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Testing for local adaptation in adult male and female fitness among populations evolved under different mate competition regimes

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Mating/fertilization success and fecundity are influenced by sexual interactions among individuals, the nature and frequency of which can vary among different environments. The extent of local adaptation for such adult fitness components is poorly understood. We allowed 63 populations of *Drosophila melanogaster* to independently evolve in one of three mating environments that alter sexual interactions: one involved enforced monogamy, while the other two permitted polygamy in either structurally simple standard fly vials or in larger "cages" with added complexity. Adult male and female reproductive fitness were measured after 16 and 28 generations, respectively, via full reciprocal transplants. In males, reciprocal local adaptation was observed between the monogamy and simple polygamy treatments, consistent with the evolution of reproductively competitive males under polygamy that perform poorly under monogamy because they harm their only mate. However, males evolved in the complex polygamy treatment performed similarly or better than all other males in all mating environments, consistent with previous results showing higher genetic quality in this treatment. Differences in female fitness were more muted, suggesting selection on females was less divergent across the mating treatments and echoing a common pattern of greater phenotypic and expression divergence in males than females.

KEY WORDS: Environmental complexity, experimental evolution, mating success, monogamy, polygamy, sexual conflict.

Local adaptation is typically studied in the context of understanding how natural selection varies across space (Schluter 2000; Kawecki and Ebert 2004), and the emphasis of such studies most often concerns the success of genotypes at dealing with particular extrinsic ecological conditions. For example, an experiment examining local adaptation with respect to viability may ask whether local or foreign genotypes are better at surviving local temperatures or predation regimes. However, adult fitness components like mating (fertilization) success and fecundity also depend, sometimes quite strongly, on sexual interactions occurring in these environments. How important is local adaptation for such fitness components?

It is intuitive why local adaptation is expected for fitness components that are the direct outcome of interfacing with some extrinsic ecological factor (e.g., avoiding predation by crypsis in environments that differ in background coloration). Naïvely, one might assume that there is no opportunity for local adaptation for fitness components that are strongly influenced by sexual interactions if sexual interactions are viewed as an "intrinsic" property of the species that is not influenced by the environment. In reality, the environment may nevertheless be important because it can affect the frequency and nature of sexual interactions, creating the

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opportunity for local adaptation. For example, lighting conditions can have an immediate impact on which male colors are most apparent to females, and thus which male phenotypes are most attractive (Endler 1983; Boughman 2001). Changes in extrinsic ecology can also lead to changes in the social setting. For example, factors reducing density may reduce the opportunity for female choice and multiple mating, or resource availability or clumping may affect intra- and intersexual interactions. In sand gobies, for example, the availability of nesting resources affects the relative importance of male–male competition and female choice (Forsgren et al. 1996; Svensson and Forsgren 2003). Alternative social settings may also shift the importance of male–male competition versus female choice (Price and Rodd 2006), affecting what phenotypes are most successful. As discussed below, one important difference between populations experiencing different social settings can be the extent to which the reproductive interests of males and females are codependent; among-population variation in this codependence may affect the potential for local adaptation.

Recent work has drawn attention to the interesting, but often overlooked, parallels between local adaptation and intersexual differences, as well as their intersection (Connallon et al. 2018 and references therein). Models have explored situations where the optimal phenotype for each sex varies over space because of changes in the extrinsic environment alone (e.g., Connallon 2015) or in response to changes in the other sex (e.g., Day 2000). These models illuminate factors affecting local adaptation, even though their precise quantitative predictions are particular to their assumptions. Such models demonstrate the simple but robust point that the sexes will often differ in their degree of local adaptation. Although the degree of local adaptation is expected to depend on a variety of factors (e.g., sex-specific variances, intersexual covariances, and migration rates), perhaps the most important is the degree to which selection varies across environments. Thus, the sex for which selection differs most should have the highest degree of local adaptation, holding all else equal. Although empirical studies of local adaptation are numerous, a recent review by Svensson et al. (2018) found that very few have attempted to measure local adaptation in nature for both males and females for major fitness components other than viability. In the two studies they found that examined local adaptation for both male and female traits (Hedderson and Longton 2008, Li et al. 2015), the fitness components, and thereby local adaptation measures, were not comparable between sexes.

In our study, we consider replicate lab populations of *Drosophila melanogaster* that have evolved in three different mating environments that alter the nature of sexual interactions, one of which involves enforced monogamy and the other two involve polygamy. In one polygamous treatment, mating interactions occur in a smaller, and hence higher density environment, with low spatial complexity (a standard fly vial); in the other polygamous treatment, mating interactions occur in a larger and thus lower density, more structurally complex environment (a small "cage"). Previous experiments using a single fly population have shown that mating interactions are more frequent, and male harm is more severe, when assayed in the simpler environment (Yun et al. 2017). Thus, we suspect the two polygamy treatments differ with respect to the codependence of male and female fitness and hence the importance of sexual conflict. Here, we measure adult male and adult female fitness for each population, assayed in each of the three mating environments. In each assay, the focal sex from an evolved population is tested in a particular mating environment with individuals of the opposite sex from the ancestral population. As such, these assays reflect the success of genotypes of the focal sex shortly after the ancestral population colonizes this new environment. (There is another potential layer of local adaptation of the focal sex to genotypes of the opposite sex that have evolved in that environment, which we do not consider here.) We examine variation in adult fitness to gain insight into two issues concerning local adaptation.

First, does local adaptation occur equally for adult male and female fitness? Unlike juvenile viability, which is likely to have a similar genetic basis between the sexes (Chippindale et al. 2001), different traits contribute to adult male and female fitness and the reproductive interests of the sexes may not align, causing selection to differ between them. Adult male fitness usually depends strongly on sexual interactions, so if the physical environment modulates such interactions, then different male phenotypes are likely to be favored in different environments. Compared to adult male fitness, adult female fitness is often less strongly tied to sexual interactions and, instead, may be more dependent on available food sources (Chapman and Partridge 1996). Thus, on average, we might expect less divergence for traits affecting adult female fitness among populations that differ with respect to sexual interactions and, consequently, less potential for reciprocal local adaptation of adult female fitness than male adult fitness under such circumstances. Of course, the environment can affect female fitness by altering sexual interactions, especially in systems with male harm. Thus, it would be misleading to assume there is no local adaptation for adult female fitness, although we might expect less than for males. Local adaptation therefore needs to be quantified separately for each sex. Local adaptation is expected when selection differs across environments and the appropriate genetic variation exists to respond to that selection. If local adaptation is stronger in one sex than the other, then this implies that either selection is more divergent across environments for that sex and/or that sex has more genetic variation to respond to the divergent selection it experiences.

A second focus of this study involves the contrast between social environments with and without mate competition, that is, polygamy and monogamy, respectively. In many polygamous

species, traits evolve in males that enhance their own fitness but have negative effects on the females with whom they interact (i.e., interlocus sexual conflict; Arnqvist and Rowe 2005). Harmful effects of males on females are well documented in *D. melanogaster* (Fowler and Partridge 1989; Partridge and Fowler 1990; Liddle et al. 1995), making it a model system for the study of sexual conflict. Although such "harmful" males are presumed to perform well in their home social setting, they are expected to perform poorly in environments where fitness is more codependent between mates. Consequently, males are expected to evolve to be less harmful in monogamous populations. Indeed, in a classic evolution experiment in *D. melanogaster*, Holland and Rice (1999) showed that females had higher survival and reproductive output when housed with males evolved in a monogamy treatment rather than from a polygamy treatment; in other words, monogamy males evolved to be less harmful to their mates. Holland and Rice (1999) did not perform a reciprocal transplant experiment to assess male fitness of monogamous versus polygamous males in each mating environment, but one would predict a pattern of reciprocal local adaptation in this case. Does such a pattern occur? And does one always find a pattern of reciprocal local adaptation when comparing monogamous and polygamous populations?

As outlined above, reciprocal local adaptation is expected for male fitness under the assumption that polygamy is characterized by strong sexual conflict resulting in the evolution of male harm. However, sexual conflict is not necessarily strong in all polygamous situations. For example, male harm is thought to be largely absent from taxonomic groups in which females largely control sexual interactions (Zuk et al. 2014). Even within taxa in which sexual conflict and male harm are known to occur, the environment can play a key role in determining the extent of conflict in any particular population (Magurran and Seghers 1994; Rowe et al. 1994; Edward and Gilburn 2007; Fricke et al. 2010; Karlsson et al. 2010; Arbuthnott et al. 2014; Yun et al. 2017). Our two alternative polygamy treatments differ in the extent of male harm (Yun et al. 2017), providing an opportunity to test whether reciprocal local adaptation between monogamous and polygamous populations is more likely when the polygamous population has evolved under conditions where we expect sexual conflict to be strong versus weak.

Material and Methods

EXPERIMENTAL POPULATIONS

A stock population of *D. melanogaster* was originally collected from Similkameen Valley, British Columbia, Canada in 2005 by S. Yeaman. Since fall 2010, this population had been maintained in standard *Drosophila* culture bottles containing 40 mL of cornmeal medium at 25°C, 12L:12D photoperiod, and 50% relative humidity at a large population size (~3000 adults) with discrete, nonoverlapping 2-week generations. In September 2014, 63 separate experimental populations, consisting of 140 males and 140 females each, were derived from this "ancestral" stock. These experimental populations were divided equally into three "larval adaptation" sets (21 populations per set), each set involving different and novel larval rearing conditions: Set 1 featured a cornstarch (rather than cornmeal) larval medium and a 2-hour heat shock in a 37°C water bath to 3-day old larvae, set 2 had the ancestral cornmeal larval medium supplemented with 10% ethanol and included a 2-hour cold shock in a 4°C fridge to 3-day old larvae, and in set 3, the ancestral cornmeal medium was supplemented with 5% salt and larvae were exposed to a constant 28°C (rather than the standard 25°C). To promote continued adaptation to these larval environments, after the sixth generation, we increased the salt concentration to 6% (set 3) and the duration of the heat and cold shocks to 4 hours in sets I and II, respectively. Each adaptation set was maintained on a 3-week nonoverlapping generation, with each set offset from the others by 1 week. These are the same populations in which Yun et al. (2018) quantified egg-to-adult survivorship and inbreeding depression. In the current experiment, we are not examining local adaptation with respect to these different larval rearing conditions. Rather, our reciprocal transplant is a comparison among populations from different mating regimes (described below) but within the same larval adaptation set.

Within each adaptation set, the 21 replicate populations were divided equally among three mating treatments (seven populations/treatment) that manipulated the opportunity for mate competition among adults and the abiotic environment in which this occurred. (Outside of the mating treatments, all populations within a given adaptation set were maintained in the same manner and experienced the same environment.) The first mating treatment removed mate competition via enforced monogamy (MC_{absent}), which was implemented by randomly creating 140 individual male–female mating pairs per population each generation and separately holding them in wide plastic straws (radius = 6.35 mm; height = 88.9 mm), the bases of which were inserted into a 3 oz. wax paper cup filled with 25 mL of ancestral food with 1–2 pellets of yeast added to the surface of the food within each straw. Straws were used to reduce the space and maintenance costs associated with this treatment. The second mating treatment allowed mate competition in a small and structurally simple environment (MC_{simple}) by placing 35 males and 35 females together in a standard *Drosophila* culture vial (28.5 mm × 95 mm) filled with 10 mL of ancestral food with abundant yeast sprinkled on top. Four vials were created for each population yielding 140 adults of each sex each generation. The third mating treatment also allowed mate competition but in a larger and more spatially complex environment ($MC_{complex}$). In this case, 35 males and 35 females were placed in a 1.65 L cylindrical plastic Ziploc® food storage container (hereafter "cages") containing five separate food sources

(three 3 oz. wax paper cups containing 25 mL of ancestral food, the surface of which was divided into two by a plastic barrier into the food, and two smaller 1 oz. cups containing 7.5 mL of ancestral media) with abundant yeast sprinkled on top. Each cage also had two pipe cleaners protruding from the lid into the interior. The cages are pictured in figure S1 of Yun et al. (2017). Four cages were setup for each population, totaling 140 adults of each sex each generation. The MC_{simple} and $MC_{complex}$ treatments are the same mating environments used in Yun et al. (2017) and Singh et al. (2017). We refer to vials and cages as "simple" and "complex" environments but note that they differ in additional ways including volume (and hence fly density) and the availability of food and egg-laying sites.

Adult flies were held in their respective mating environment for a 6-day "interaction phase" every generation. On the third day of this interaction phase, flies were transferred via light CO_2 anesthesia to a fresh mating environment of the same type to avoid adult mortality resulting from increasing liquefaction of the food (caused by developing larvae hatching from eggs laid by the females). At the end of the interaction phase, flies were anaesthetized, males were discarded, and 105 females were randomly chosen from each population and were evenly distributed among seven standard culture vials for egg laying. These adult females were discarded after approximately 24 hours. Egg density among oviposition vials was standardized to approximately 200 eggs/vial by physically removing (via scraping) excess eggs from the food surface. To achieve a 3-week generation time to simplify the maintenance schedule, newly emerged adults from the oviposition vials were collected 11 days later and were stored for 3 days in holding vials, separately by sex (35 flies per vial), before being placed in their respective mating environment as described above.

In March 2015, we created a marked "competitor" population for use in our subsequent fitness assays by introgressing an autosomal *DsRed* marker into a sample of the ancestral population, as described in Yun et al. (2018). *DsRed* heterozygous and homozygous flies are indistinguishable from wild-type flies under standard lighting, but appear red under appropriate fluorescent lighting.

MALE REPRODUCTIVE FITNESS ASSAY

A competitive assay of adult male reproductive fitness was conducted at generation 16 separately for each adaptation set. Within each set, a reciprocal transplant was performed in which males from all 21 experimental populations from within the same larval adaptation set, and the ancestor, were all assayed in each of the three mating environments (i.e., the mating environment in which they had been evolving as well as the other two mating environments). We refer to the mating environment in which the flies are tested as the "assay mating environment" (i.e., single-pair straws, multi-fly vials, and multi-fly cages) to distinguish this from the mating environment in which they had been evolving (i.e., their "evolutionary mating treatment": MC_{absent}, MC_{simple}, or $MC_{complex}$). In all cases, females in these assays came from the ancestral population; as noted above, these assays thus reflect the success of male genotypes as if appearing in the ancestral population shortly after it colonized the new environment.

Within a given adaptation set, all "focal" (i.e., experimental) males were raised for one generation in a common environment prior to conducting the assay. To do this, 100 males and 100 females were collected from each experimental population and were spread equally among 10 vials to lay eggs for 24 hours, after which the adult flies were discarded, and the food surface was scraped to crudely approximate 100 eggs/vial. All vials contained the appropriate larval food and experienced environmental conditions appropriate to the given adaptation set. A total of 150 newly emerged adult males were collected from these vials 11 days later and were stored in 15 holding vials (10 males/vial) for 3 days prior to use in the assay, mimicking the maintenance protocol of the experimental populations. Ancestral males were also separately raised under the conditions of each larval adaptation set following a similar protocol to that above and were tested alongside all of the experimental populations from each set. Ancestral females and *DsRed* competitor males were collected from flies raised on the ancestral food and were also stored for 3 days (35 females/vial, 25 *DsRed* competitor males/vial) prior to use in the assay. All of the flies (experimental males, *DsRed* competitor males, and ancestral males and females) were collected on the same day within a 6-hour time period.

After the 3-day holding period, adult flies were placed into an assay mating environment (i.e., a single-pair straw, multi-fly vial, or multi-fly cage) for a 6-day interaction phase, mimicking the normal life-cycle for the experimental populations. For each of the three assay mating environments, we created five replicates for each of the 21 experimental populations and 30 replicates of the ancestor. For the multi-fly vial and multi-fly cage assay mating environments, each replicate consisted of 10 focal males together in a single vial or a single cage with 25 *DsRed* males and 35 ancestral females, matching the densities used during their normal maintenance. For the single-pair straw assay mating environment, each replicate consisted of 10 focal males and 25 *DsRed* competitor males, each male being housed in a separate straw together with a single ancestral female. In all three assay mating environments, after the 6-day interaction period, the surviving females were collected from each replicate using light CO_2 anesthesia (pooling the females from the 35 straws in a given single-pair straw replicate) and these females were then evenly distributed among three vials containing 10 mL of ancestral food to lay eggs for 4 hours, after which they were discarded. We used a shorter duration of egg laying and ancestral food to maximize offspring

survival so that variation in the number of emerging adults would primarily reflect male reproductive success.

Adult offspring that subsequently emerged from the egg laying vials were phenotyped (i.e., *DsRed* vs. wild-type) and the reproductive fitness of the focal males from a given replicate was calculated as the overall proportion of offspring sired by focal males across the three vials representing that replicate (i.e., the total number of wild-type offspring produced across the three vials divided by the total number of offspring produced across the same three vials). These proportions were then averaged across replicates to yield a single value for the male reproductive fitness of each population when tested in each assay mating environment. Variation in male reproductive fitness was analyzed, separately by adaptation set, using a partially nested linear mixed model, fit via restricted maximum likelihood, with assay mating environment, evolutionary mating treatment, and their interaction as fixed effects. Random effects included population nested within evolutionary mating treatment and its interaction with assay mating environment. Given a significant assay mating environment × evolutionary mating treatment interaction, the effect of evolutionary mating treatment was subsequently tested via one-way ANOVAs separately by assay mating environment. Results are qualitatively unchanged if we instead fit a generalized linear mixed model with a binomial distribution and logistic link function to the individual replicates (Table S1), so we present the parametric ANOVA results for simplicity. The ancestor was excluded from all analyses because it was unreplicated at the population level.

FEMALE REPRODUCTIVE FITNESS ASSAY

On generation 28, female reproductive fitness (i.e., fecundity) was assayed separately by adaptation set. Analogous to the male fitness assay above, within each set, a reciprocal transplant was performed in which females from all 21 populations (i.e., seven from each of the three evolutionary mating treatments) and the ancestral population were all assayed in each of the three assay mating environments. In all cases, males in these assays came from the ancestral population.

Within a given adaptation set, prior to conducting the assay, all focal (i.e., experimental and ancestral) females were raised for one generation in a common environment (the larval environment appropriate to the given adaptation set) following the same protocol as in the male assay above. Adult focal females were collected 11 days later and were stored in holding vials (10 individuals/vial). Ancestral males and *DsRed* females for use in the assay were collected from flies raised on the ancestral food and were also stored for 3 days (35 males/vial, 25 *DsRed* females/vial) prior to use in the assay. All the flies were collected on the same day and within a 6-hour period.

After the 3-day holding period, adult flies were assigned to one of three assay mating environments for a 6-day interaction phase, mimicking the normal life-cycle of the experimental populations. For both the multi-fly vial and multi-fly cage assay mating environments, we created three replicates from each of the 21 experimental populations and 25 replicates of the ancestor. Each replicate consisted of 10 focal females together with 25 *DsRed* females and 35 ancestral males, matching the densities used during their normal maintenance. After their 6-day interaction period, five focal females were randomly sampled from among the survivors for each replicate and were put singly into special vials for 24 hours of egg laying, after which they were discarded. These egg laying vials were modified 50 mL Falcon^{TM} tubes that had their bottom cut off and plugged with a foam stopper and which were then inverted and had a small dish of the appropriate larval food for the given adaptation set placed inside on top of the original lid. Females would oviposit on the food dish that was subsequently removed, and eggs were counted under a dissecting microscope. For the single-pair straw assay mating environment, 30 straws were set up for each experimental population, each containing one focal female and one ancestral male. After 6 days, 20 focal females were randomly sampled from among the survivors for each population using light CO_2 anesthesia and were put singly into the modified Falcon tubes described above for egg laying. These tubes contained the appropriate food for their adaptation set. These females were discarded after 24 hours and the number of eggs was then counted.

Female reproductive fitness was calculated as the average number of eggs across all replicates for a given population in a given assay mating environment. Variation in female reproductive fitness was analyzed separately by adaptation set using a using a partially nested linear mixed model, fit via restricted maximum likelihood, with assay mating environment, evolutionary mating treatment, and their interaction as fixed effects. Random effects included population nested within evolutionary mating treatment and its interaction with assay mating environment. Given a significant assay mating environment × evolutionary mating treatment interaction, the effect of evolutionary mating treatment was subsequently tested via one-way ANOVAs separately by assay mating environment, treating populations as replicates. The ancestor was excluded from these analyses because it was unreplicated at the population level.

Results

MALE REPRODUCTIVE FITNESS

There was a significant interaction between assay mating environment and evolutionary mating treatment in all three adaptation sets (set 1: $F_{8,54} = 8.23$, $P < 0.001$; set 2: $F_{8,54} = 8.90$, $P < 0.001$; set 3: $F_{8,54} = 18.82$, $P < 0.001$), indicating that the

Table 1. Results from one-way ANOVAs testing the effects of evolutionary mating treatment on male reproductive fitness separately by adaptation set and assay mating environment and treating populations as replicates.

Adaptation set	Assay mating environment	$F_{2,18}$	P-value
1 (Cornstarch/ heatshock)	Single-pair straw	9.65	0.0014
	Multi-fly vial	29.05	<0.0001
	Multi-fly container	31.65	<0.0001
2 (EtOH/ coldshock)	Single-pair straw	15.74	0.0001
	Multi-fly vial	44.39	<0.0001
	Multi-fly container	11.98	0.0005
3 (NaCl/28°C)	Single-pair straw	43.96	<0.0001
	Multi-fly vial	44.89	<0.0001
	Multi-fly container	17.49	<0.0001

effect of evolutionary mating treatment differed by assay mating environment. For all three adaptation sets, male reproductive fitness differed significantly among the evolutionary mating treatments in all three assay mating environments (one-way ANOVAs: $P < 0.01$ in all cases; Table 1).

Within each assay mating environment, the effects of the evolutionary mating treatments were similar across the three adaptation sets (Fig. 1). Recall that in the single-pair straw mating environment, variation in fitness among males is due to variation in fecundity of their mates as there is no variation in number of mates. In this mating environment, MC_{absent} males, which had evolved in this environment, tended to sire more offspring than did MC_{simple} males; this difference was significant in two of the three adaptation sets (Fig. 1D and G) and the pattern was present, but not significant, in the third (Fig. 1A). Males evolved with mate competition in the complex environment (i.e., $MC_{complex}$) also sired significantly more offspring than did MC_{simple} males in all three adaptation sets in the straw mating environment. Surprisingly, $MC_{complex}$ males sired a similar proportion as the MC_{simple} males in two of the three adaptation sets (Fig. 1A and G) and significantly more in the third (Fig. 1D). In the multi-fly vial assay mating environment, results were qualitatively the same across all three adaptation sets, with $MC_{complex}$ males siring significantly more offspring than MC_{simple} males, which in turn sired more offspring than MC_{absent} males (Fig. 1B, E, and H). Finally, in the multi-fly container assay mating environment, $MC_{complex}$ males again performed best in all three adaptation sets, significantly so in two of these (Fig. 1C and I) but not in the third (Fig. 1F). MC_{simple} males performed similarly to MC_{absent} males in two of the three adaptation sets (Fig. 1C and I), but significantly better in the third (Fig. 1F).

FEMALE FECUNDITY ASSAY

There was a significant interaction between assay mating environment and evolutionary mating treatment in all three adaptation sets (set 1: $F_{4,36} = 6.52$, $P = 0.0005$; set 2: $F_{4,36} = 8.83$, $P < 0.0001$; set 3: $F_{4,36} = 6.32$, $P = 0.0006$). Hence, we analyzed the data separately for each assay mating environment (Table 2). In the single-pair straw mating environment, there was significant variation in female fecundity among the evolutionary mating treatments in only one of the three adaptation sets (set 2), in which MC_{absent} females had higher fecundity than $MC_{complex}$ females (Fig. 2D). In the multi-fly vial mating environment, variation in female fecundity among the evolutionary mating treatments was nonsignificant in all three adaptation sets (Fig. 2B, E, and H). In contrast, in the multi-fly container mating environment, there was significant variation among evolutionary mating treatments in all three adaptation sets (Fig. 2C, F, and I), with $MC_{complex}$ females producing significantly more eggs than both MC_{absent} and MC_{simple} females in all cases.

Discussion

There is a long tradition of using reciprocal transplant experiments as a means to study the power of natural selection to improve the fit of organisms to their local environment. The implicit or explicit perspective of many such studies is that fitness is increased via adaptation of traits that interface directly with local ecological factors extrinsic to the focal species (e.g., matching background coloration, thermal physiology, and resistance to the local array of natural enemies). In this study, we focus on fitness components that are likely heavily affected by sexual interactions with conspecifics rather than directly with the "extrinsic" environment. Populations can vary in the frequency and nature of sexual interactions, which in turn may be due to variation in extrinsic ecological factors, and differences in sexual interactions may generate selection for different phenotypes involved in maximizing mating/fertilization success and fecundity. Less attention has been given to quantifying the extent of local adaptation to environments that alter sexual interactions and whether this differs between the sexes. We conducted reciprocal transplant assays measuring adult male and female fitness among sets of populations adapted to three different mating environments that are known to alter sexual interactions (Supporting Information; Yun et al. 2017).

EVIDENCE FOR AND AGAINST LOCAL ADAPTATION IN MALES

We detected clear evidence of reciprocal local adaptation when comparing males evolved under monogamy (MC_{absent}) to those evolved under polygamy in a simple environment (MC_{simple}). When tested in their native assay mating environment (i.e.,

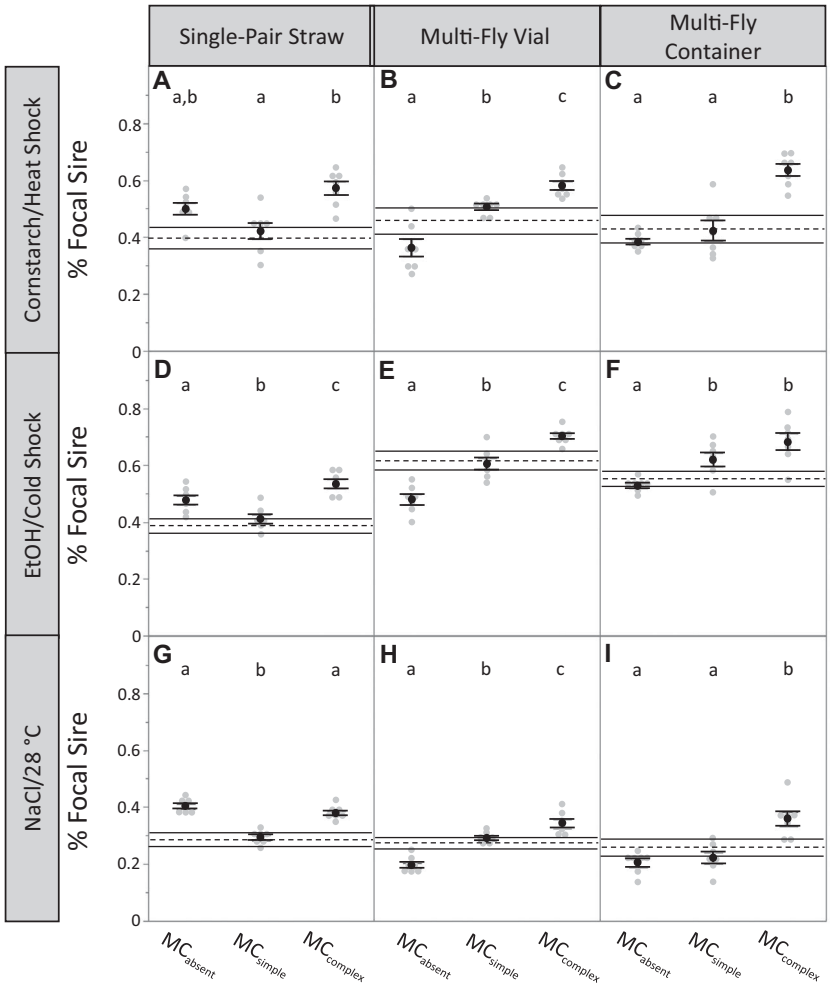

Figure 1. **Reproductive fitness of experimental males when tested in each of the three assay mating environments. Rows are adaptation sets (i.e., larval abiotic environments; see Methods) and columns are the three assay mating environments. Gray points indicate values for individual populations. Black points represent average siring success (± 1 SE) across the seven populations within a given treatment. Horizontal dashed lines represent the average fitness of ancestral males ($n = 30$); horizontal solid lines depict bootstrap 95% confidence intervals. Different letters indicate significantly different means in a post hoc comparison (Tukey's HSD) performed separately by adaptation set and assay mating environment.**

single-pair straws), MC_{absent} males outperformed MC_{simple} males in all three adaptation sets (Fig. 1A, D, and G), with this difference being significant in two sets. Conversely, MC_{simple} males consistently sired significantly more offspring compared to MC_{absent} males when tested in their native mating environment (i.e., multi-fly vials; Fig. 1B, E, and H). This result is consistent with the idea that environments differing in the opportunity for sexual conflict can give rise to a pattern of reciprocal local adaptation. If there is a low degree of codependence between the sexes in reproductive interests, sexual selection may favor traits in males that enhance their own fitness in competition with other males, even at the expense of female fitness (Parker 1979; Rice and Holland 1997; Arnqvist and Rowe 2005). Conversely, in an environment that causes a high degree of codependence, selection will act against traits in a male that harm his only mate (Rice and Holland 1997; Holland and Rice 1999; Martin and Hosken 2003; Tilszer et al. 2006; Crudgington et al. 2010; Hollis et al. 2019). In such a scenario, polygamous males would incur fitness costs under monogamy because they harm the female upon which their reproductive success is entirely dependent, causing her to produce fewer offspring. Alternatively, such "harmful" males are expected to father more offspring in their native social environment in comparison to monogamous males, because they harbor traits that enhance their mating/fertilization success in a competitive setting.

In their classic paper, Holland and Rice (1999) used experimental evolution in *D. melanogaster* to examine the outcome of evolution in the presence versus absence of enforced monogamy

Table 2. Results from one-way ANOVAs testing the effects of evolutionary mating treatment on female fecundity separately by adaptation set and assay mating environment and treating populations as replicates.

Adaptation set	Assay mating environment	$F_{2,18}$	*P*-value
1 (Cornstarch/ heatshock)	Single-pair straw	1.19	0.3268
	Multi-fly vial	2.32	0.1267
	Multi-fly container	7.24	0.0049
2 (EtOH/ coldshock)	Single-pair straw	5.71	0.0120
	Multi-fly vial	2.59	0.1026
	Multi-fly container	4.94	0.0194
3 (NaCl/28°C)	Single-pair straw	1.97	0.1679
	Multi-fly vial	3.32	0.0591
	Multi-fly container	21.96	<0.0001

Female fecundity was calculated for each population as the average number of eggs laid across all experimental replicates.

(analogous to our MC_{absent} and MC_{simple} treatments, respectively). They showed that females had higher survival and reproductive output when housed with males evolved in the monogamy treatment rather than from the polygamy treatment; in other words, monogamy males evolved to be less harmful to their mates. Holland and Rice (1999) did not perform a reciprocal transplant experiment to assess the fitness of monogamous versus polygamous males in each mating environment. Nonetheless, our results are logically consistent with their findings with regard to male harm. Several other groups have also created experimental *Drosophila* populations analogous to our MC_{absent} and MC_{simple} treatments (e.g., Promislow et al. 1998; Crudgington et al. 2005; Rundle et al. 2006; Hollis and Kawecki 2014), although we are not aware of any other reciprocal transplant experiments. Crudgington et al. (2005) found little support for the evolution of reduced harm in monogamy-evolved males in their study with *Drosophila pseudoobscura*, so one might not expect a pattern of local adaptation in this case (at least not for the reasons described above). Monogamy versus polygamy experimental evolution has been performed in other taxa too (e.g., dung flies: Martin and Hosken 2003; bulb mites: Tilszer et al. 2006; seed beetles: Fricke and Arnqvist 2007; and flour beetles: Lumley et al. 2015), although again without reciprocal transplant experiments. Nonetheless, in their experiment with bulb mites, Tilszer et al. (2006) found evidence that males from monogamous populations had evolved to be less harmful to females and had also evolved lower reproductive competitiveness, which strongly suggests local adaptation.

Although we observed a simple pattern of reciprocal local adaptation when considering populations from the MC_{absent} and MC_{simple} mating treatments, a different pattern emerges when we consider the $MC_{complex}$ populations. As expected, in their native environment (i.e., multi-fly cages), $MC_{complex}$ males outperformed both MC_{absent} and MC_{simple} males (Fig. 1C, F, and I). Contrary to reciprocal local adaptation, however, these $MC_{complex}$ males also consistently and significantly outperformed males from all other experimental populations in the standard multi-fly vial assay mating environment, including the MC_{simple} males that had evolved in this mating treatment (Fig. 1B, E, and H). In addition, these $MC_{complex}$ males also sired as many or more offspring in the single-pair straw assay mating environment as the MC_{simple} males that had evolved in this mating environment (i.e., under monogamous conditions; Fig. 1A, D, and G).

Given the observed differences between MC_{simple} and $MC_{complex}$, our results are inconsistent with a simple "monogamy versus polygamy" perspective. The MC_{simple} and $MC_{complex}$ treatments both allow for mate competition and polygamy, but we have previously shown these mating environments differ with respect to the importance of sexual conflict (Yun et al. 2017; MacPherson et al. 2018; Supporting Information). Compared to vials, male–female interactions in cages are less frequent and are less biased toward high-quality females. In vials, increased exposure to males is more harmful to females than it is in cages and it tends to weaken selection on female quality (as a consequence of a cost of attractiveness to high-quality females; Long et al. 2009), whereas in cages increased male exposure strengthens selection on females. The strong performance of $MC_{complex}$ males in their two "non-native" mating environments reported here is intriguing but we can only speculate on potential explanations.

Broadly speaking, male fitness depends on two different (but potentially interacting) factors: general health or vigor as well as specific mating/fertilization strategies. Typically, we expect vigor to be important in all environments, whereas different mating strategies are likely favored in different mating environments. $MC_{complex}$ males are likely healthier and more vigorous (i.e., higher genetic quality); we have previously shown more efficient purging of deleterious mutations, reduced overall mutation load (evidenced by lower inbreeding depression), and faster adaptation with respect to egg-to-adult viability in these novel larval habitats in populations evolved in a complex polygamous mating environment compared to those evolved in a simple polygamous mating environment (Colpitts et al. 2017; Singh et al. 2017; Yun et al. 2017). Evidence indicates that higher quality genotypes likely evolve in cages because sexual conflict does not diminish natural selection on females in cages as it does in vials (Colpitts et al. 2017; Yun et al. 2017; Malek and Long 2019), and likely because total sexual selection on males is stronger in cages than in vials (see Maclellan et al. 2009).

Although it is easy to imagine how this "superior genetic quality" hypothesis could explain why $MC_{complex}$ males outperform MC_{simple} males in vials, it is less obvious why $MC_{complex}$

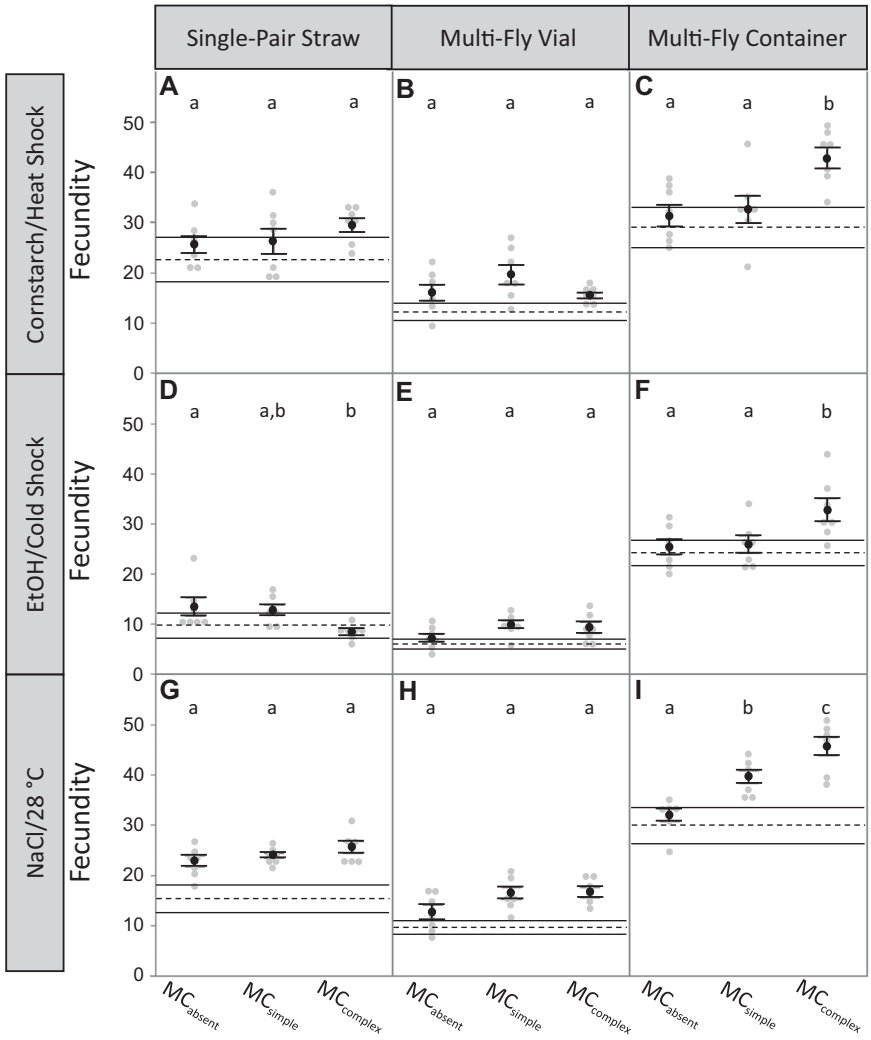

Figure 2. **Reproductive fitness of experimental females when tested in each of the three assay mating environments. Rows are adaptation sets (i.e., larval abiotic environments; see Methods) and columns are the three assay mating environments. Gray points indicate values for individual populations. Each black point represents average female fecundity (± 1 SE) across the seven populations within a given treatment. Horizontal dashed lines are the average fitness of ancestral females (n = 30 replicates); horizontal solid lines depict bootstrap 95% confidence intervals. Different letters indicate significantly different means in a post hoc comparison (Tukey's HSD) performed separately by adaptation set and assay mating environment.**

males also outperformed MC_{simple} males in a monogamous setting. In discussing the pattern of reciprocal local adaptation of MC_{simple} and MC_{absent} males, we posited direct selection against harm in MC_{absent} males, whereas MC_{simple} males evolve to be harmful to females as a by-product of competition for mates. Our $MC_{complex}$ results suggest that male harm is not a necessary outcome of competition for mates: $MC_{complex}$ males do well when facing mate competition in either vials or cages, and yet do not appear to be harmful to females as evidenced by the high fitness they achieved in single-pair straws. In cages, we suspect females can more easily escape unwanted male sexual attention, possibly leading to selection against precopulatory harassment by males in the $MC_{complex}$ treatment because a male fruitlessly pursuing a disinterested female in an environment where coercion is unsuccessful misses the opportunity to find and court a more receptive female. Further, we have evidence that mating rates are much lower in cages than in vials (supplemental material), which should cause $MC_{complex}$ males to experience less postcopulatory competition. If a female is unlikely to re-mate, then there should be selection on males to reduce levels of harmful seminal fluid proteins they are known to produce (Chapman et al. 1995; Mueller et al. 2007; Sirot et al. 2014). Although these reasons could account for why $MC_{complex}$ males outperform MC_{simple} males in the monogamy assay, it is more difficult to explain why $MC_{complex}$ males do as well or better than MC_{absent} males in this mating environment. It is possible that any harm caused by $MC_{complex}$ males is more

than offset by their superior genetic quality, yielding a net benefit. An alternative possibility, suggested by William R. Rice (pers. comm.), is that the ancestral females used in these assays increase their reproductive output in response to mating with higher quality $MC_{complex}$ males relative to lower quality MC_{absent} males.

A third alternative possibility, arising from comments by an anonymous reviewer, is based on the idea that female fecundity during the critical egg-laying period is determined, at least in part, by male stimulation (e.g., via seminal fluid proteins that stimulate egg-laying and female sensitivity to this stimulation; Ravi Ram and Wolfner 2007). Perhaps, the alignment of fitness interests between the sexes in the MC_{absent} treatment has resulted in coevolution between males and females to maximize female fitness, resulting in less harmful males and females that are more sensitive (i.e., less resistant) to male stimulation. However, our male fitness assay used ancestral females that may be more resistant to stimulation than MC_{absent} females; perhaps MC_{absent} males do not provide sufficiently strong stimulation to ancestral females for these females to achieve maximal fitness under the monogamous assay conditions. $MC_{complex}$ males may provide stronger stimulation (without being as excessively harmful as MC_{simple} males), allowing ancestral females to achieve high fitness in the monogamous assay.

LOW DIVERGENCE AMONG FEMALES

In contrast to males, in females there was no clear pattern of reciprocal local adaptation when comparing females evolved under monogamy (MC_{absent}) to those evolved under polygamy in a simple environment (MC_{simple}). As in males, there was evidence of nonreciprocal local adaptation in that $MC_{complex}$ females outperformed other females in their native mating environment. Perhaps the most striking result from the female data is the low level of among-mating treatment divergence compared to that observed in males (the exception being the aforementioned $MC_{complex}$ females in their native environment). This echoes the commonly observed pattern in nature of greater phenotypic and expression divergence in males compared to females (Eberhard 1985; Andersson 1994; Meiklejohn et al. 2003). In our study, we do not know whether the heightened divergence is due to selective environments being more divergent for males than females or there is simply more genetic variation for male traits, although we suspect the former.

We also assayed the fitness of males and females from the ancestral population when reared in each of the novel larval environments. The ancestor is unreplicated at the population level so we have an unknown level of uncertainty regarding the effect of genetic drift on it. Moreover, the mating environment of the ancestral population is different from any of the evolved treatments, but is likely more similar to some (i.e., MC_{simple}) than others. Finally, the ancestor is the only case in which the fitness assay for each sex was done using opposite sex individuals from the same (i.e., their own) population (because all fitness assays used ancestral individuals for the nonfocal sex). These factors complicate the interpretation of the ancestral results, but it is worth considering them as a point of reference. In the female fitness assays, ancestral females tended to perform worse than females from almost any experimental treatment in any mating assay environment (Fig. 1). This is unsurprising as the ancestor is not adapted to any of the novel larval environments and thus females raised in these environments are expected to produce adults of comparatively poor condition, resulting in low fitness. Results in males are less clear, however. Ancestral males tended to perform less well than evolved males when tested in the evolved males native mating environment, but the ancestor often outperformed evolved males when assayed in other (i.e., non-native) mating environments.

Collectively, the comparisons among mating treatments and with the ancestor are consistent with the following ideas. Phenotypic selection on females does not vary drastically among mating treatments and much of the variation in female fitness is determined by their condition, the major component of which is determined by whether females are adapted to the novel larval environment in which they are reared (explaining why evolved females outperformed ancestral females under almost all test conditions). On the other hand, phenotypic selection on males varies considerably among mating treatments resulting in divergent male "mating strategies" evolving in the different mating environments (e.g., harmful but competitive vs. benign but uncompetitive); variation in condition also contributes to variation in male fitness, but this effect is obscured in some cases by variation in male mating strategies (explaining why evolved males do not consistently outperform ancestral males).

Above we have speculated about different mating strategies being successful in different environments but we have no direct evidence of this, only inferences from patterns in the fitness data. Much additional work would be necessary to understand what behavioral strategies and phenotypic traits make for a successful male in each environment. In other systems, it is clearer that different strategies are used in different circumstances. For example, in guppies, the frequency of forced copulations versus courtship induced copulations is higher in higher predation environments and this is due to declines in female receptiveness to courtship under high predation risk (Farr 1975; Dill et al. 1998; Evans et al. 2002). In the acarid mite *Caloglyphus berlesei*, armored males that fight and kill their rivals are highly successful at low density, whereas unarmored nonfighting males are more successful via scramble competition at high density (Radwan 1993). Emlen and Oring (1977) summarized how the distribution of resources and/or females—in combination with the extent to which males can monopolize them—is expected to play a key role in favoring different male mating strategies. Indeed, there are numerous cases where ecological variation in resource distribution (or

density, which affects defensibility) is associated with variation in mating strategies both among populations and species (Emlen and Oring 1977; Ryan 1982; Langbein and Thirgood 1989; Grant 1993). However, in most cases, direct evidence is lacking for either fitness trade-offs across different environments of different male phenotypes or a genetic basis to the phenotypic differences.

Adult fitness components are often affected by sexual interactions that themselves are shaped by the external environment. Our results show how environments that alter such interactions can be an important factor governing local adaptation (or its absence). Although the generality of our results remains to be determined, we suspect such effects are common across a variety of taxa. The extent of sexual conflict, for example, has been shown to be sensitive to various aspects of the environment not only in *Drosophila* but in diverse taxa as well, including guppies, odonates, and water striders (Arnqvist 1992; Rowe 1992; Krupa and Sih 1993; MaGurran and Seghers 1994; Gosden and Svensson 2009; Karlsson et al. 2010). How common local adaptation is for adult fitness components and whether this differs consistently between the sexes are open questions. The rapid divergence of male secondary sexual traits and genitalia among closely related taxa (Eberhard 1985; Arnqvist 1998; Schluter 2000) suggests that selection is often divergent on male phenotypes related to mating, favoring different mating strategies that may produce reciprocal local adaptation. Less attention is often given to divergence in females, however, so direct tests of these ideas are an important avenue for future research.

AUTHOR CONTRIBUTIONS

A.F.A., H.D.R., and L.Y. conceived the experiments and L.Y. collected the data with help from M.B., S.Y., and P.J.C. L.Y., H.D.R., and A.F.A. contributed to data analysis and interpretation, and A.F.A. and H.D.R. wrote the manuscript with input from L.Y. H.D.R. and A.F.A. contributed equally.

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DATA ARCHIVING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Average number of mating pairs per observation in vials vs. cages. Error bars are ± 1 SE treating individual vials and cages as replicates.
Table S1. Results from generalized linear mixed models, fit at the individual replicate level, testing the effects of evolutionary mating treatment on male reproductive fitness separately by adaptation set and assay mating environment