CrossMark

Misalignment of natural and sexual selection among divergently adapted *Drosophila melanogaster* populations

Devin Arbuthnott*, Howard D. Rundle

Department of Biology, University of Ottawa, Ottawa, ON, Canada

ARTICLE INFO

The effect of sexual selection on nonsexual fitness is a major unanswered question in evolutionary biology that may have important implications for adaptation, diversification and the evolution of mate preferences. If reproductive success is condition dependent, the resulting sexual selection will tend to align with natural selection, promoting adaptation. One prediction under such a scenario is that adaptation to a novel environment should increase male mating success and hence sexual fitness. Environmentally induced plasticity in mate preferences could also contribute to an alignment of natural and sexual selection if the changes cause females to prefer locally adapted males as mates. We tested for both forms of alignment using a set of 10 independent populations of *Drosophila melanogaster* that were adapted to one of two environments. Competitive mating trials were performed between pairs of populations adapted to these two environments, with the trials designed to separate the effects of local adaptation on male mating success from plasticity of female mate preferences in response to these environments. Contrary to expectations under an alignment of natural and sexual selection, males did not have higher mating success when competing in the environment to which they were adapted. Furthermore, there was no evidence that females altered their mate choice based on their rearing environment, indicating the absence of any adaptive plasticity in mate preferences. Overall, despite previous evidence of reciprocal adaptation to these different environments, increased nonsexual fitness did not translate into higher mating success, indicating a lack of any alignment with natural selection of this component of male sexual fitness.

© 2013 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Natural selection can arise both from variation in nonsexual fitness (e.g. longevity, fecundity) and from variation in sexual fitness (i.e. differential success in mating and fertilization among reproductive individuals), with the latter process commonly known as sexual selection. In his original description of sexual selection, Darwin (1859) hypothesized that, because the most vigorous and best adapted males will tend to acquire the most mates, sexual selection may act to promote adaptation. More recently, theory suggests that the extent to which these two forms of selection align may also have major implications for population mean fitness (Lorch, Proulx, Rowe, & Day, 2003; Whitlock & Agrawal, 2009), niche diversification (van Doorn, Edelaar, & Weissing, 2009; Proulx, 1999, 2001, 2002) and the evolution of sex (Agrawal, 2001; Siller, 2001), among other things.

Building on Darwin's (1859) original idea, it has been suggested that sexual selection may align with selection arising from variation in nonsexual fitness (hereafter natural selection, for simplicity)

because successful reproduction requires a substantial investment of time and effort, and includes direct and/or indirect competition with other members of the same sex (Andersson, 1994). Therefore, an individual's sexual fitness is likely to depend on their condition, as is expected for all major fitness components (Rowe & Houle, 1996; Whitlock & Agrawal, 2009). Individuals of high genetic quality (i.e. individuals well adapted to their environment) should acquire and use resources more efficiently to achieve a higher condition and should therefore have higher sexual fitness as well (Whitlock & Agrawal, 2009). As a consequence, condition-dependent sexual fitness should promote the transmission of locally adapted alleles.

Despite this expectation, data on the potential alignment of natural and sexual selection are mixed, with some studies finding evidence for alignment (Dolgin, Whitlock, & Agrawal, 2006; Hollis, Fierst, & Houle, 2009; Promislow, Smith, & Pearse, 1998; Radwan, 2004; Sharp & Agrawal, 2008), while others do not (Arbuthnott & Rundle, 2012; Correia, Yeaman, & Whitlock, 2010; Holland & Rice, 1999; Long, Pischedda, Stewart, & Rice, 2009; Martin & Hosken, 2004; Rundle, Chenoweth, & Blows, 2006). Sexual selection also generates the opportunity for sexual conflict arising from divergent reproductive interests of the sexes and can lead to the evolution of

* Correspondence: D. Arbuthnott, Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON K1N 6N5, Canada.
E-mail address: devin.arbuthnott@uottawa.ca (D. Arbuthnott).

traits with sexually antagonistic fitness effects (Arnqvist & Rowe, 2005). The costs associated with such conflict may therefore reduce or even outweigh the benefits of mate choice and/or intrasexual competition in some systems (e.g. Long et al., 2009; Stewart, Hannes, Mirzatuny, & Rice, 2008; Stewart, Morrow, & Rice, 2005), potentially explaining some of the variation among studies (Rowe & Day, 2006; Whitlock & Agrawal, 2009).

Here we address the effects of adaptation on male mating success. While much attention has been given to studying adaptation to novel environments with respect to nonsexual fitness (e.g. Losos, Jackman, Larson, de Queiroz, & Rodriguez-Schettino, 1998; Sandoval, 1994; Schluter, 1993), its effects on male reproductive success are poorly understood. In many species, mating success is a major component of male sexual fitness that is likely to depend on the health or vigour of the males (i.e. is likely to be condition dependent). If sexual selection arising from variation in mating success tends to align with natural selection, one simple prediction is that individuals should have higher mating success in the environment to which they are adapted (i.e. in which they have higher nonsexual fitness; Dolgin et al., 2006). Surprisingly, there are few direct tests of this. Dolgin et al. (2006) and Correia et al. (2010) tested this prediction using independent sets of *Drosophila melanogaster* populations from two separate long-term selection experiments. In both cases, half of the populations were adapted to a cold temperature (18 °C or 16 °C, respectively) and the other half were adapted to a hot temperature (25 °C in both cases). Results were mixed, with increased mating success of males in the environment to which they were adapted in one set of populations (Dolgin et al., 2006) but not in the other set (Correia et al., 2010). The interpretation of these data were also complicated by the choice of environments (i.e. divergent temperatures) because, during mating trials, females must necessarily be exposed to the environment to which one of the two types of males are adapted. If female mate preferences are plastic with respect to these environments, increased mating success of males in their adapted environment could have arisen from divergent sexual selection generated by these different preferences rather than by the higher condition of locally adapted males, as recognized by Dolgin et al. (2006). More generally, plasticity of mate preferences may cause sexual selection to differ among environments, potentially altering its alignment with natural selection.

Female mate preferences can be affected by a diversity of factors, including age and reproductive experience (Bateman, Gilson, & Ferguson, 2001; Judge, 2010; Mautz & Sakaluk, 2008; Moore & Moore, 2001), season (Borg, Forsgren, & Amundsen, 2006; Milner, Detto, Jennions, & Backwell, 2010; Qvarnström, Pärt, & Sheldon, 2000), a female's condition (Cotton, Small, & Pomiankowski, 2006; Hunt, Brooks, & Jennions, 2005), predation risk (Csada & Neudorf, 1995; Hendrick & Dill, 1993) and learning (Amcoff, Lindqvist, & Kolm, 2013). While such plasticity has been observed under various conditions, its impacts on offspring fitness among environments have rarely been considered. This is surprising given the potential impact of plasticity on adaption to new environments and the alignment of natural and sexual selection (Price, 2006; Qvarnström, 2001). If female preferences are consistent across environments and target condition-dependent male displays, then the preferences themselves will be adaptive to females, causing them to mate with the best adapted males in any given environment. Under such a scenario, the preferred males may vary among environments due to changes in their underlying sexual displays as a consequence of their nonsexual fitness in a given environment. Alternatively, if female preferences change across environments, targeting different traits or different values of the same trait(s), then such plasticity will only be adaptive to females if it results in an increased preference for traits reflecting local adaptation in

males. Importantly, such adaptive plasticity in mate preferences could contribute to the alignment of natural and sexual selection independently of condition-dependent male mating success (West-Eberhard, 2003). In addition, whether adaptive to females or not, males may evolve increased mating success during adaptation to a particular environment in response to the divergent sexual selection created by such plastic changes in female mate preferences, rather than because of their higher nonsexual fitness. Therefore, changes in male mating success and female preference in response to novel environments may have important implications for our understanding of the alignment of natural and sexual selection.

We evaluated the potential alignment of natural and sexual selection by examining male mating success across two novel environments in a way that allowed us to separate the effects of local adaptation of the males (i.e. their nonsexual fitness) and plasticity of female mate preferences in these environments. Our experiment took advantage of a long-term evolution experiment involving 10 replicate *D. melanogaster* populations, with five being adapted to the presence of salt in their food medium and the other five being adapted to the addition of the heavy metal cadmium. When assayed after 20 generations, these populations showed evidence of reciprocal adaptation such that nonsexual fitness was always higher for populations when tested in the environment in which they were evolved as compared to the other environment (Long, Rowe, & Agrawal, 2013). Using males from these different populations, we performed mating trials in a common garden experiment conducted separately within each environment. In this design, salt- and cadmium-adapted males competed for mates when both were raised in a salt environment and when both were raised in a cadmium environment, with females always being raised in a consistent (ancestral food) environment. If sexual selection arising from variation in male mating success aligns with natural selection, then males should have higher mating success when raised and tested in the environment to which they are adapted. In addition, to gain insight into plasticity of female mate preferences, we also used ancestral, nonadapted females in mate choice trials to estimate their preference for salt- versus cadmium-adapted males (each raised in their own environment) when these females were raised for a single generation in each of the two environments. If female mate choice is plastic, the outcome of these trials will vary based on the females' rearing environment, and if adaptive, females will more strongly prefer males raised in the same environment as them. Overall, we found no evidence for the alignment of natural and sexual selection, as neither male mating success nor female mate preference varied consistently based on the test environment. The lack of such alignment suggests that sexual selection, at least that arising from variation in mating success and the female preferences underlying this, will not necessarily enhance natural selection nor promote adaptation to new environments.

METHODS

Drosophila *Populations*

Our experiment used a set of replicate *D. melanogaster* populations from an evolution experiment began by A. Wang, C. C. Spencer, Y. Huang and A. F. Agrawal at the University of Toronto. For a detailed description of the history of these populations, see Long et al. (2013). These populations were established from a laboratory stock population, originally collected in 2005 in the Similkameen Valley, British Columbia, Canada (Yeaman, Chen, & Whitlock, 2010), that has been maintained at large population size on standard cornmeal/sugar/killed-yeast/agar medium. Following transfer to the University of Toronto, separate populations were exposed to

experimental selection, with some raised on food with added salt (8% NaCl, mass by volume), and others raised on food that included the heavy metal cadmium (70 μg/ml CdCl$_2$) for 36–56 generations. In 2009, these separate populations were mixed and 10 replicate populations were then established from the pooled stock and assigned to one of the two environments, with five populations raised on the salt food and five populations raised on the cadmium food. This divergence, pooling and re-establishment of new populations in the two environments was performed as part of a separate study. While the initial response to selection of any population in a novel environment will often involve the sorting of existing genetic variation, this was likely particularly important in these populations given their evolutionary history. However, we can think of no reason why these populations would not be representative of others with respect to inferences concerning the alignment of natural and sexual selection during adaptation.

Following their re-establishment, the new experimental populations were maintained on a 2-week cycle with nonoverlapping generations in vials. Throughout the entire time, the stock population was maintained on a standard medium at a large census size in cages. After 20 generations in their respective environments, a reciprocal transplant experiment measuring egg-to-adult survival showed strong evidence of divergence and reciprocal adaptation of these 10 populations to their respective environments in both survival and fecundity. Cadmium-adapted populations showed approximately 15% higher survival than salt-adapted populations in cadmium medium, and salt-adapted populations showed approximately 45% higher survival than cadmium-adapted populations in the salt medium (Long et al., 2013). Similarly, cadmium-adapted females produced significantly more eggs than did salt-adapted females when both were raised in the cadmium environment, while the reverse was true in the salt environment (Long et al., 2013).

In September of 2012, a large sample of each of these populations was transferred to the University of Ottawa and maintained under the same conditions (25 °C, 12:12 h light:dark cycle) on their respective media. Because cadmium-adapted populations have very low survival in the 8% salt medium, we decreased the salt concentration to 6% when rearing individuals from all populations (cadmium and salt) for use in the assays below. Salt-adapted populations were therefore not raised in the same conditions to which they were adapted, although previous survival assays using a 6% salt concentration found reciprocal adaptation among these populations (Huang & Agrawal, 2010), demonstrating that 8% salt-adapted populations outperformed cadmium-adapted populations when tested in 6% salt. When our assays were conducted, the experimental populations had been evolving in their respective environments for 80–90 generations.

Male Mating Success

We first sought to determine whether males would have higher mating success in the environment to which they were adapted as compared to the other environment. To do this, we conducted mating trails in which multiple cadmium-adapted and salt-adapted males competed for matings with stock females within a small cage. Trials were performed when both types of males were raised in cadmium and when both types were raised in salt, with females always raised on the standard food. Each of the five cadmium-adapted and salt-adapted populations were paired randomly such that males from one cadmium-adapted population were always competing for females against males from one salt-adapted population, resulting in five independent cadmium–salt population pairs. Because female population and rearing environment were the same for all trials, any differences in mating success can be attributed directly to the males.

To collect males raised in each environment, 20–30 adults from each experimental population (reared in their normal selective environment) were allowed to lay eggs for 24 h in vials containing either salt or cadmium food. Stock females were collected by placing food-containing vials in population cages and allowing females to lay eggs for 1–2 h. All vials were controlled for egg density such that no vial contained more than 50 individuals. Virgin females/males were collected from their respective vials using light CO$_2$ anaesthesia within 8 h of emergence. Males were held in vials containing food matching their rearing environment at a density of seven per vial, without edible yeast, for 2–3 days. Stock females were held at a density of 10 females per vial, with excess live yeast, for 3 days prior to the mating trials. Virgin flies were used to ensure that variance in reproductive history did not influence mating behaviour, including mate choice. Four hours prior to the mating trials, males were transferred to vials containing yeast paste that was coloured with either red or blue food colouring (McCormick Canada, London, ON). Males ate this coloured yeast, and the colour was visible through their abdomen, temporarily marking them and making it possible to identify their population of origin. Males were coloured in a balanced design such that each population was blue for half of the trials and red for the other half. Previous use of such markings in mating trials has shown no effects on mate choice (Mooers, Rundle, & Whitlock, 1999; Rundle, 2003; Rundle, Mooers, & Whitlock, 1998, Rundle, Odeen, & Mooers, 2007), and we found no evidence for differential mating by males of one colour in this assay (one-sample t test: $t_{96} = 0.0168$, $P = 0.99$) nor the female mate preference assay below ($t_{77} = 1.32$, $P = 0.19$).

We performed 20 replicate mating trials for each population pair: 10 when both types of males were raised in 6% salt and 10 when both types were raised in cadmium. The only exception was population pair 4, for which we performed 14 trials rather than 20 due to a limited number of males. Within each trial, 21 cadmium-adapted males and 21 salt-adapted males were placed together with 20 stock females in a translucent plastic cage (14 × 14 × 14 cm). We introduced both types of males first and subsequently released the females. Cages were covered with white paper to limit external disturbances and then left for 8 min, after which the first 10 mating pairs were removed by aspiration. If fewer than 10 pairs were collected, we repeated this procedure at 8 min intervals until 10 mating pairs were sampled. Males from the mating pairs were scored for colour using a dissecting microscope under CO$_2$ anaesthesia. If a male's colour could not be identified, it was discarded (approximately 6% of males overall). To determine whether the rearing environment of males influenced their mating success, we used a linear mixed model with the proportion of matings achieved by cadmium-adapted males in a given cage as the response variable, male rearing environment as a fixed effect and population pair as a random effect. In this model, we used population pair, rather than the number of trials, as the unit of replication. The model was fitted via restricted maximum likelihood and significance of the random effect of population pair was tested using a likelihood ratio test.

Female Mating Preference

We next sought to determine whether female mate preference changed with rearing environment by raising stock females in both cadmium and salt media and then assaying their preference using mating trials like those above. The same population pairs were used such that within a given trial, stock females raised in one of the two environments had the choice of mating with either a cadmium-adapted male raised in cadmium or a salt-adapted male raised in salt. Because male populations and rearing environment were constant across trials, an effect of female rearing environment on

the difference in mating success of the two types of males cannot be attributed to changes in male competitive ability. Rather, an effect of female rearing environment would be consistent with plasticity in female mate preferences, although in theory it could also arise from an interaction between female rearing environment and male mate choice (e.g. if cadmium-adapted males were more attracted to females reared in cadmium as opposed to salt). While male mate preferences have been demonstrated in *D. melanogaster*, they have been found to target female body size, most likely because of the direct fecundity benefit this provides to males (Bondquriansky, 2001; Long et al., 2009), suggesting that such an interaction is unlikely. More importantly, the absence of an effect of female rearing environment (see Results) argues against this possibility.

Stock females for use in the trials were raised in both cadmium and salt by allowing females in population cages to lay eggs in vials containing one of the two food types (only one food type was presented to females at any one time), with egg density again controlled at 50/vial. Following development, emerging females were collected as virgins and subsequently held at 10 individuals per vial, with excess live yeast, for 5 days. Cadmium-adapted and salt-adapted populations were raised in their adapted environment (i.e. cadmium populations in cadmium, salt populations in 6% salt) using the same egg collection protocols described above, and emerging males were collected as virgins and held at seven individuals per vial, without live yeast, for 4–5 days. Males were marked in a balanced design and trials were conducted as described above. Seven to nine replicate mating trials were performed for each population pair per female rearing environment, yielding 78 trials overall. To test whether female rearing environment changed their preference for the two types of males, we used a linear mixed model as described above but with female (instead of male) rearing environment as a fixed effect.

RESULTS AND DISCUSSION

If condition-dependent mating success contributes to the alignment of natural and sexual selection during adaptation to new environments, then males should have higher mating success in their adapted environment than in a nonadapted environment. Contrary to this hypothesis, the mating success of cadmium-adapted and salt-adapted males did not differ overall based on male rearing environment ($F_{1,4} = 0.04$, $P = 0.860$), providing no evidence of higher mating success of locally adapted males (Fig. 1). While differences among population pairs in the relative mating success of the two types of males approached significance (likelihood ratio test: $\chi^2_1 = 3.0$, $P = 0.083$), and three population pairs showed the expected pattern of males having higher mating success in their adapted environment, the other two showed the opposite pattern and in no case were any of these differences significant (two-sample *t* test: $P > 0.08$ in separate tests of all five pairs). Therefore, despite confirmed adaptation with respect to nonsexual fitness in these populations, and strong trade-offs between environments, this did not translate into increased mating success of locally adapted males.

Natural and sexual selection may also align if mate preferences are plastic such that females raised in a given environment are more likely to mate with locally adapted males than are females raised in a different environment. By generating divergent sexual selection, such plasticity could also result in higher mating success of locally adapted males without any alignment of sexual and natural selection, provided males show an evolutionary response to this selection. Inconsistent with any plasticity in our populations, female mate preferences for cadmium-adapted versus salt-adapted males did not differ significantly when ancestral stock females were raised for a single generation with cadmium or salt food
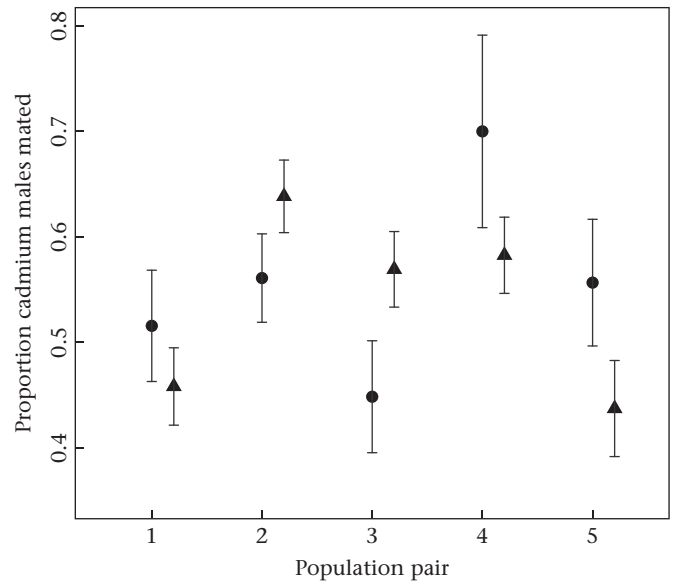
Figure 1. Proportion of cadmium-adapted males among mating pairs in trials measuring male mating success. Points represent the average (±SE) of the 10 replicate cages for each unique population pair when all males were raised either in cadmium (circles) or in salt (triangles) media. A proportion of 0.5 represents equal mating success of cadmium-adapted and salt-adapted males. A higher mating success of locally adapted males would cause the circles to have larger values than triangles.

($F_{1,4} = 0.84$, $P = 0.412$; Fig. 2). Results did not vary significantly among the five population pairs ($\chi^2_1 = 1.4$, $P = 0.237$), although two showed the predicted pattern of females more strongly preferring males adapted to the female's rearing environment. The other three pairs showed the opposite pattern, however, and one of these (pair 4) was the only one in which the difference was significant (two-sample *t* test: $t_{13.99} = -5.13$, $P < 0.001$; $P > 0.4$ for the other four pairs). Therefore, our results provide no evidence of any
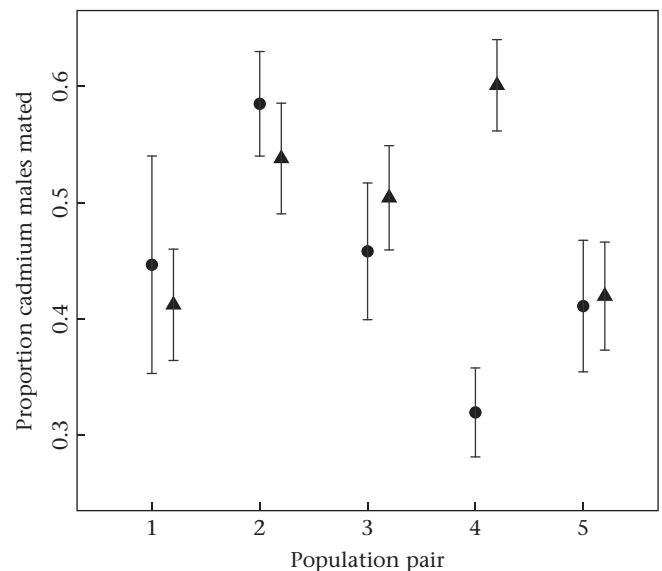
Figure 2. Proportion of cadmium-adapted males among mating pairs in trials measuring female mate choice. Points represent the average (±SE) of the replicate cages for each unique population pair when females were raised either in cadmium (circles) or salt (triangles) media. All males were raised in their adapted environment. If females preferred to mate with males adapted to their rearing environment, the circles would have higher values than the triangles.

consistent plasticity of female mate preferences with respect to their choice between these two types of males.

Condition dependence of the traits underlying sexual fitness has been proposed as a general mechanism to explain the maintenance of unexpectedly high genetic variance in these traits in the face of strong directional sexual selection (Rowe & Houle, 1996). The condition dependence of sexual displays is well supported (Cotton, Fowler, & Pomiankowski, 2004; see Delcourt & Rundle, 2011, for a more recent discussion), at least with respect to environmental manipulations, and this should contribute to the alignment of natural and sexual selection (Whitlock & Agrawal, 2009). Increased reproductive success of high-condition males has been found in some cases (e.g. Maclellan, Whitlock, & Rundle, 2009; Sharp & Agrawal, 2008), although these studies made use of single deleterious mutations of large effect that may not be representative of the genetic basis of adaptation to new environments. There are few investigations of male mating success in particular among divergently adapted populations, despite the relevance of such studies to our understanding of the effects of sexual selection on adaptation to novel environments, and the two that have been done have provided mixed results despite employing the same species and almost identical environments (i.e. high and low temperature in *D. melanogaster*: see Correia et al., 2010; Dolgin et al., 2006). Our finding that local adaptation does not necessarily result in increased male mating success suggests that at least this component of sexual selection may not reinforce natural selection, consistent with Correia et al.'s (2010) results. Given the limited number of experiments that address such alignment, as well as their conflicting results, we echo Correia et al.'s (2010) call for additional studies across a range of species that use different environments to determine the generality of this result. The sensitivity of other components of sexual fitness to local adaptation also remains a key question.

The lack of alignment between sexual and nonsexual fitness is surprising, especially given strong evidence of the adaptive divergence of these populations (Long et al., 2013), and the underlying explanation is therefore of interest. Much attention of late has been given to sexual conflict's ability to obscure the benefits of condition-dependent reproductive success (Bonduriansky & Chenoweth, 2009; Long et al., 2009; Pischedda & Chippindale, 2006; Stewart et al., 2008; Whitlock & Agrawal, 2009). However, our approach tested only for a benefit of sexual selection and did not consider its overall fitness consequences. The operation of sexual conflict itself cannot therefore explain the lack of alignment that we observed.

The nature of our environments could, in theory, contribute to the lack of an alignment. In particular, our environments altered the larval food media and this may not have impacted adults directly. One would expect that adaptation to these environments would entail not only increased survival, but also as an increased ability to gain and use resources, leading to increased condition of surviving adults. However, it is possible that, when raised in the 'other' (i.e. nonadapted) environment, the adults that do successfully emerge are of similar condition to locally adapted males and thus perform equally well in terms of intrasexual mate competition. Inconsistent with this, differences in adult body mass mirrored patterns of survival and fecundity when measured in a reciprocal transplant using the same food environments as our experiments (Huang & Agrawal, 2010), with adapted males being heavier on average than nonadapted males, and male mass being lower when individuals were raised on nonadapted versus adapted food (Supplementary Table S1). This suggests that adult males were of lower condition when raised in the environment to which they were not adapted.

Another possibility is that we may have underestimated the condition dependence of male mating success by using a 6% salt environment in our assays, despite the salt populations being adapted to 8% salt. We used 6% salt in order to collect sufficient numbers of cadmium-adapted males as few, if any, of these males survived when raised in 8% salt. Therefore, the condition of salt-adapted males may have been lower than it would have been had they been raised in their evolved environment, although as noted in the Methods, salt-adapted males were larger and had significantly higher survival than cadmium-adapted males in an assay using a 6% salt environment.

An alternative explanation for the lack on an alignment involves the differential allocation of condition. In particular, while males reared in a nonadapted environment may be of lower average condition, and thus have fewer resources overall, these males may allocate disproportionately more of these resources to mating success at the expense of other life history traits, thereby maintaining this fitness component at the cost of others. Such differential allocation has been previously demonstrated in male *D. melanogaster* (Rundle et al., 2007), but has received limited attention in sexual selection research.

Finally, our use of ancestral rather than salt- and/or cadmium-adapted females in our mating trials may have contributed to the lack of any detectable alignment. If female mate preferences evolved in each population to target well-adapted males, but these changes in preference were specific to the population and/or environment such that they targeted different male traits (possibly because different male traits are indicative of high condition in different environments), then local males would only have higher mating success when tested with females with the appropriate preference (i.e. that evolved in the same population or environment), and not with stock females. We used stock females to avoid confounding the effects of local adaptation of the males with plasticity of female mate preferences. Our results suggest that such plasticity did not occur, so further experiments using females adapted to each of the two environments could be used to test for such population or environment-specific effects that could contribute to an alignment of natural and sexual selection.

We focused here on male mating success because it is likely a particularly important component of sexual fitness in these populations given their maintenance routine involving nonoverlapping generations and severely truncated adult life spans. Male mating success has also been the subject of past studies with respect to natural selection (e.g. van Doorn et al., 2009; Proulx, 1999, 2001), including direct tests concerning the effects of local adaptation on it (Correia et al., 2010; Dolgin et al., 2006). The lack of alignment we observed with respect to mating success does not preclude the possibility that sexual selection arising from other components of male sexual fitness (e.g. postcopulatory performance) may align with natural selection and thereby promote adaptation. It is also possible that virgin females may be less discriminatory than previously mated females, and our use of them therefore failed to detect an alignment that may occur in these populations. Like past studies (Correia et al., 2010; Dolgin et al., 2006), we used virgin females to control for female mating history. In other insects, there is some evidence that nonvirgin females show stronger mate preferences than virgin females (Bateman et al., 2001; Judge, 2010), although others did not find this pattern (Ivy & Sakaluk, 2007). However, in each of these studies, mate preferences were observed in virgins, and females preferred similar traits regardless of mating history, suggesting that studying mate choice in virgins should be representative of mate choice of all females. We are not aware of any evidence for decreased mate discrimination by virgin, as compared to mated, *D. melanogaster*, and given the short adult phase of our populations (4 days on average), a substantial portion

of the matings in our unmanipulated populations likely involve virgin females, making mating success with such females an important component of a male's sexual fitness.

Turning to the plasticity of female mate preferences, such changes could have large impacts on sexual selection and adaptation (Price, 2006; Qvarnström, 2001) and yet have received little attention within the context of adaptation to new environments (Price, Qvarnström, & Irwin, 2003). There is some evidence for the condition dependence of mate preferences themselves (Cotton et al., 2006) as well as other forms of preference plasticity (e.g. Amcoff, Lindqvist, & Kolm, 2013; Grace & Shaw, 2004; Hendrick & Dill, 1993; Qvarnström, 2000), but the effect of environmentally induced preference variation on adaptation to novel environments has not been directly addressed. Plastic changes in mate preferences may alter the direction and strength of sexual selection rapidly upon colonization of a new environment, and the nature of such plasticity would determine its adaptive benefit (or not) with respect to nonsexual fitness. Plasticity in preference could also cause males to have higher mating success in their local environment without requiring any alignment of natural and sexual selection, and its existence is therefore of interest in such studies. In our experiment, no increase in mate preference for cadmium-adapted or salt-adapted males was observed when stock females were raised in each of these respective environments, indicating the absence of any adaptive plasticity that would contribute to an alignment of natural and sexual selection. Although not an issue given our results, it is worth noting that had we observed such a pattern, it would not necessarily have indicated adaptive plasticity. Our experimental design used cadmium-adapted and salt-adapted males, so if males evolved higher mating success during experimental evolution in response to the divergent sexual selection generated by plasticity, these males would be expected to be more attractive to the females expressing this preference (i.e. those raised in that environment).

In conclusion, despite significant divergence among populations and reciprocal adaptation to their different environments, we found no effect of rearing environment on male mating success or female mate preferences. In particular, although the nonsexual fitness of populations differed greatly when raised in their local as opposed to the other environment, this did not serve to increase a male's ability to acquire mates, suggesting that at least this component of sexual selection does not reinforce natural selection in these environments. Other studies have also failed to find alignment between natural and sexual selection in experimental populations (Correia et al., 2010; Holland & Rice, 1999; Martin & Hosken, 2004), and the presence of sexual selection did not speed adaptation to a new environment in *Drosophila serrata* (Rundle et al., 2006) nor *D. melanogaster* (Holland, 2002). Sexual selection on some loci will likely be sex specific, generating intra- and/or interlocus sexual conflict that may hamper natural selection and reduce nonsexual fitness (Bondurjansky & Chenoweth, 2009; Long et al., 2009; Pischedda & Chippindale, 2006; Stewart et al., 2008). Overall, the costs associated with sexual conflict may outweigh the potential benefits of sexual selection, causing the net effect to be deleterious (Arbuthnott & Rundle, 2012; Rice et al., 2006; Stewart et al., 2005, 2008). Recent work has suggested that the potential alignment of natural and sexual selection may depend on a population's location within a fitness landscape, such that alignment is more likely when the population is far from a fitness peak, where benefits are more likely to outweigh costs of sexual selection (Fricke & Arnqvist, 2007; Long, Agrawal, & Rowe, 2012). Our current results suggest that, even aside from any potential costs of sexual conflict, sexual selection arising from variation in mating success may not promote adaptation to novel environments.

Supplementary Material

Supplementary material for this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2013.10.005.

References

Agrawal, A. F. (2001). Sexual selection and the maintenance of sexual reproduction. *Nature, 411*, 692–695.

Amcoff, M., Lindqvist, C., & Kolm, N. (2013). Sensory exploitation and plasticity in female mate choice in the swordtail characin. *Animal Behaviour, 85*, 891–898.

Andersson, M. (1994). *Sexual selection*. Princeton, NJ, USA: Princeton University Press.

Arbuthnott, D., & Rundle, H. D. (2012). Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. *Evolution, 66*, 2127–2137.

Arnqvist, G., & Rowe, L. (2005). *Sexual conflict*. Princeton, NJ, USA: Princeton University Press.

Bateman, P. W., Gilson, L. N., & Ferguson, J. W. H. (2001). Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Animal Behaviour, 61*, 631–637.

Bondurjansky, R. (2001). The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biology Reviews, 76*, 305–339.

Bondurjansky, R., & Chenoweth, S. F. (2009). Intralocus sexual conflict. *Trends in Ecology & Evolution, 24*, 280–288.

Borg, A., Forsgren, E., & Amundsen, T. (2006). Seasonal change in female choice for male size in the two-spotted goby. *Animal Behaviour, 72*, 763–771.

Correia, L., Yeaman, S., & Whitlock, M. C. (2010). Local adaptation does not always predict high mating success. *Journal of Evolutionary Biology, 23*, 875–878.

Cotton, S., Fowler, K., & Pomiankowski, A. (2004). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society B, 271*, 771–783.

Cotton, S., Small, J., & Pomiankowski, A. (2006). Sexual selection and condition-dependent mate preferences. *Current Biology, 16*, R755–R765.

Csada, R. D., & Neudorf, D. L. (1995). Effects of predation risk on mate choice in female *Acheta domesticus* crickets. *Ecological Entomology, 20*, 393–395.

Darwin, C. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: J. Murray.

Delcourt, M., & Rundle, H. D. (2011). Condition dependence of a multicomponent sexual display trait in *Drosophila serrata*. *American Naturalist, 177*, 812–823.

Dolgin, E. S., Whitlock, M. C., & Agrawal, A. F. (2006). Male *Drosophila melanogaster* have higher mating success when adapted to their thermal environment. *Journal of Evolutionary Biology, 19*, 1894–1900.

van Doorn, G. S., Edelaar, P., & Weissing, F. J. (2009). On the origin of species by natural and sexual selection. *Science, 326*, 1704–1707.

Fricke, C., & Arnqvist, G. (2007). Rapid adaptation to a novel host in a seed beetle (*Callosobruchus maculatus*): the role of sexual selection. *Evolution, 61*, 440–454.

Grace, J. L., & Shaw, K. L. (2004). Effects of developmental environment on signal-preference coupling in a Hawaiian cricket. *Evolution, 58*, 1627–1633.

Hendrick, A. V., & Dill, L. M. (1993). Mate choice by female crickets is influenced by predation risk. *Animal Behaviour, 46*, 193–196.

Holland, B. (2002). Sexual selection fails to promote adaptation to a new environment. *Evolution, 56*, 721–730.

Holland, B., & Rice, W. R. (1999). Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences, United States of America, 96*, 5083–5088.

Hollis, B., Fierst, J. L., & Houle, D. (2009). Sexual selection accelerates the elimination of a deleterious mutant in *Drosophila melanogaster*. *Evolution, 63*, 324–333.

Huang, Y., & Agrawal, A. F. (2010). *Adaptation and rearing environmental effects on* Drosophila melanogaster *survival and mass*. Unpublished data.

Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition-dependent life-history strategy. *American Naturalist, 166*, 79–92.

Ivy, T. M., & Sakaluk, S. K. (2007). Sequential mate choice in decorated crickets: females use a fixed internal threshold in pre- and postcopulatory choice. *Animal Behaviour, 74*, 1065–1072.

Judge, K. A. (2010). Female social experience affects the shape of sexual selection on males. *Evolutionary Ecology Research, 12*, 389–402.

Long, T. A. F., Agrawal, A. F., & Rowe, L. (2012). The effect of sexual selection on offspring fitness depends on the nature of genetic variation. *Current Biology, 22*, 204–208.

Long, T. A. F., Pischedda, A., Stewart, A. D., & Rice, W. R. (2009). A cost of sexual attractiveness to high-fitness females. *PLoS Biology, 7*, e1000254.

Long, T. A. F., Rowe, L., & Agrawal, A. F. (2013). The effects of selective history and environmental heterogeneity on inbreeding depression in experimental populations of *Drosophila melanogaster. American Naturalist, 181*, 532–544.

Lorch, P. D., Proulx, S., Rowe, L., & Day, T. (2003). Condition-dependent sexual selection can accelerate adaptation. *Evolutionary Ecology Research, 5*, 867–881.

Losos, J. B., Jackman, T. R., Larson, A., de Queiroz, K., & Rodriguez-Schettino, L. (1998). Contingency and determinism in replicated adaptive radiations of island lizards. *Science, 279*, 2115–2118.

Maclellan, K., Whitlock, M. C., & Rundle, H. D. (2009). Sexual selection against deleterious mutations via variable male search success. *Biology Letters, 5*, 795–797.

Martin, O. Y., & Hosken, D. J. (2004). Reproductive consequences of population divergence through sexual conflict. *Current Biology, 14*, 906–910.

Mautz, B. S., & Sakaluk, S. K. (2008). The effects of age and previous mating experience on pre- and post-copulatory mate choice in female house crickets (*Acheta domesticus* L.). *Journal of Insect Behavior, 21*, 203–212.

Milner, R. N. C., Detto, T., Jennions, M. D., & Backwell, P. R. Y. (2010). Experimental evidence for a seasonal shift in the strength of a female mating preference. *Behavioral Ecology, 21*, 311–316.

Mooers, A.Ø., Rundle, H. D., & Whitlock, M. C. (1999). The effects of selection and bottlenecks on male mating success in peripheral isolates. *American Naturalist, 153*, 437–444.

Moore, P. J., & Moore, A. J. (2001). Reproductive aging and mating: the ticking of the biological clock in female cockroaches. *Proceedings of the National Academy of Sciences, United States of America, 98*, 9171–9176.

Pischedda, A., & Chippindale, A. K. (2006). Intralocus sexual conflict diminishes the benefits of sexual selection. *PLoS Biology, 4*, e356–e360.

Price, T. D. (2006). Phenotypic plasticity, sexual selection and the evolution of colour patterns. *Journal of Experimental Biology, 209*, 2368–2376.

Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B, 270*, 1433–1440.

Promislow, D. E., Smith, E. A., & Pearse, L. (1998). Adult fitness consequences of sexual selection in *Drosophila melanogaster. Proceedings of the National Academy of Sciences, United States of America, 95*, 10687–10692.

Proulx, S. R. (1999). Matings systems and the evolution of niche breadth. *American Naturalist, 154*, 89–98.

Proulx, S. R. (2001). Female choice via indicator traits easily evolves in the face of recombination and migration. *Evolution, 55*, 2401–2411.

Proulx, S. R. (2002). Niche shifts and expansion due to sexual selection. *Evolutionary Ecology Research, 4*, 351–369.

Qvarnström, A. (2001). Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution, 16*, 5–7.

Qvarnström, A., Pärt, T., & Sheldon, B. C. (2000). Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature, 405*, 344–347.

Radwan, J. (2004). Effectiveness of sexual selection in removing mutations induced with ionizing radiation. *Ecology Letters, 7*, 1149–1154.

Rice, W. R., Stewart, A. D., Morrow, E. H., Linder, J. E., Orteiza, N., & Byrne, P. G. (2006). Assessing sexual conflict in the *Drosophila melanogaster* laboratory model system. *Philosophical Transactions of the Royal Society B, 361*, 287–299.

Rowe, L., & Day, T. (2006). Detecting sexual conflict and sexually antagonistic coevolution. *Philosophical Transactions of the Royal Society B, 361*, 277–285.

Rowe, L., & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society B, 263*, 1415–1421.

Rundle, H. D. (2003). Divergent environments and population bottlenecks fail to generate premating isolation in *Drosophila pseudoobscura. Evolution, 57*, 2557–2565.

Rundle, H. D., Chenoweth, S. F., & Blows, M. W. (2006). The roles of natural and sexual selection during adaptation to a novel environment. *Evolution, 60*, 2218–2225.

Rundle, H. D., Mooers, A.Ø., & Whitlock, M. C. (1998). Single founder-flush events and the evolution of reproductive isolation. *Evolution, 52*, 1850–1855.

Rundle, H. D., Odeen, A., & Mooers, A.Ø. (2007). An experimental test for indirect benefits in *Drosophila melanogaster. BMC Evolutionary Biology, 7*, 36.

Sandoval, C. P. (1994). Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae: Timemidae) and its consequences for host range. *Biological Journal of the Linnean Society, 52*, 341–356.

Schluter, D. (1993). Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology, 74*, 699–709.

Sharp, N. P., & Agrawal, A. F. (2008). Mating density and the strength of sexual selection against deleterious alleles in *Drosophila melanogaster. Evolution, 62*, 857–867.

Siller, S. (2001). Sexual selection and the maintenance of sex. *Nature, 411*, 689–692.

Stewart, A. D., Hannes, A. M., Mirzatuny, A., & Rice, W. R. (2008). Sexual conflict is not counterbalanced by good genes in the laboratory *Drosophila melanogaster* model system. *Journal of Evolutionary Biology, 21*, 1808–1813.

Stewart, A. D., Morrow, E. H., & Rice, W. R. (2005). Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution. *Proceedings of the Royal Society B, 272*, 2029–2035.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York, NY, USA: Oxford University Press.

Whitlock, M. C., & Agrawal, A. F. (2009). Purging the genome with sexual selection: reducing mutation load through selection on males. *Evolution, 63*, 569–582.

Yeaman, S., Chen, Y., & Whitlock, M. C. (2010). No effect of environmental heterogeneity on the maintenance of genetic variation in wing shape in *Drosophila melanogaster. Evolution, 64*, 3398–3408.