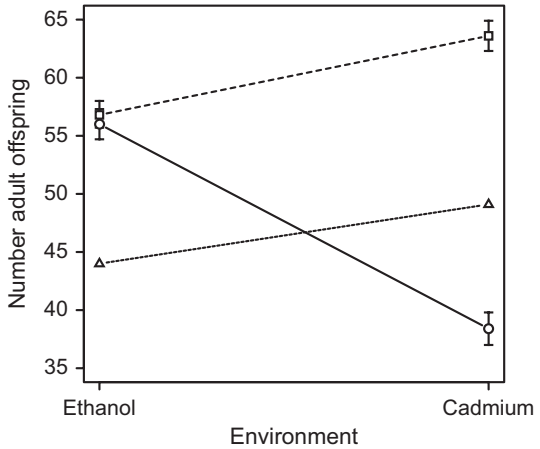

Figure 1 Number of adult offspring produced by single *Drosophila melanogaster* male–female pairs in each of two environments (ethanol vs. cadmium containing food) for each of ten ethanol-adapted (circles, solid line) and ten cadmium-adapted (squares, dashed line) populations. The ancestral stock population is also shown for reference (triangles, dotted line). Points represent the mean (± among-population SE) of the ten replicate populations within each environment. Forty replicate male–female pairs were created per population per test environment

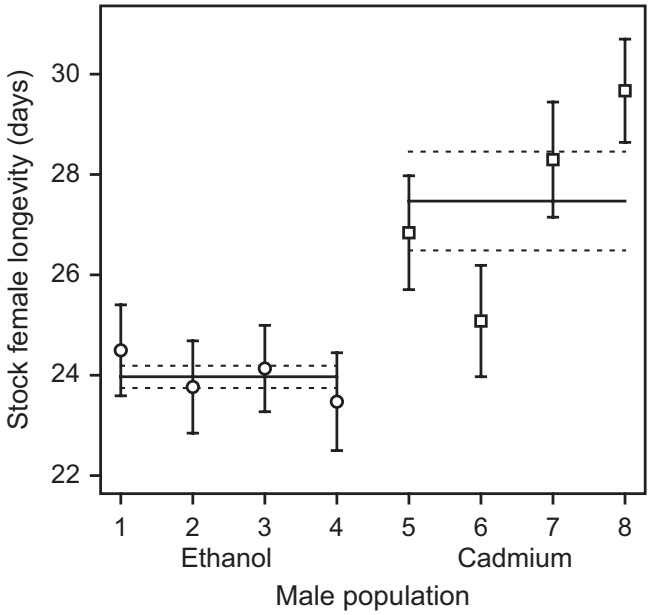

Figure 2 Average longevity (± SE) of stock *Drosophila melanogaster* females when exposed to evolved males adapted to either ethanol (populations 1–4, circles) or cadmium (populations 5–8, squares; 30 vials per population). Solid lines show the means (± SE, dashed lines) for the two types of males, treating populations as replicates.

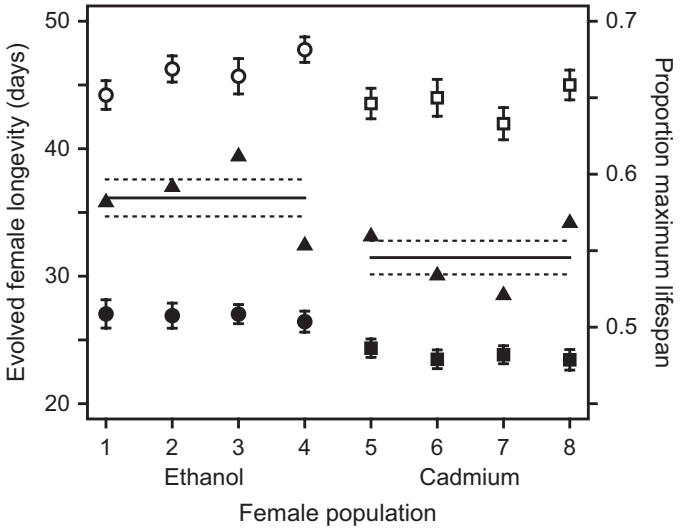

Figure 3 Average (± SE) longevity of *Drosophila melanogaster* females from each of the eight selection lines (ethanol: populations 1–4, circles; cadmium: populations 5–8, squares) under treatments involving high (filled) and low (open) exposure to stock males (30 replicate vials per female population per treatment). Triangles (right axis) represent the proportion of the low exposure treatment lifespan achieved by high exposure treatment females for each population, with among-population means and SE for each environment denoted by the solid/dashed lines respectively.

$P = 0.129$). However, a large component of this variation was associated with environment (58% overall), with females exposed to ethanol-adapted males dying an average of 3 days sooner than females exposed to cadmium-adapted males (Fig. 2). This generated a significant environment effect overall ($F_{1,6} = 11.96$, $P = 0.013$), demonstrating ecologically dependent parallel evolution of male-induced harm in correlation with environment.

Female resistance to male harm

We next tested for variance in female resistance to male harm among the eight selection lines. As previously observed for stock females, increased male exposure had a large and significant effect on the longevity of evolved females overall, reducing it by 19 days on average across the eight lines ($F_{1,6} = 1415.6$, $P < 0.0001$). Female longevity also varied significantly between environments, with ethanol-adapted females living longer than cadmium-adapted females across the male exposure treatments ($F_{1,6} = 27.2$, $P = 0.002$). The reduction in female longevity between male exposure treatments did not differ significantly between females adapted to ethanol vs. cadmium when measured on an absolute scale of days (treatment × environment interaction: $F_{1,6} = 0.50$, $P = 0.501$; Fig. 3). However, because cadmium-adapted females had significantly shorter lifespans overall, a given reduction in absolute lifespan represented a greater proportion of the maximum lifespan of these females as compared with ethanol-adapted females (Fig. 3), generating a difference in female resistance on a relative scale that approached significance ($t_{5.94} = 2.39$, $P = 0.055$).

Mortality parameters may differ even in the absence of differences in average longevity, and we therefore also analysed these for each female population × male treatment combination. The estimated baseline mortality (α) did not differ between male exposure treatments ($F_{1,10} = 1.1$, $P = 0.32$), between females adapted to the cadmium vs. ethanol environments ($F_{1,10} = 0.05$, $P = 0.83$), nor was there evidence of an exposure treatment × environment interaction ($F_{1,10} = 1.1$, $P = 0.33$). In contrast, senescence rates (β) were significantly higher in the high vs. low male exposure treatments ($F_{1,10} = 188.2$, $P < 0.0001$), and for cadmium as compared to ethanol-adapted females ($F_{1,10} = 6.48$, $P = 0.029$), mirroring the differences in average lifespan. However, the effect of female environment depended on male exposure, generating a significant exposure treatment × environment interaction ($F_{1,10} = 5.47$, $P = 0.038$) in which senescence rates were similar for cadmium and ethanol-adapted females under low male exposure, but greater for cadmium than ethanol-adapted females under high male exposure (Fig. 4). Ethanol-adapted females were therefore more resistant to male-induced increases in senescence rate, on average, than were cadmium-adapted females.

Factorial assay of selection lines

Finally, to test the consequences of male-induced harm within and among selection lines, we created all 64 possible combinations of males and females among the eight selection lines. Consistent with unique evolutionary trajectories of separate populations under sexual conflict, we found significant effects of both male and female population on female longevity (LRT: $\chi^2_1 = 39.5$, $P < 0.0001$ and $\chi^2_1 = 67.3$, $P < 0.0001$ respectively).

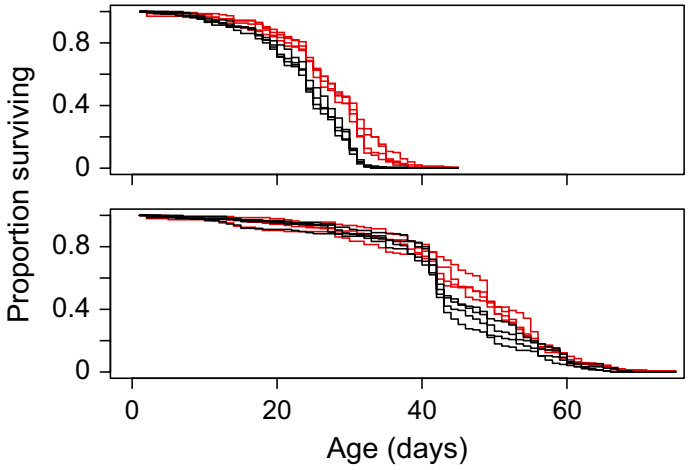

Figure 4 Proportion survival over time of *Drosophila melanogaster* females from four ethanol-adapted populations (red lines) and four cadmium-adapted populations (black lines) under treatments of high (top) and low (bottom) exposure to stock males. Each population started with 150 females.

Longevity was also greater for ethanol-adapted females overall ($F_{1,6} = 93.19$, $P < 0.0001$), as previously observed when these females were exposed to stock males. There was also a trend for ethanol-adapted males to be more harmful than cadmium-adapted males, as previously observed, although this was not significant ($F_{1,6} = 1.49$, $P = 0.27$). There was no interaction between male and female environment (Fig. 5; $F_{1,603} = 1.3$, $P = 0.26$).

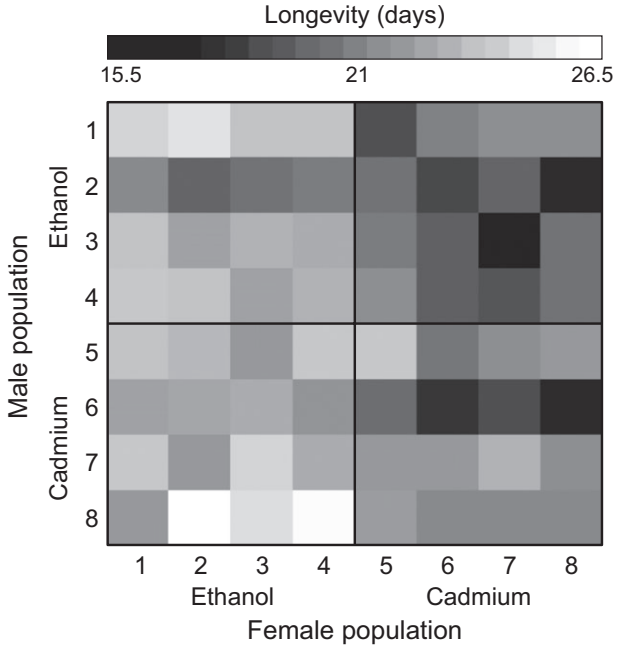

Figure 5 Average longevity of *Drosophila melanogaster* females from each of the eight selection lines (*x*-axis) when paired with males from each of the eight populations (*y*-axis). Ten replicate vials were established for each female × male population combination. Populations 1–4 are adapted to ethanol and populations 5–8 are adapted to cadmium. Darker shades represent lower female longevity as shown in the legend.

We found little indication that females were more resistant to their coevolved males among our crosses (Fig. 5): the male × female population interaction was non-significant overall ($\chi^2_1 = 1.6$, $P = 0.20$) and, when considering only within-environment crosses, there was no significant difference in longevity when females were paired with their own vs. foreign males (ethanol populations: $F_{1,148} = 0.27$, $P = 0.60$; cadmium populations: $F_{1,141} = 1.10$, $P = 0.30$). Therefore, the among-population differences in male and female effects on female longevity, including those associated with environment, are relatively consistent irrespective of the identity of the other sex.

DISCUSSION

In line with numerous past studies (Fowler & Partridge 1989; Partridge & Fowler 1990; Friberg 2005; Edward *et al.* 2011), we found that males impacted females through a substantial reduction in their longevity. Though we did not measure female offspring production and therefore do not know the impact on net female fitness, that these reductions are harmful appears likely given the magnitude of the effect (19 days on average) in populations evolving with overlapping generations. Male-induced reductions in female longevity have previously been shown to be associated with reductions in fitness (Edward *et al.* 2011), though further tests would be required to confirm such reductions. Consistent with previous theory (Holland & Rice 1998; Arnqvist & Rowe 2005), we also found significant variation in the magnitude of male harm and female resistance among independently evolved populations, including within environments (e.g. Fig. 2), confirming that isolated populations can follow unique evolutionary trajectories under sexual conflict. However, our results also revealed that a significant portion of this variation was predictable based on the environment to which a population was adapted, such that the magnitude of both male harm and female resistance depended to a large extent on the ecological histories of both sexes. Specifically, ethanol-adapted males were more harmful, and ethanol-adapted females were more resistant to this harm, than were cadmium-adapted flies, demonstrating ecologically dependent parallel evolution of these traits. The evolution of such traits in correlation with ecology demonstrates that sexual conflict should not be considered as an entirely non-ecological promoter of divergence (Coyne & Orr 2004; Rundle & Nosil 2005), and also suggests that the common division of models of diversification into ecological vs. non-ecological may be overly simplistic.

Ecology may affect sexual conflict in several (non-mutually exclusive) ways including imposing limits on trait exaggeration via antagonistic coevolution, biasing coevolutionary pathways or even overriding sexually antagonistic selection entirely by favouring particular values of harmful or resistant traits outside of their role in sexual conflict. While we do not know the particular mechanism by which ecology caused the divergence of male harm and female resistance among our populations, our results suggest that these traits have been exaggerated to a greater extent in the ethanol environment than in the cadmium environment. In our simulations (Supporting Information), we found that the exaggeration of traits under sexual conflict tended to be limited in environments in which natural selection

on these traits was stronger, whereas environments with weaker natural selection allowed for greater trait exaggeration (see also Rowe *et al*. 2003). This pattern led to ecologically dependent parallel evolution of increased male harm and female resistance in populations evolving in environments with weak natural selection on the traits mediating sexual conflict. From this perspective, one interpretation of our results is that the cadmium environment acted as a harsh and constraining habitat that limited the evolution of male harm and female resistance, while sexual conflict was less constrained in the populations evolving in ethanol. An alternative is that the ethanol environment selects for more robust genotypes than does cadmium, resulting in males capable of causing more harm and females capable of greater resistance. Regardless, it is clear that ecological history has a marked influence, through both sexes, on sexual conflict measured as the effect of males on female survival.

Fricke *et al*. (2010a) measured the divergence of sexual conflict traits (including male harm and female resistance) among replicate seed beetle populations that had evolved in an ancestral vs. novel food environment. In this experiment, populations adapting to a new environment showed less trait divergence, demonstrating that the selective environment can influence sexual conflict and suggesting that stronger selection acting on populations can limit the extent of sexual conflict trait exaggeration. Similarly, male mate guarding and harassment in freshwater isopods was exaggerated to a greater extent in environments with less predation (Karlsson *et al*. 2010). Our fly populations, which were all under directional selection, experimentally demonstrate that the particular environment to which populations are adapting can have large impacts on sexual conflict trait values, as well as the level of among-population divergence.

Contrary to previous predictions (Holland & Rice 1998; Andrés & Arnqvist 2001), we did not find that females were best defended against the harm of their own (i.e. local) males. The evidence for females having greatest resistance to harmful effects of coevolved males is mixed (Andrés & Arnqvist 2001; Brown & Eady 2001; Chapman *et al*. 2003; Long *et al*. 2006; Wigby & Chapman 2006), and as Rowe *et al*. (2003) mathematically demonstrated, it is just as easy to make the opposite prediction that males should be best adapted, and thus most harmful to, their own females. Obviously, both predictions cannot be simultaneously true and the realized outcome of sexually antagonistic interactions depends on a variety of details. What is remarkable in our current results is the extent to which ecology predicted divergence among populations of both male harm and female resistance over and above such details that could have produced population-specific outcomes. Our results highlight the variation that could arise in crosses among natural populations with different (and possibly poorly understood) histories of ecological selection.

Ecologically dependent selection plays a large role in species radiations, often driving the parallel evolution and/or divergence of phenotypes and reproductive isolation (e.g. Rundle *et al*. 2000; Schluter 2000; Nosil *et al*. 2002). A role for ecology in the evolution of traits under sexual conflict could have large impacts on how populations adapt to new environments, potentially also influencing patterns of reproductive isolation. The joint influence of ecology and sexual conflict on phenotypic diversification and speciation is therefore an important topic for future work.

ACKNOWLEDGEMENTS

We are grateful to J. Sztepanacz, A. White, S. Gershman, M. Stoeva, K. Henbest, E. Toumishey and A. Groulx for significant help with laboratory work. L. Rowe and members of the Rundle laboratory provided useful feedback, and R. Sargent gave invaluable advice on mathematical modelling and computer simulations.

AUTHORSHIP

DA, AFA and HDR designed research; DA and EMD performed research; DA, EMD and HDR analysed data; and DA, EMD, AFA and HDR wrote the manuscript.

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Editor, Andrew Bourke
Manuscript received 8 July 2013
First decision made 14 August 2013
Manuscript accepted 22 October 2013