

Local Adaptation of Male Sexual Fitness in *Drosophila melanogaster*

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Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
M.Sc. degree at the Ottawa-Carleton Institute of Biology

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Abstract

Darwin first proposed sexual selection as a process to explain the evolution of extravagant morphological traits in males. Despite being potentially detrimental to individual survival, such traits evolve because they increase a male's reproductive success, and provide a net benefit to their fitness. Mate competition is the source of sexual selection, and healthier, more vigorous males are likely to be superior competitors. Because most genes are likely to impact an individual's health/vigour, sexual selection should act across much of the genome to favour the same alleles as natural selection, thereby promoting adaptation. On the other side of the coin, adaptation to an environment should enhance male sexual fitness, since it is likely to increase the overall health/vigour of individuals within a population, though tests of this prediction are rare and results are mixed. Taking advantage of a long-term evolution experiment involving replicate populations of *Drosophila melanogaster*, I performed a reciprocal transplant in which the sexual fitness of males was compared when raised in an environment to which they are well adapted and in one to which they are not. I improved on past tests via a comprehensive measure of male sexual fitness that included pre- and post-copulatory reproductive success in a competitive assay under conditions that closely mirrored those to which the populations have been evolving. I found that sexual fitness was higher in locally-adapted males from these experimental populations, a result that was consistent across environments that also manipulated the context in which mate competition occurred.

Résumé

Darwin a été le premier à proposer la sélection sexuelle comme processus pour expliquer l'évolution des traits morphologiques extravagants chez les mâles. Bien qu'ils soient potentiellement nuisibles à la survie de l'individu, ces traits évoluent parce qu'ils augmentent le succès reproducteur du mâle et apportent un avantage net à sa valeur sélective. La compétition pour des partenaires est la source de la sélection sexuelle et les mâles en meilleure santé et les plus vigoureux sont susceptibles d'être des concurrents supérieurs. Puisque la plupart des gènes sont susceptibles d'avoir un impact sur la santé ou la vigueur d'un individu, la sélection sexuelle devrait agir sur une grande partie du génome pour favoriser les mêmes allèles que la sélection naturelle, favorisant ainsi l'adaptation. De même, l'adaptation à un environnement devrait améliorer le succès reproducteur des mâles, puisqu'elle est susceptible d'augmenter la santé/vigueur globale des individus au sein d'une population. Les tests de cette prédiction sont rares et les résultats des quelques tests qui ont été effectués sont mitigés. Profitant d'une expérience d'évolution à long terme impliquant des populations répliquées de *Drosophila melanogaster*, j'ai effectué un essai de transplantation réciproque dans laquelle le succès reproductif des mâles a été comparé lorsqu'ils sont élevés dans un environnement auquel ils sont bien adaptés et dans un autre où ils ne le sont pas. J'ai amélioré les tests précédents par une mesure complète de succès reproducteur des mâles qui incluait les aspects pré- et post-copulatoire dans un essai compétitif dans des conditions qui reflètent étroitement celles auxquelles les populations ont évoluées. J'ai constaté que le succès reproducteur était plus élevé chez les mâles de ces populations expérimentales adaptés aux conditions locales, un résultat qui a également été observé dans des environnements qui manipulaient le contexte dans lequel se produisait la compétition entre partenaires.

Acknowledgements

I would like to thank my supervisor, Dr. Howard Rundle, for offering me a spot in his lab – allowing me to conduct this research. I am appreciative of his help with the conception and execution of this project, especially in light the complications that emerged as a result of the pandemic. My research would also not have been possible without support from his lab’s funding sources. I would also like to thank the members of my committee Dr. Aneil Agrawal (University of Toronto) and Dr. Julie Morand-Ferron (University of Ottawa) for their guidance.

Thank you to Li Yun for beginning the long-term evolution experiment upon which my project was based, and to Kevin Kwok for teaching me how to maintain the constituent *Drosophila* populations. I would also like to thank the other members of my lab, Tristan Ducharme and Will Jarvis, for their help with maintenance and data collection. Finally, I would like to extend a big thanks to Tia Chen, without whom this research would not have been possible. Her continual support through every aspect of this project, from preliminary assays, to the main experiment, to the writing of this manuscript, was indispensable.

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Introduction

Darwinian selection arises from variation in fitness, which can be broken down into sexual and non-sexual components. Here I restrict the term ‘natural selection’ to that arising from variation in non-sexual fitness – i.e., viability and fecundity combined. As populations adapt to novel environments, traits that increase overall health and vigour (i.e., condition) are selected for through this process and become more prevalent. Additionally, in most sexually reproducing species, competition for mates generates differences in sexual fitness that causes sexual selection (Darwin 1859; Andersson 1994). Herein I use the term ‘reproductive success’ synonymously with ‘sexual fitness’, as opposed to the broader sense that equates it with lifetime fitness (which includes non-sexual components). Exaggerated morphological displays such as the peacock’s tail or a buck’s antlers are well-understood traits that benefit male sexual fitness; they evolve because they increase a male’s reproductive success despite potentially hindering his ability to survive. However, differential reproductive success between individuals of the same sex need not be caused solely by costly ornaments/armor and/or courtship behaviour. Any trait that affects an individual’s reproductive success may be acted on through sexual selection (Whitlock and Agrawal 2009). Healthier, more vigorous (i.e., higher condition) males may be better able to search for mates, outcompete rivals pre- and/or post-copulation, and/or appear more desirable to females compared to their lower condition counterparts (Jennions et al. 2001; Andersson 1994). Furthermore, individuals that are well-adapted to a particular environment should be healthier on average compared to those that are not, and hence have higher reproductive success (Rowe and Houle 1996). Sexual selection should therefore align with natural selection across most of the genome to promote adaptation and purging of deleterious alleles (Whitlock and Agrawal 2009).

Some alleles are known to have contrasting effects on male and female fitness. For instance, intralocus sexual conflict can arise from alleles that increase male reproductive success but are harmful to females as a by-product (Bonduriansky and Chenoweth 2009), though such alleles appear to be rare relative to those with sexually concordant effects for which alignment can occur (Bonduriansky and Chenoweth 2009). Darwin (1859) was the first to suggest that sexual and natural selection should act to reinforce one another, writing that “... sexual selection will give its aid to [natural] selection, by assuring to the most vigorous and best adapted males the greatest number of offspring.” Since most genes affect the overall health/vigour of an individual, mutations should be acted on in the same direction by both forms of selection (Rowe and Houle 1996). In other words, if healthier and more vigorous males indeed have higher reproductive success than their lower-condition counterparts, then sexual selection should align with natural selection to favour alleles that increase health and vigour. This hypothesis of ‘alignment’ has received much attention (Hughes 1995; Promislow et al. 1998; Whitlock and Bourget 2000; Whitlock and Agrawal 2009), where some studies have found support for it (e.g., Partridge 1980; Radwan 2004; Fricke and Arnqvist 2007; Hollis et al. 2009; Lumley 2015; Yun et al. 2018) while others have not (e.g., Holland 2002; Rundle et al. 2006; reviewed in Cally et al. 2019, Rowe and Rundle 2021). The other side of this coin is that, as populations adapt to novel environments (i.e., as non-sexual fitness increases through natural selection), sexual fitness (reproductive success) should improve due to increased male health/vigour.

Surprisingly, to the best of my knowledge, only three studies have tested this relatively straightforward prediction of alignment (Dolgin et al 2006; Correia et al 2010; Arbuthnott and Rundle 2014), and have conflicting results. These studies used experimental evolution in which replicate *Drosophila* populations were independently evolved in one of two environments which

differed in either food source or temperature (termed ‘adaptation sets’). Arbuthnott and Rundle (2014), for instance, used experimental food supplemented with either 8% m/v salt or the heavy metal cadmium (70 µg/ml). Dolgin et al. (2006) and Correia et al. (2010), on the other hand, manipulated the temperature at which experimental populations were raised, with one set of populations evolved at a colder temperature (18C or 16C respectively) and another set at a warmer temperature of 25C. Male mating success was then compared when males from both adaptation sets were raised, and then competed sexually, in both environments. Correia et al (2010) and Arbuthnott and Rundle (2014) found no evidence that local adaptation improved male sexual fitness, while Dolgin et al (2006) showed that males had significantly higher mating success on average in the environment to which they were adapted compared to males that were adapted to the other environment. Dolgin’s finding that males adapted to the local environment outcompeted those who are not for access to mates is the result expected under alignment (i.e., if mutations favoured by natural selection are also favoured by sexual selection).

There are some issues with these studies, however, that hamper interpretation. First, the assays measured mating success only – i.e., a component of pre-copulatory sexual fitness. Since post-copulatory components (e.g., sperm competition, cryptic female choice, etc.) were not included, the effect of local adaptation on total sexual fitness remains unknown. This is of particular concern because pre- and post-copulatory fitness may trade-off (Andersson 1994). Furthermore, mating trials were performed under conditions that differed somewhat from those to which the flies were adapted – for instance, in arenas and/or at temperatures which the flies had not previously experienced. If fitness is measured differently than how it is determined naturally, then male mating strategies may be non-optimal, and mating success may be less dependent on condition. Finally, in the case of at least one of these studies, weak local

adaptation, potentially due to a limited number of generations of experimental evolution, limiting genetic variation, and/or for other reasons, may have made any signal of alignment difficult to detect (Correia et al. 2010).

In order to better test the effect of local adaptation on male reproductive success, I used a subset of the populations from a long-term evolution experiment (LTEE) in *D. melanogaster*, started in 2014 by Li Yun and colleagues (see Yun et al. 2018). The experiment was originally designed to test the effects of mate competition on adaptation and purging of deleterious alleles and, as far as I am aware, it is the largest evolutionary manipulation of mating systems performed to date. The experiment included three distinct larval environments that differed in both food media and temperature conditions to which 21 populations each were adapting (63 populations in total), with all populations derived from a common ancestor. These populations were further split equally among one of three mating treatments that manipulated the opportunity for mate competition and the abiotic environment in which it occurred. One mating treatment removed mate competition by enforcing monogamy, a second allowed for mate competition in highly simplified environment consisting of standard *Drosophila* vials at high population density, and the third allowed for mate competition in somewhat larger, lower density containers with added structural complexity. Some of these populations were later discontinued to reduce the cost and effort of maintenance, but I took advantage of populations from two of the three larval environments, and from both mating treatments that allowed for mate competition, that were still being maintained (five populations from each larval environment and mating treatment; 20 populations in total). These populations had undergone 110+ generations of experimental evolution by the start of my experiment.

Adaptation of these populations to their different larval environments was confirmed in generations 14 and 50 via measures of larval viability. Populations from all three mating treatments had significantly higher average egg-to-adult survivorship compared to the ancestral stock when tested in their respective larval environments, at least a 1.40-fold increase after 14 generations and 1.46-fold increase after 50 (Yun et al. 2018). These populations also feature a life cycle in which adults spend 6 days in their mating treatment each generation, after which females are transferred to new vials for ~24 hours to lay eggs. These conditions (under which the populations have been evolving) can be mirrored quite closely when measuring male sexual fitness, including both pre- and post-copulatory components. This was done by Yun et al. (2019) to test for local adaptation of adults to their mating treatments. Here I assayed male sexual fitness using a reciprocal transplant among larval environments, wherein males from each of the two mating treatments were raised in both the larval environment to which they are adapted and in the one to which they are not. Relative siring success was then compared against mutant competitor males using an assay design based on that used by Yun et al. (2019).

Previous studies of these populations demonstrated that, relative to the treatment in which mate competition was absent (i.e., monogamy), mate competition in the more complex mating environment promoted adaptation to their novel larval conditions, and more efficient purging of deleterious mutations, across the three larval environments (Yun et al. 2018). However, mate competition in the simplified mating environment did not, demonstrating the importance of the ecological context in which reproduction occurs. There are several potential factors that could have contributed to this result and which are not mutually exclusive. For example, there is evidence that sexual conflict, in the form of harm inflicted by males, can be disproportionately targeted toward higher quality females, thereby reducing the variance in female fitness and

weakening natural selection on them (Long et al. 2009). This phenomenon occurs in these populations in the simple mating environment, but is absent, and may in fact reverse, in the complex mating environment (Yun et al. 2017; MacPherson et al. 2018). Additionally, male reproductive success may be more condition-dependent in the more spacious and structurally complex mating environment. For example, using different *D. melanogaster* populations, MacLellan et al. (2009) showed that sexual selection against deleterious mutations tended to be stronger on average in a larger, lower density mating environment, presumably because additional components of male sexual fitness like search effort and territoriality come into play. Condition-dependent aspects of male sexual fitness may therefore be more important in the LTEE populations in the complex mating treatment, such that sexual selection on male health/vigour is stronger, and this could help explain why mate competition promoted adaptation and purging in this treatment. This hypothesis has not been addressed to date, but it makes a clear and testable prediction: if greater adaptation and purging in the complex environment occurred at least in part because male sexual fitness was more condition-dependent, then local adaptation to the larval environment should have a greater effect on male sexual fitness in the complex compared to the simple mating treatments.

There is much ongoing interest in the effect of sexual selection on adaptation and purging, but results are highly variable, and ecological aspects of the mating environment could be key. Here I took advantage of an unparalleled resource to test a fundamental prediction of the alignment hypothesis, not only improving on previous tests but also shedding light on whether and how the ecology of the mating environment may impact alignment.

Materials and Methods

Study Populations

A stock population of *D. melanogaster* was collected from Similkameen Valley, British Columbia in 2005 by S. Yeaman. Since 2010, this population had been maintained in *Drosophila* culture bottles with 40 mL of cornmeal based medium at 25C, 50% relative humidity, and a 12L:12D photoperiod at large population size (~3000 adults) with discrete, non-overlapping 2-week generations. In September 2014, 63 separate experimental populations were derived from this stock by Li Yun. These experimental populations were divided equally into three larval adaptation sets (21 populations per set), each involving distinct and novel rearing conditions. I used two of these adaptation sets, and so I focus here on them; descriptions of the other set can be found in Yun et al. (2018 and 2019). One set featured a cornstarch based (rather than the more nutritious cornmeal) larval medium and a 2-hour heat shock at 37C to 3-day old larvae, and the second set had the ancestral cornmeal based larval medium supplemented with 5% salt, with larvae being reared under a constant 28C (rather than the standard 25C). To promote continued adaptation to these larval environments, after the sixth generation the duration of the heat shock was increased to 4 hours in the first set, and the salt concentration increased to 6% in the second. I refer to these adaptation sets as “cornstarch” and “salt” respectively (referring to the larval medium) for the sake of brevity, but note that the temperature conditions also vary between them. Each adaptation set was maintained on a 3-week nonoverlapping generation, as described below.

Within each adaptation set, the 21 replicate populations were split equally among three mating treatments (i.e., seven populations per larval environment/mating treatment combination) that manipulated the opportunity for mate competition and, when present, the abiotic

environment in which it occurred. Outside of the mating treatments, all populations within a given adaptation set were maintained in the same manner and experienced the same conditions. I used populations from the two mating treatments that permitted mate competition. The first of these allow mate competition in a small and structurally simple environment (referred to as MC_{simple} for mate competition in a simple environment) by placing groups of 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. The second mating treatment allowed mate competition in a larger and more spatially complex environment (MC_{complex}). In this case, groups of 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 surfaces of which were broken into two by a plastic divider placed 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 for added structural complexity. Four vials or four cages were set up for each population, totaling 140 adults of each sex each generation. I refer to vials and cages as “simple” and “complex” mating environments, but note that they differ in additional ways including volume/fly density and the availability of food and egg-laying sites. The third mating treatment involved the absence of mate competition via enforced monogamy. These populations had been discontinued prior to the start of this experiment, though details concerning this treatment can be found in Yun et al (2018 and 2019).

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 CO₂ anesthesia to a fresh mating environment of the same type to avoid adult mortality

resulting from liquefaction of the food (caused by developing larvae). At the end of the interaction phase, flies were anaesthetized, males were discarded, and 105 females were randomly chosen from each population and evenly distributed among seven standard culture vials (featuring the appropriate larval medium) for egg laying. These adult females were then 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 8 holding vials per population, separately by sex (35 flies per vial), before being placed in their respective mating environment as described above.

Within each adaptation set, two of the seven replicate populations for each mating treatment were later discarded to reduce maintenance effort and cost, thus leaving five replicate populations within each. I took over maintenance of these populations at generation 104. Here I used all five populations from the MC_{simple} and the MC_{complex} mating treatments from each of the two adaptation sets (i.e., cornstarch and salt), yielding 20 replicate populations overall. In December 2020, I created two new “competitor” populations for use in the fitness assay. The first of these came from combining all 10 populations adapted to the simple mating treatment (MC_{simple}) and the second from all 10 populations adapted to the complex mating treatment (MC_{complex}). This way, the competitor populations are each adapted to the 6-day interaction phase and the vial or cage environment in which this occurs, and are more similar genetically to what the experimental males have evolved to compete with. These two ‘mixed’ populations were then ‘marked’ through introgression with a recessive autosomal *bw* mutation. Flies that are homozygous for this *bw* marker have brown eyes that can be easily distinguished from wild-type

red eyes, but are otherwise identical. Introgression was performed through two crosses between each of the competitor populations and a stock *bw* population, one round in each ‘direction’. In the first cross, males from each of the mixed populations were mated with virgin *bw* females, and brown-eyed homozygotes were collected from among the resulting F2 offspring. Then, *bw* stock males were mated with brown-eyed virgin females from the mixed populations, and brown-eyed homozygotes were again collected from among the resulting F2 offspring. The competitor populations were maintained with the appropriate mating environment (i.e., either vials or cages) for their interaction phase, and following the exact same lifecycle as the experimental populations, with their larval environment consisting of standard food and standard conditions. Each of these competitor populations was maintained at twice the population size as each of the experimental populations (280 individuals of each sex per population per generation).

Assaying Adaptation and Male Reproductive Fitness

Yun et al (2019) performed a reciprocal transplant *between mating environment* to test for local adaptation of adult male and female fitness of populations *within an adaptation set*. My assay of adult male reproductive fitness closely followed that used by Yun et al. (2019), but consisted of a reciprocal transplant *between adaptation sets* (i.e., rearing populations in each of the two larval environments) separately *within* the simple and *within* the complex mating treatments. A transplant of this type allowed me to test for local adaptation (using egg-to adult survival as a measure) and its consequences for male reproductive success (i.e., sexual fitness) at the same time. Within a given mating treatment, males from all 10 experimental populations (i.e., five from each of the two adaptation sets) competed against a common set of marked competitor males for access to marked females (within their appropriate mating environment), and their reproductive success was measured when raised in both larval environments. I refer to the larval

environment in which the flies were reared as the “assay environment” (i.e., cornstarch or salt) and the larval environment to which the flies are adapted as “adaptation set.” In all cases, focal males were assayed in the mating environment to which they were adapted. The mass of experimental males and females was also measured.

A reciprocal transplant between adaptation sets had, to date, never been performed with these experimental populations. I therefore performed some preliminary assays to ensure flies could survive to maturity when reared in the environment to which they were not adapted. The reproductive success of a dead male is necessarily zero, making the prediction of increased reproductive success due to local adaptation trivially true, whereas I wanted to compare the reproductive success of males that could survive, but were more or less locally adapted. Based on the results of this preliminary work, I reduced the stressors in each larval environment to a 2-hour heat shock and 5% salt in adaptation sets 1 and 2 respectively for the purposes of the fitness assay (i.e., the initial conditions that the first few generations of the LTEE began adapting to), as the 4-hour heat shock and 6% salt conditions proved lethal for too many of the non-adapted flies. In addition to lowering mortality of non-adapted ‘focal’ males, and hence making it possible to collect a sufficient number of individuals to assay, I chose to reduce the stressors so that these males would not benefit disproportionately from lack of competition for resource availability as a result of high mortality within the larval vials. Reduced intraspecific competition could result in offspring of higher condition than they otherwise would be, confounding any effect of local adaptation.

All experimental populations were raised for one generation in a common environment prior to conducting the assays. To do this, following the egg-laying period as part of the normal lifecycle on which the populations were being maintained, females were transferred to fresh vials

consisting of ancestral food. From here, they were allowed to oviposit for another 24 hours instead of being discarded immediately. The adult flies that emerged became the parents from which larvae would be obtained to raise in the two larval assay environments. I used a larval picking technique to control density when raising individuals from each population in each assay environment. To do so, the adults (i.e., parents) were transferred to large plexiglass cages for several days to mate, each containing plates (petri dishes) filled with ancestral medium and abundant yeast. Plates consisting of a grape-juice agar medium subsequently replaced these food plates, and females were allowed to lay eggs on them for 2-hour periods per batch (plates could be removed and replaced with fresh ones). Larvae were collected from these plates approximately 24 hours later and placed into their assay environment (i.e., vials containing either the cornstarch or salt food). Exactly 100 larvae were placed in each vial, and these larvae experienced the conditions of their assay environment (i.e., a 2-hour heat shock at 37°C on day three, or a constant 28°C with either cornstarch or salt food respectively) during their development. Flies experienced the same conditions regardless of whether they were adapted to that environment or not, (i.e., cornstarch/heat shock adapted larvae received a 2-hour heat shock instead of 4 hours, just like the salt/28°C adapted larvae).

Multiple vials of 100 larvae were set up per population in each assay environment, and newly emerging adults from each set were pooled and collected daily over a five-day period. These adults were not necessarily virgin (female reproductive fitness was not being measured, only dry mass) and males were all handled in a consistent manner. Females were desiccated and their dry mass was subsequently measured. Males were placed into holding vials (10 focal males/vial) for use in the assay, and each vial consisted of males that had emerged in the same 24-hour window. Adult males and females from each of the *bw* competitor populations were also

collected at an appropriate stage during their normal maintenance and kept in holding vials (25 males/vial and 35 females/vial). 15 days following the larval picks, adult flies were placed into their respective mating environment (vial or cage) for a 6-day interaction phase, mirroring the normal life-cycle for the experimental populations.

For both assay environments, I created five replicates for each of the 20 experimental populations, each consisting of 10 focal males together in a single vial or a single cage with 25 *bw* males and 35 *bw* females, mimicking the densities used during regular maintenance. Due to differences in the rate at which adapted versus non-adapted populations matured, egg-laying and larval picks were staggered by 24 hours, giving the non-adapted flies one day longer to develop. Replicates within an assay/mating environment combination consisted of focal males that were the same adult age, and were selected roughly in proportion to the day on which the males emerged (i.e., the number obtained across the five collection days). After the 6-day interaction phase, surviving males were counted from each replicate, and focal males were desiccated and weighed. 21 randomly selected surviving females were also collected from each replicate using light CO₂ anesthesia and distributed equally among three vials containing ancestral food to lay eggs for 2 hours before being discarded. Use of ancestral food and an attenuated oviposition time (compared to normal maintenance of these populations) was intended to maximize survival of offspring so that variation in the proportion of *wt* (as opposed to *bw*) flies emerging is more likely to reflect differences in focal male reproductive success.

Adult offspring that emerged from the three egg laying vials of a given replicate were phenotyped (*bw* vs. *wt*). The total number of brown eye vs wild-type flies was summed across the five replicates of each population separately for each assay environment, and the reproductive fitness of the focal males from a given population was calculated as the proportion

of offspring sired by them across the five replicates (i.e., the number of *wt* offspring divided by the total number of offspring). The assay was completed over four completely balanced blocks in which replicates from populations of each of the four treatment combinations were assayed in the same generation, preventing any confounding of block with the experimental treatments.

Variation in larval survival, male reproductive fitness, and dry mass of both males and females were separately analyzed using a linear mixed model, with assay environment, adaptation set, mating environment, and their interactions as fixed effects. Population and block were included as random effects, though block was dropped from every model due to lack of significance. Given a significant assay environment \times adaptation set interaction, the effect of adaptation set was subsequently tested by two-way ANOVAs, separately by assay environment, with adaptation set, mating environment, and their interactions as fixed effects. Results were qualitatively unchanged if generalized linear mixed models were fit instead.

Results

Adaptation (egg-to-adult survival)

There was evidence of local adaptation in terms of non-sexual fitness in both adaptation sets. A greater proportion of larvae survived to adulthood when raised in the environment to which they were adapted, and this was the case in populations from both mating treatments (Fig. 1). This generated a significant interaction between adaptation set and assay environment ($F_{1,16} = 33.00$, $P < 0.0001$), indicating that the effect of evolutionary adaptation set differed by assay environment, and this result was consistent across mating treatments (i.e., non-significant effects of mating treatments and its interactions; Table 1). Fitting simpler models separately by assay environment showed a significant effect of adaptation set in both the cornstarch ($P = 0.0225$) and salt ($P = 0.0006$) assay environments, with no effect of mating treatments nor interaction thereof (Table 2).

Male Reproductive Success

Males had greater reproductive success when raised in the environment to which they were adapted in populations from both mating treatments (Fig. 2), generating a significant interaction between adaptation set and assay environment for the proportion of offspring sired by focal males ($F_{1,32} = 24.99$, $P < 0.0001$). This result does not differ between the mating environments (i.e., non-significant three-way interaction with mating environment; Table 1), although there is a significant interaction between mating environment and assay environment ($P = 0.0281$), likely arising because, in the simple but not complex environment, salt adapted males do better when raised in cornstarch than in salt (but are nevertheless outperformed in cornstarch by cornstarch-adapted males). It is also likely for this reason that there is a significant main effect of mating environment ($P = 0.0007$), with higher average success in cornstarch than in salt. Analyzing

separately by assay environment reveals a significant effect of adaptation set on focal sire mating success in both the cornstarch ($P = 0.0234$) and salt ($P = 0.0004$) assay environments, and no interaction with mating environment. There is a main effect of mating environment in the cornstarch assay environment ($P = 0.0003$), likely arising because simple males do better overall than complex males (Table 2)

Body Mass

Salt-adapted flies tend to be larger than cornstarch-adapted flies in all cases (Fig. 3), although this effect was greater and significant in females, and smaller and non-significant in males (main effect of adaptation set; Table 1). None of the interactions were significant. There was also a main effect of assay environment in females, with salt-raised flies being larger, on average, compared to cornstarch-raised flies (Table 1; Fig. 3).

Discussion

Here I performed a reciprocal transplant experiment using populations from a long-term evolution experiment involving different larval environments. In a competitive fitness assay using these flies, I tested whether local adaptation to a given larval environment enhanced male reproductive success when raised in that environment, and whether this effect differed between populations adapted to two different adult mating treatments. Enhanced reproductive success as a result of local adaptation is expected if aspects of sexual fitness, including pre- and post-copulation competitive success, are greater in healthier, more vigorous males (i.e., they are condition dependent; Andersson 1994, Rowe and Houle 1996 Whitlock and Agrawal 2009, Rowe and Rundle 2021). In other words, I tested if natural selection adapting populations to their local environment tended to favor alleles of high sexual fitness, as would occur if these two selective processes were aligned across the genome. Interest in whether this effect differed between populations from the two mating treatments was to gain insight into whether this could explain faster adaptation and more efficient purging of deleterious alleles previously observed in the complex compared to simple populations (Yun et al. 2018). I found that the experimental populations were adapted to their respective larval environments (Fig. 1), consistent with results from earlier generations (Yun et al. 2018), although there was no longer any indication that complex populations were better adapted than simple populations. I also found that reproductive success was higher in locally-adapted males (Fig. 2), and that these effects did not differ between the simple vs. complex adult mating treatments.

Results from past studies testing this prediction of enhanced reproductive success via local adaptation are mixed (Dolgin et al. 2006, Correia et al. 2010, Arbuthnott and Rundle 2014). This could be due to a few shortcomings of these studies, which my experiment improved upon.

First, my study populations were from a long-term evolution experiment for which there was a strong signal of differential adaptation to the larval environments at the time of the experiment. Again, this was consistent with earlier results from these populations (Yun et al. 2018). In contrast, earlier studies relied on assaying lineages of differentially evolved flies, but differential adaptation was not confirmed (Dolgin et al. 2006), or only a weak signal was observed (Correia et al. 2010). Arbuthnott and Rundle (2014) used populations that had shown differential local adaptation, but did not assay the populations at the time of the experiment (instead relying on results from assays performed 60 generations prior). Second, I measured total male reproductive fitness (i.e., both pre- and post-copulatory factors), and did so in an assay that closely mirrored the conditions under which males had evolved during the evolutionary experiment. This means that I was assaying the condition-dependence of reproductive strategies that males normally express. In all three previous experiments, mating trials involved conditions that less closely matched those the populations normally experienced, so evolved reproductive strategies may not have been adaptive under the changed conditions. Lastly, I obtained dry mass data for adults, which provided additional insight into the results of my fitness assays.

While I did find evidence that local adaptation enhances male reproductive success, there was no substantial difference in the magnitude of this effect between the two mating environments (Fig. 2). This suggests that greater condition-dependence of male sexual fitness, and hence stronger sexual selection favouring local adaptation, did not contribute to the faster adaptation and greater purging of deleterious alleles previously observed in the complex populations (Yun et al. 2018). However, local adaptation to the larval environments was similar in the two mating treatments in the current assay, despite being greater in the MC_{complex} compared to the MC_{simple} in assays in earlier generations (Yun et al. 2017, 2018). It is therefore

possible that the MC_{simple} populations have ‘caught up’ to the MC_{complex} , such that local adaptation no longer differs between them. In the absence of a current difference in local adaptation, similar impacts on male reproductive success are expected. In other words, we wouldn’t expect a difference in reproductive success, in contrast to the situation in which local adaptation to larval environments was still stronger in the complex populations. Current evidence of alignment of natural and sexual selection in the MC_{simple} populations suggests that sexual selection was favouring more rapid adaptation and more efficient purging of deleterious alleles in this mating treatment. This implies that healthier, more vigorous males may have higher reproductive success even in situations in which a net benefit of sexual selection to population fitness is negated by other factors, such as targeted male harm of high fitness females, which earlier studies indicate occurs in MC_{simple} but not MC_{complex} populations (Yun et al. 2017, 2021; MacPherson et al. 2018).

While I did observe a consistent pattern that local adaptation enhances male reproductive success, the benefit of local adaptation to cornstarch appears somewhat less than that of adaptation to salt, most notably in populations evolved in the complex mating environment (Fig. 2). Changes in body size provide some insight into this result. There is some evidence that body size is positively associated with reproductive success in males; a larger male can better defend high-value laying sites females are likely to frequent, can produce more sperm, and tend to have greater mating success (Ewing 1961, Partridge and Farquhar 1988). Adaptation to salt results in increased body size, and this persists even when flies are raised in the cornstarch environment (i.e., salt-adapted males are larger than cornstarch-adapted males even when raised in cornstarch; Fig. 3). Although not significant in males (Table 1), this body-size effect was strongest in the

complex populations. Therefore, the benefit to male reproductive success of local adaptation to cornstarch may have been offset, in part, by the increased body size of the salt-adapted males.

Finally, via preliminary assays I chose larval environments that were likely to produce a signal of local adaptation, but were not so stressful as to be lethal when populations were raised in their non-adapted environment. This involved reducing the duration of heat shock from 4h to 2h in the cornstarch adaptation set, and the salt concentration from 6% to 5% in the salt adaptation set, in both cases mirroring conditions at the start of the LTEE when the experimental populations first experienced these novel environments. This was done for the practical reason of obtaining sufficient individuals to perform the assay, but was also motivated conceptually since the prediction of increased reproductive success as a result of local adaptation is necessarily true if non-adapted individuals cannot survive. Separating non-sexual (i.e., survival) from sexual components of fitness is not necessarily straightforward, however. While all males were alive at the start of my reproductive success assays, there were treatment differences in survival across the six-day interaction phase (prior to females laying eggs). In particular, in the simple (but not the complex) mating environment, locally adapted males tended to have higher survival than non-adapted males in both assay environments (Fig. 4). This may have contributed to the increased reproductive success for the adapted compared to non-adapted males; there were relatively more of them around to sire offspring. Whether survival through the interaction phase is considered a component of sexual fitness is a matter of interpretation, but such differential survival was not observed in the complex environment and thus did not contribute to variation in reproductive success in that case.

In summary, local adaptation enhanced a comprehensive measure of male reproductive success in *D. melanogaster* populations adapted to two distinct ecological contexts for adult

mating (i.e., simple vs complex environments). Furthermore, heterogeneity between the two larval environments, perhaps due to selection for particular phenotypes (e.g., larger body size in the salt environment), demonstrates the importance of testing a range of environments to provide insight into the average effects of local adaptation on reproductive success. Experimental tests for the alignment of natural and sexual selection provide mixed results, and understanding the conditions that make this more or less likely is an important goal for future work.

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Tables

Table 1. Fixed effect results from linear mixed models testing the effects of assay environment (AE), adaptation set (AS), adult mating environment (ME) and their interactions on larval survival, male reproductive success, and dry mass in both sexes. Population was included as a random effect. Bold denotes significant ($P < 0.05$) effects.

Factor	Larval survival		Male reproductive success		Female mass		Male mass	
	F _{1,16} ^a	P	F _{1,32}	P	F _{1,32}	P	F _{1,16}	P
Assay Env. (AE)	6.445	0.0219	31.34	<0.0001	5.438	0.0262	0.7902	0.3872
Adaptation Set (AS)	1.242	0.2814	2.883	0.0991	15.65	0.0004	0.4352	0.5188
Mating Env. (ME)	1.054	0.3198	14.05	0.0007	0.0157	0.9012	0.0144	0.9060
AE × AS	33.00	<0.0001	24.99	<0.0001	1.406	0.2445	2.915	0.1071
AE × ME	0.0017	0.9679	5.293	0.0281	0.6796	0.4158	0.1044	0.7507
AS × ME	0.2679	0.6119	3.872	0.0578	1.311	0.2606	0.7978	0.3850
AE × AS × ME	0.1306	0.7226	0.0338	0.85534	0.4641	0.5006	0.7347	0.4040

^aF-value, numerator df, denominator df

Table 2. Fixed effect results from linear mixed models performed separately by assay environment (AE). Adaptation set (AE), mating environment (ME) and their interaction are main effects, with population as a random effect. Bold denotes significant ($P < 0.05$) effects.

Factor	Larval survival in cornstarch		Larval survival in salt		Male reproductive success in cornstarch		Male reproductive success in salt	
	F ₁	P	F ₁	P	F ₁	P	F ₁	P
AS	6.373	0.0225	18.45	0.0006	6.2833	0.0234	19.80	0.0004
ME	0.7274	0.4063	0.6385	0.4360	21.09	0.0003	0.9254	0.3504
AS × ME	0.4008	0.5356	0.0404	0.8433	1.835	0.1943	2.044	0.1721

Figures

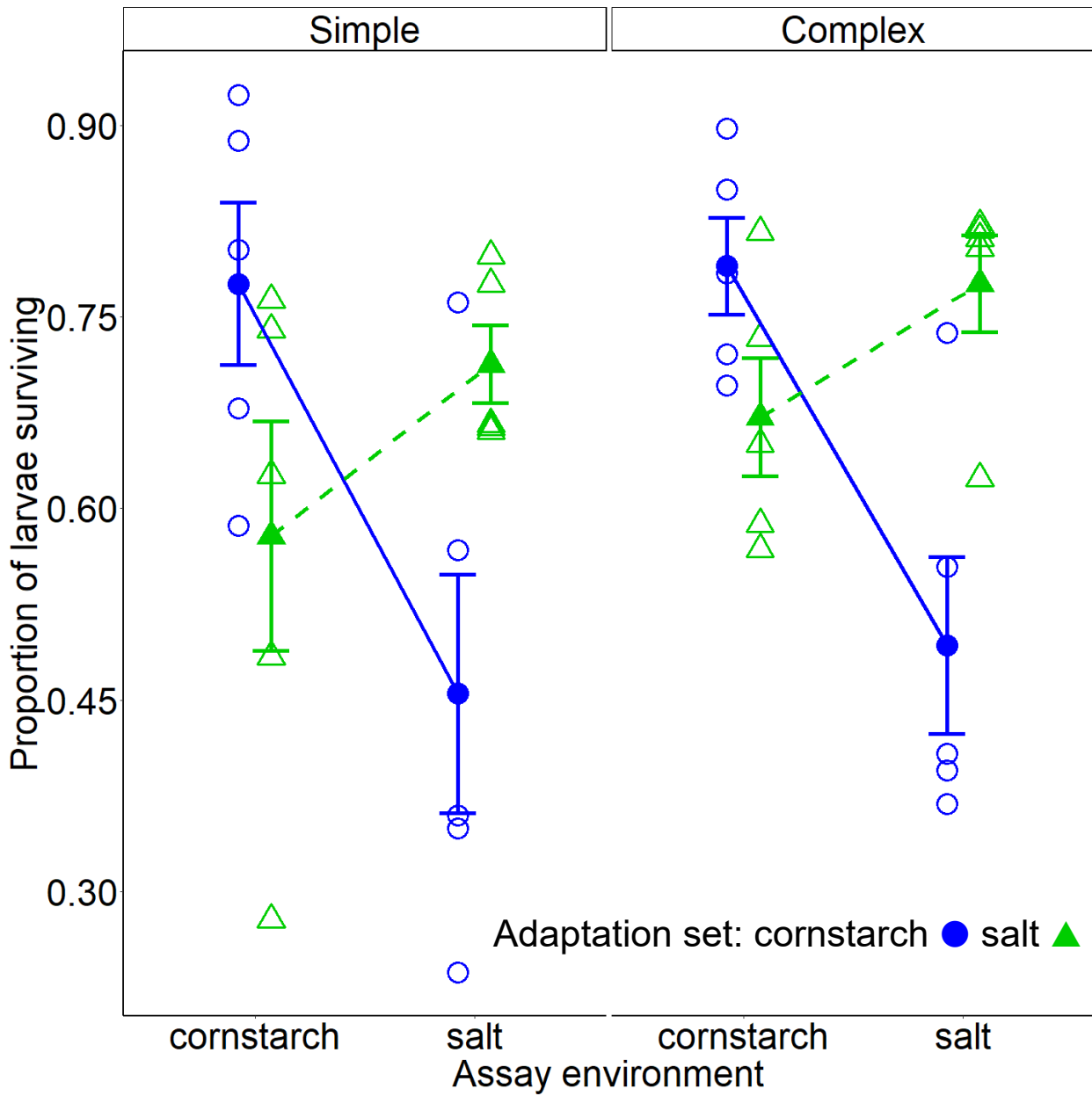


Figure 1. Egg-to-adult survival of experimental populations from the cornstarch (blue circles) and salt (green triangles) adaptation sets when reared in each of the two assay (i.e., larval abiotic) environments, separately for the simple (left panel) and complex (right panel) mating treatments. Open points are individual populations; filled point are means (± 1 SE) across the five populations within a given treatment combination.

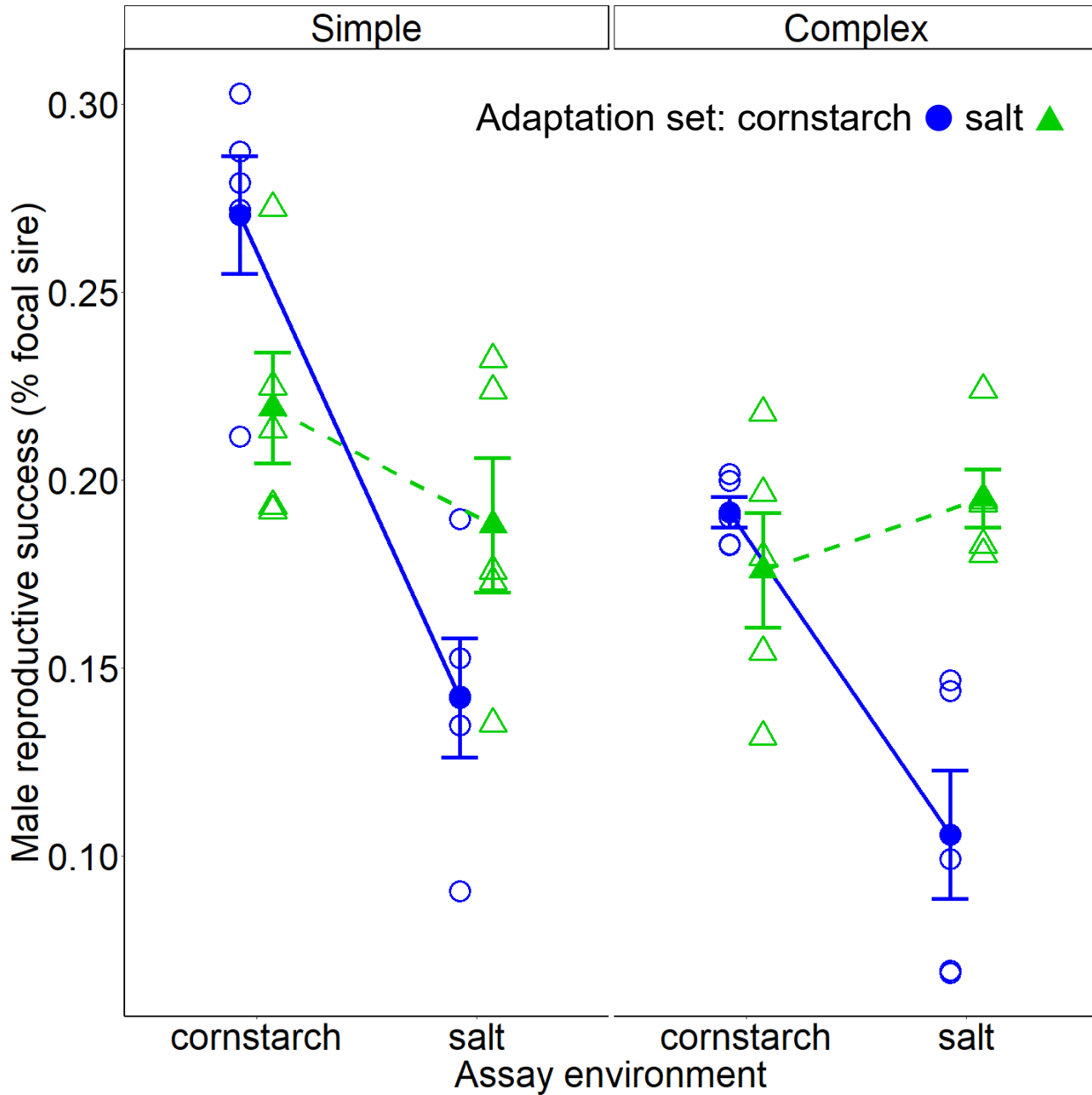


Figure 2. Reproductive success (i.e., sexual fitness) of focal males from the cornstarch (blue circles) and salt (green triangles) adaptation sets when reared in each of the two assay (i.e., larval abiotic) environments, separately for the simple (left panel) and complex (right panel) mating treatments. Open points are individual populations; filled point are means (± 1 SE) across the five populations within a given treatment combination

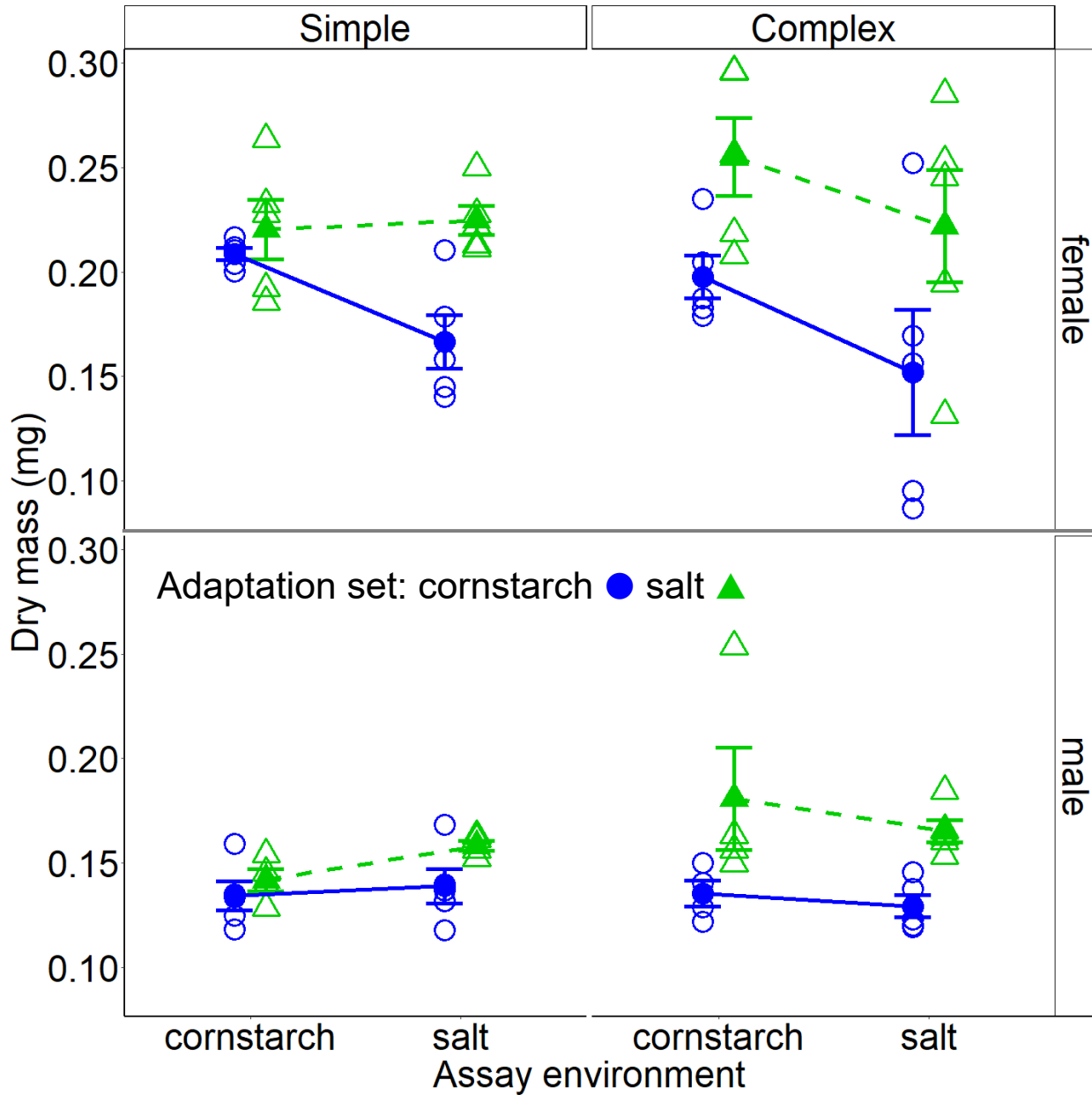


Figure 3. Dry mass of experimental females (top) and males (bottom) when reared in each of the two assay (i.e., larval abiotic) environments, separately for simple and complex mating treatments. Cornstarch and salt larval adaptation sets are shown in blue and green respectively. Open points indicate values for individual populations, filled points represent the average siring success (± 1 SE) across the five populations within a given treatment combination.

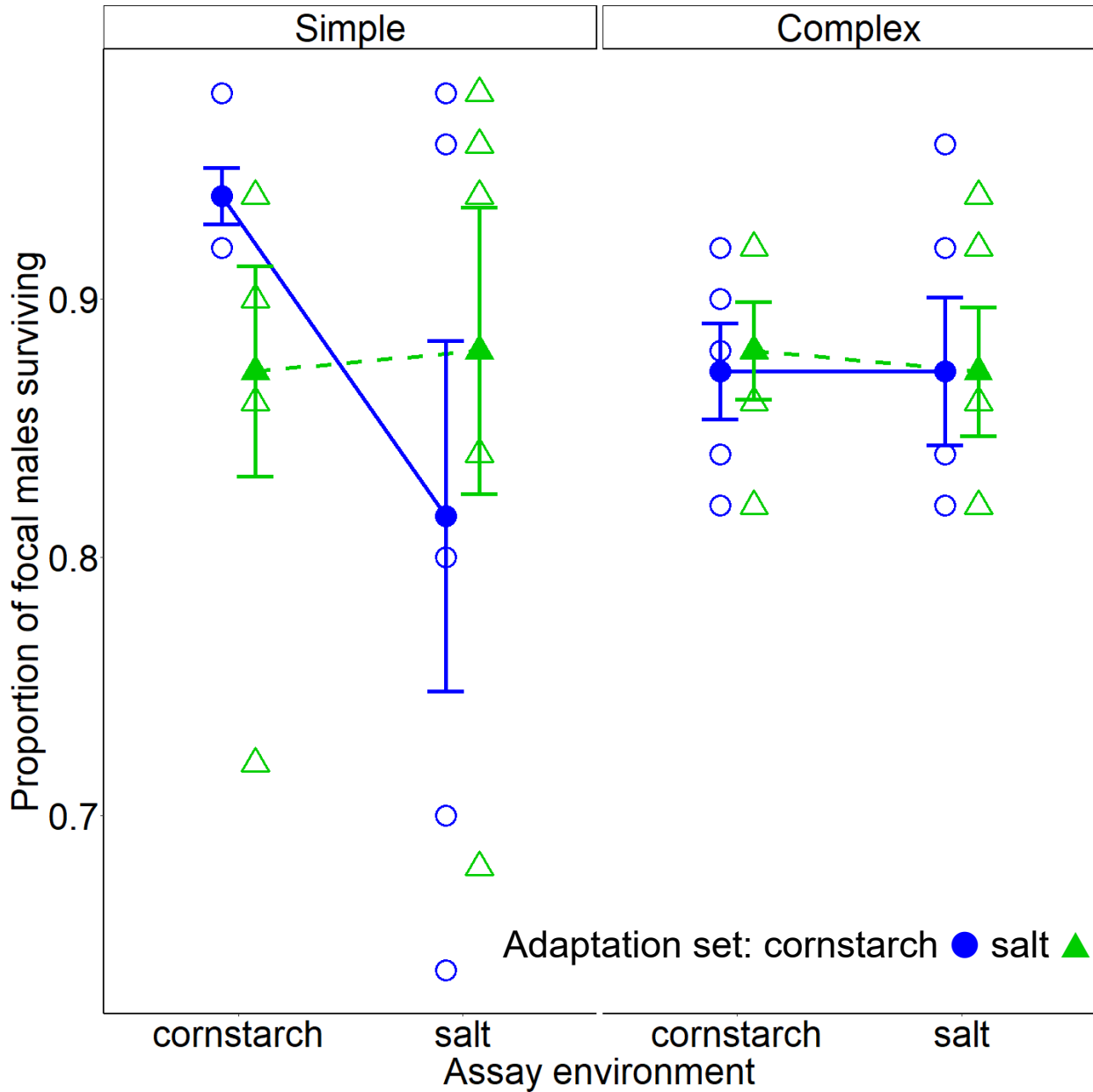


Figure 4. Survival through the six day interaction phase of experimental males from the cornstarch (blue circles) and salt (green triangles) adaptation sets when reared in each of the two assay (i.e., larval abiotic) environments, separately for the simple (left panel) and complex (right panel) mating treatments. Open points are individual populations; filled point are means (± 1 SE) across the five populations within a given treatment combination.