

Drift and selection entwined: asymmetric reproductive isolation in an experimental niche shift

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ABSTRACT

Question: Host races of phytophagous insects originate when a population adapts to a novel resource (host) while other populations remain resident on their ancestral host. The derived and ancestral host race populations will be subject to unequal selection intensity and genetic drift. Is this asymmetry responsible for asymmetric reproductive isolation observed in some populations?

Hypothesis: Unequal intensity of selection and/or genetic drift between populations may lead to asymmetric reproductive isolation.

Methods: We reared populations of *Tribolium castaneum* flour beetles on wheat or corn flour, which represent ancestral and suboptimal novel resources respectively. After approximately 43 generations, we assayed the fitness of wheat- and corn-evolved beetles on wheat and corn flour, and measured reciprocal pre-mating, pre-zygotic, and post-zygotic reproductive isolation between the ecotypes.

Results: Three of our four corn-evolved populations went extinct. The one surviving corn population exhibited evidence of adaptation to corn half way through the experiment, but by the final generation we found little evidence of adaptation. Instead, the corn-evolved population had lower survival than the wheat-evolved population, independent of rearing environment. This result is consistent with maladaptation due to fixation of deleterious alleles via genetic drift. Pre-mating and post-zygotic reproductive isolation were both asymmetric, favouring the higher-fitness ancestral population. Females from both wheat- and corn-evolved populations avoided mating with corn-evolved males. This bias improved female fitness, because corn-evolved males had offspring with lower survival, regardless of female genotype or rearing medium.

Conclusion: Strong selection in the derived (corn) population appears to have decreased population size to the extent that genetic drift led to the fixation of deleterious alleles that reduced corn-evolved male fitness. We posit that asymmetric pre-mating isolation arose because of this drift-induced maladaptation. Although our study is limited to a single pair of derived and ancestral populations, the results of our extensive pre- and post-mating reproductive

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isolation tests are consistent with this conclusion. This study highlights the importance of recognizing the fundamental entangling of drift and selection in the evolution of reproductive isolation, which complicates the frequently invoked dichotomy of selection and drift as distinct alternative forces in speciation.

Keywords: ecological speciation, founder effects, inbreeding depression, niche shift, phytophagous insects, post-zygotic isolation, pre-mating isolation, pre-zygotic isolation.

INTRODUCTION

Speciation due to host shifts by phytophagous insects (Feder *et al.*, 1988, 2003; Bush *et al.*, 1989; Funk, 1998; Nosil *et al.*, 2002), in which a few individuals may colonize a new host plant, often entails an evolutionary asymmetry in which a derived population is subjected to both intense selection and drift while another related population using the ancestral resource remains essentially unchanged. This asymmetry in selective regimes may cause asymmetric pre-mating reproductive isolation, which is common in nature (Watanabe and Kawanishi, 1979; Kaneshiro, 1980; Bordenstein *et al.*, 2000; Shine *et al.*, 2002; Lin *et al.*, 2012). Asymmetric post-mating isolation is also widely documented (Tiffin *et al.*, 2001), but has been attributed strictly to genetic rather than adaptive mechanisms (Turelli and Moyle, 2007). We posit that asymmetric reproductive isolation may arise from asymmetric fitness in the ancestral and novel conditions. Selection may cause the new population to adapt, in the sense that it can now persist in the new habitat. However, if initial fitness in the new habitat is extremely low, genetic drift due to founder effects or strong selection may cause maladaptation by fixing deleterious alleles. Due to asymmetric selection and drift, the derived population may have lower survival or fecundity than the ancestral population. The ancestral population, being generally more fit, should discriminate against mates from the derived population. The derived population, being less fit, might actually prefer mates from the ancestral population because the outbreeding would increase fitness.

Considering the joint effect of selection and drift may help to explain patterns of asymmetric reproductive isolation, and more generally may highlight the necessity of considering these processes jointly rather than separately. The relative importance of natural selection and genetic drift in speciation has been hotly debated since the New Synthesis (Wright, 1931, 1932; Rice and Hostert, 1993; Coyne *et al.*, 1997, 2004). Natural selection can drive the fixation of hybrid incompatibility genes (Dobzhansky, 1936; Muller, 1942; Orr, 1995), select against migration between populations (Nosil *et al.*, 2005), or drive pre-mating isolation due to divergent mating cues (Ryan, 1990; Proctor, 1991, 1992). In principle, genetic drift may also fix incompatibility genes, or drive divergence in traits used for mate choice. Thus, a major question in evolutionary biology is whether reproductive isolation arises more frequently from divergent natural selection [e.g. ecological speciation (Schluter, 2001; Rundle and Nosil, 2005)] or genetic drift [e.g. founder effect speciation (Mayr, 1942, 1963)].

This dichotomy between selection- and drift-based speciation is a simplification, however, because the two evolutionary mechanisms interact in complex ways. Selection reduces effective population size and thereby increases the role of genetic drift in the fixation of alleles (Fig. 1). Conversely, genetic drift may drive maladaptive changes that undermine the effect of selection (Fig. 1). Under certain circumstances, this maladaptation may cause a population to shift off a local adaptive peak, thereby allowing natural selection to

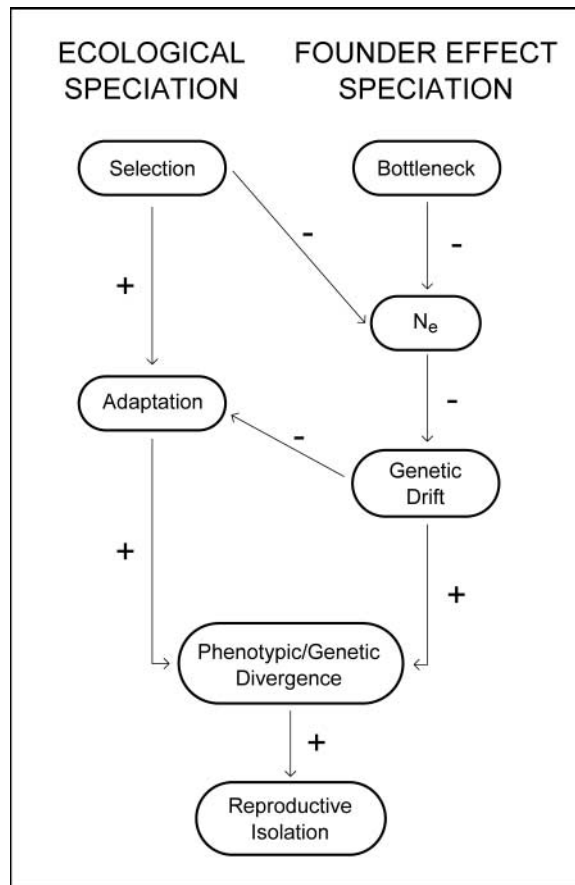


Fig. 1. A conceptual diagram of the fundamental entangling of selection and drift and their potentially interacting effect on reproductive isolation between populations.

drive populations towards more global optima [e.g. Wright's shifting balance theory (Wright, 1931, 1932)].

A number of laboratory evolution experiments have attempted to experimentally (Mooers *et al.*, 1999; Rundle, 2003) or statistically (reviewed in Rice and Hostert, 1993; Coyne and Orr, 2004; Fry, 2009) isolate the effects of selection and drift. Such experiments have found that natural selection may drive adaptive divergence between populations, pleiotropically generating reproductive isolation ('ecological speciation'). However, as noted above, such selection can also increase the rate of genetic drift in fixing deleterious alleles. Consequently, very strong natural selection can simultaneously drive and (through bottlenecks) undermine adaptation, while also promoting founder effect speciation. It may therefore be important to consider the interacting effect of selection and drift in speciation experiments, especially when selection is strong enough to drive strong founder effects.

Here, we present the results of an experiment in which we maintained populations of *Tribolium castaneum* flour beetles on an ancestral resource (wheat) and a suboptimal novel resource (corn). Corn is a nutritionally poor resource (Via, 1991; Agashe, 2009), imposing

extremely strong selection on beetle populations that caused most populations to go extinct within a few generations (Agashe *et al.*, 2011). We assayed the effect of this strong selection on reproductive isolation between the derived corn and ancestral wheat beetles, and found asymmetric pre-mating and post-zygotic reproductive isolation. Upon measuring relative fitness on corn and wheat, we also found a corresponding asymmetry in fitness. We speculate that these results arise from a strong maladaptive effect of genetic drift during the process of adaptation to survive on corn, combined with mate preferences for more fit genotypes.

METHODS

Experimental lines

The *Tribolium castaneum* populations described in this study were part of a previous, larger experiment to determine the impact of genetic variation on population dynamics and adaptation (Agashe, 2009; Agashe *et al.*, 2011). Populations were derived from wild-type strains obtained from the Beeman Lab (Biological Research Unit, Grain Marketing and Product Research Center, Kansas) reared strictly on wheat flour (~250 generations). We obtained a sample of ~50 individuals of each strain in April 2006, and reared them under ancestral conditions (organic white wheat flour + 5% yeast mixture at 33°C and 60% relative humidity). After 5 months, we extracted adults from these stock populations and initiated 42 populations of 120 beetles each, on a suboptimal novel resource, corn flour (Agashe, 2009). The corn populations had an average of 94% fewer individuals than populations in wheat during bimonthly censuses [mean $N = 21$ and 323 for corn and wheat populations, respectively (Agashe, 2009)]. All corn populations experienced an initial decline in population size, after which many (45%) went extinct within a year (Agashe *et al.*, 2011). Most of the surviving populations had high founding genetic variation (i.e. were initiated with individuals derived from multiple strains). Only two of the surviving populations were derived from single strains (one each of strains Pak-3 and Col-2), corresponding to the low genetic variation treatment. Individuals from these populations had increased fecundity, survival, and growth rate on corn flour relative to their ancestors (Agashe *et al.*, 2011).

Here, we focus on one of these surviving single-strain corn populations (Col-2) and its wheat-evolved counterpart (for logistical reasons, other wheat populations were terminated at the end of the original experiment described above). Of four replicate Col-2 corn populations, three went extinct during the original experiment described above. Individuals from the surviving population exhibited adaptation to corn after approximately 18 generations [median development rate was 6 weeks to reach adulthood (Agashe *et al.*, 2011)]. The surviving corn population and the wheat population from which it was derived were then maintained for an additional 3 years (~43 generations in total). At this point, we again assayed adaptation of corn-evolved and wheat-evolved beetles to each resource, and tested for reproductive isolation between these populations. We will refer to individuals as coming from either wheat-evolved (WE) or corn-evolved (CE) populations.

Note that because we had only a single surviving corn population, we did not have replicate CE populations and therefore could not assay whether reproductive isolation was stronger between the WE and CE populations than among either the WE or CE populations, as would typically be required in a test of ecological speciation (Coyne and Orr, 2004; Ostevik *et al.*, 2012). We therefore acknowledge that the results reported below cannot be

specifically ascribed to divergent selection, though this seems likely. Instead, we present a very extensive analysis of pre-mating and post-mating reproductive isolation between the two populations.

Tests of (mal)adaptation

If the surviving CE populations adapted to corn, we would expect that CE individuals reared on corn would have higher fecundity or survival than WE individuals reared on corn. Conversely, if genetic drift induced maladaptation, we might observe decreased mean fitness (survival or fecundity) over time within the CE line independent of the resource they are fed. Survival rates were recorded both for the original stock population (in 2006) and at the end of this experiment. We can thus measure (mal)adaptation as the change in survival over time, or the fitness of contemporary CE versus WE beetles.

To assay fecundity, 50 adults from each population (CE, WE) were placed in 80 g of either wheat or corn flour. After 2 weeks for mating and oviposition, adults were removed from each treatment and larvae were allowed to continue development until pupation. Pupae were placed in sex-specific containers (one pupa per gram of flour) and allowed to eclose over a period of 14 days. This ensured that all beetles used were virgins of similar age. We refer to beetles reared on wheat or corn as WR and CR respectively, and we factorially crossed evolved type with rearing type generating four treatments (CE/CR; CE/WR; WE/CR; WE/WR). Eclosed virgin males and females were then paired with individuals of the same ancestry and rearing environment (WE/WR, $n = 20$ pairs; CE/WR, $n = 14$; WE/CR, $n = 14$; CE/CR, $n = 27$) in 0.35 g of flour corresponding to the female's rearing flour (corn for CR, wheat for WR). Eggs were sifted from the flour and counted every 48 h for 8 days. We used a quasi-poisson generalized linear model (GLM) to test whether fecundity depends on evolved and reared flour types and their interaction.

To assay survival rate, we used individuals raised as described above, but did not factorially cross ancestral and parental rearing environment (i.e. treatments were CE/CR and WE/WR). Beetles were crossed with same-type individuals in 0.35 g of flour (12 pairs for each cross type) and reared in their native flour type. Eggs were sifted from the flour every 48 h until 20 eggs were obtained from each cross. Half of the eggs were individually isolated in wheat and half in corn, so that adult evolved type was factorially crossed with egg-rearing environment. After 10 weeks, we checked whether the eggs had survived to adulthood. We used a binomial GLM to test whether survival depends on evolved flour, offspring rearing flour, and the interaction between these. For all binomial GLMs in this study, we used AIC model selection before determining effect estimates and support. Except where noted, AIC supported retention of the full model, in which case we focus on reporting statistical significance of effects and do not report AIC results.

Pre-mating isolation (I)

We used a binary choice experiment in which a virgin adult female was given a choice between two 1.5-g flour piles placed on opposite sides of a 4-inch diameter petri dish. The bottom of the petri dish was lined with a piece of paper to give the female traction when moving around the arena. We used flour that was previously scented by males from the WE or the CE populations: males were placed in fresh media at a density of one male per gram of flour for 72 h during which time the flour accumulated olfactory cues from the males. The

males were sifted out of the flour prior to the mate-choice trials. To avoid the confounding effect of flour type on female choice, any given female was presented with male-scented flour using the female's rearing flour (corn for CR, wheat for WR). A single female was placed in the centre of the petri dish, half way between the two flour piles (1 cm, or about two beetle lengths, from each pile). Petri dishes were placed in an incubator (33°C, 60% relative humidity) for 24 h, after which we noted which pile of flour the female was found in. We tested females in four different blocks of approximately 30 females each. Trials in which females did not select a flour pile were discarded (31.9% of WE and 32.8% of CE). Individual males were not included in the mate choice arena because it is unclear what behaviours of females indicate true mate choice in *T. castaneum* (Fedina and Lewis, 2008), and males are sufficiently mobile that they might have switched flour piles. Instead, we chose the above design because it allowed the female adequate time to explore the chemical cues of both types of males. Note that this design does not allow for actual mating to occur. We used a chi-square analysis to test whether the probability of choosing either WE or CE male-scented flour differed from a ratio of 50% and whether WE and CE females differ in their choice.

Pre-mating isolation (II)

As described in the Results, the choice tests detailed above found that both CE and WE females preferred flour piles scented by WE males (an effect that is statistically significant in WE females and nearly so in CE females). To determine the mechanism underlying this asymmetric pre-mating isolation, we performed two additional choice tests. The asymmetric association of WE and CE females with WE males could occur if (1) females prefer WE males, (2) females avoid CE males, or (3) females orient towards males in general but fail to detect CE males. To distinguish between these alternatives, we conducted binary choice trials with single virgin females choosing between flour piles, as above, but altered the identity of beetles generating the scent in the two flour piles. In the following experiments, all 'choosers' were virgin WE females, since CE females were scarce and did not show a significant preference in part I. The following binary choice experiments were performed as described in the previous subsection (see Fig. 2, IIA and IIB):

- WE females chose between flour scented with CE females versus flour scented with CE males. If WE females have an aversion to CE males, they should move towards CE females more often than CE males. Altogether, 28.1% of trials were discarded because females were found in neither flour pile. Note that another possibility, that WE females are averse to CE genotypes of either sex, was ruled out because in another test WE females did not avoid CE females.
- WE females chose between flour scented with WE males versus fresh flour. If WE females gravitate towards males, but fail to detect CE males, then they should move towards WE males at the same frequency in this experiment as in Experiment I (choosing between WE and CE males). In total, 26.5% of trials were discarded because females were found in neither flour pile.

If we rule out both aversion to CE males and failure to detect CE males, then by process of elimination we will infer that WE females actively prefer WE male scent cues over CE male scent cues. We used a chi-square analysis to test whether the probability of choosing either

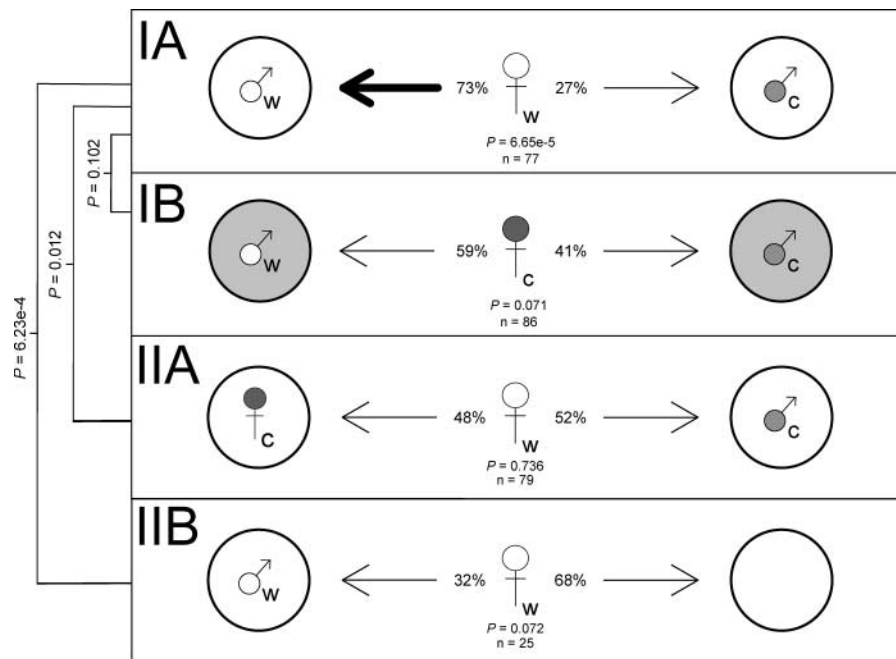


Fig. 2. Results of mate choice trials: (IA) mate preferences of WE females choosing between WE and CE males; (IB) mate preferences of CE females choosing between WE and CE males; (IIA) test of whether WE females are averse to CE males compared with CE females; and (IIB) test of whether WE females prefer WE males in the absence of an alternative male. For each test, the bold arrow indicates a significant preference. The proportion of females making each choice (out of females exhibiting a preference) is provided next to each arrow. Under each female we present the P -value from a chi-square test, and the number of females tested. The statistical significance of differences in choice between trials IA and IB, IA and IIA, IA and IIB are provided to the left side of the boxes. Grey (white) males represent corn (wheat) evolved lines. Grey and white backgrounds represent the flour type (corn and wheat, respectively) scented by the males.

flour pile was different from a ratio of 50% and whether WE females differed in their choice pattern in part I.

Pre-zygotic isolation

The pre-mating isolation assays did not involve actual male–female interactions or mating, and thus may not detect pre-zygotic isolation arising from behavioural interactions, copulation behaviour, or biased sperm usage by females. We therefore measured pre-zygotic isolation in a no-choice design that incorporated (but did not distinguish among) these isolating mechanisms. We performed a fully factorial experiment testing whether fecundity depends on the combination of male or female evolved type and male or female rearing environment. Both WE and CE males and females were reared for a single generation separately on wheat and corn, creating WE/WR, WE/CR, CE/WR, and CE/CR males and females. To create these beetles we used the same methods described previously (see p. 407). We paired individual males and females of all 16 factorial combinations of evolved and

Table 1. The mean fecundity (eggs laid per day) and standard error (s.e.) over 8 days of all 16 crosses testing for pre-zygotic isolation

Female type	Male type	<i>n</i>	Fecundity (eggs per day)	s.e.
WE/WR	WE/WR	20	6.22	0.47
WE/WR	CE/CR	20	5.68	0.37
WE/WR	WE/CR	14	5.77	0.62
WE/WR	CE/WR	20	5.87	0.43
CE/WR	WE/WR	21	4.80	0.40
CE/WR	CE/CR	19	4.86	0.45
CE/WR	WE/CR	17	6.33	0.44
CE/WR	CE/WR	21	5.89	0.33
CE/CR	WE/WR	26	3.12	0.23
CE/CR	CE/CR	27	2.24	0.19
CE/CR	WE/CR	23	2.05	0.21
CE/CR	CE/WR	21	2.42	0.27
WE/CR	WE/WR	21	3.14	0.33
WE/CR	CE/CR	19	2.61	0.36
WE/CR	WE/CR	14	1.88	0.30
WE/CR	CE/WR	15	3.08	0.47

rearing types of males and females (Table 1). Pairs were placed in 2-mL tubes with 0.35 g flour matched to the rearing environment of the female. Eggs were collected and counted every 48 h for 8 days. We used a quasi-poisson GLM to test how the number of eggs laid depends on the female's genotype, male's genotype, female rearing environment, male rearing environment, and all interactions. In general, pre-zygotic reproductive isolation should be revealed as an interaction between male and female genotype in which hybrid matings between evolved types produce fewer offspring than within evolved type matings. We also assayed adaptation by rearing the eggs to adulthood and tested whether egg survival is higher in wheat or corn flour depending on whether the sire or dam evolved on that flour (parental genotype \times rearing environment interactions).

Post-zygotic isolation

To test for post-zygotic isolation, we paired all combinations of evolved types and recorded the survival rate of their offspring on both types of flour. Beetles were removed from their evolved populations as pupae and separated by sex into containers with one beetle per gram of flour. Individuals were allowed 10 days to eclose, ensuring that all were virgins of similar age. Females of each evolved type were paired with WE or CE males, with 12 replicates of each of the four pairing combinations. All pairs were kept in 2-mL tubes filled with 0.35 g of flour. Flour type in the tubes matched the dam's rearing environment. After 48 h, mated pairs were removed and eggs were collected. Eggs were then individually isolated in 0.35 g of flour to prevent cannibalism. Half of the eggs from each collection were isolated in corn flour and half in wheat flour. Mated pairs were then placed back into fresh flour for another 48 h, after which the egg collection process was repeated. A total of 20 eggs were isolated from each pair, 10 placed in each flour type. After 10 weeks, the tubes were checked for the

presence of adults, larval remains, or pupal remains. Empty tubes were assumed to represent eggs that failed to hatch. We used a binomial GLM to test whether survival depends on female genotype, male genotype, the egg's rearing flour, and the interactions among these factors.

Lineage sex-biases

Our results from the post-zygotic isolation test reveal asymmetric male-dependent post-mating isolation (CE males sire fewer viable offspring). We hypothesized that this inviability might represent a paternally inherited (Y chromosome linked) inviability gene, which could lead to female-biased sex ratios in offspring. To test this, we factorially manipulated the rearing type of both evolved types. Five males and five females from each evolved type were placed in a petri dish with 10 g of either wheat or corn flour. Adults were removed after a week, leaving the F_1 generation eggs behind, which were allowed to develop to adulthood and then sexed (F_1 generation is thus WE/WR, WE/CR, CE/WR, and CE/CR). We used a binomial GLM to test whether a population's sex ratio depends on evolved and rearing type. We also used a chi-square analysis to test if sex ratios deviate from 50%, overall, and within each treatment group.

RESULTS

Tests of (mal)adaptation: fecundity

We found no difference between the fecundity of WE and CE females when both were reared on a wheat diet (quasi-poisson GLM: $\beta = 0.054$, $P = 0.570$) or when both were reared on a corn diet ($\beta = -0.178$, $P = 0.289$). In contrast, rearing diet (WR vs. CR) had a significant effect on fecundity ($\beta = 0.967$, $t = 8.501$, $P < 0.0001$); CR females laid an average of 2.01 eggs per day and WR females an average of 6.29 eggs per day. This is expected, since corn is a nutritionally poor resource compared with wheat (Via, 1991; Agashe, 2009; Agashe *et al.*, 2011). There was no interaction between evolved type and rearing flour ($\beta = 0.232$, $t = 1.203$, $P = 0.233$), implying a lack of local adaptation in fecundity by each population to the resource they evolved in.

Tests of (mal)adaptation: survival

Overall, comparing beetles that were reared on the same type of flour, WE and CE beetles did not differ in their survival to adulthood, although there was a non-significant trend towards higher survival of WE beetles reared in both wheat (binomial GLM: $\beta = 0.539$, $z = 1.702$, $P = 0.088$) and in corn ($\beta = 0.539$, $z = 1.702$, $P = 0.088$). The WE beetles had the same survival rate of $82.5 \pm 3.5\%$ (mean \pm S.E.) in both wheat and corn. Similarly, CE beetles had a survival rate of $73.3 \pm 4.0\%$ in both wheat and corn (standard error calculated before pooling between both medium treatments). There was no difference in survival between rearing diets ($\beta = -1.311^{-11}$, $z = 0.000$, $P = 1.000$). By pooling data between rearing environments, we found that CE beetles had a lower survival rate than WE beetles ($\beta = 0.539$, $z = 2.406$, $P = 0.016$).

Using the same methods, survival rates of the ancestral population in wheat and corn were recorded in 2006 just before the CE population was isolated. The ancestral population had a

$100 \pm 0.0\%$ survival rate in wheat and an $85.7 \pm 2.9\%$ survival rate on corn at that time (Agashe, 2009). We found that over time, individuals from the WE population had decreased survival on wheat (unpaired *t*-test: $t_{268} = 5.592$, $P < 0.0001$), and individuals from the CE population had decreased survival on corn ($t_{268} = 2.568$, $P = 0.012$). Both populations exhibited similar proportional declines in survivorship over their ~43 generations (a 14.5% reduction in survival on corn, a 17.5% reduction on wheat). In conclusion, the surviving corn population shows no clear adaptation to corn, despite having persisted after an initial population crash (and despite most other corn populations going extinct). Note that there previously was evidence for adaptation by the CE line (Agashe *et al.*, 2011), but this was lost after additional generations of evolution, so that by the end of the study we see a general decrease in survival in both WE and CE.

Pre-mating isolation (I)

The WE females given a choice between flour piles scented by WE or CE males showed a strong preference for those scented by WE males ($\chi^2_1 = 15.909$, $P < 0.0001$; Fig. 2 (IA)). The CE females presented with the same choice showed a non-significant preference for flour scented by WE males ($\chi^2_1 = 2.976$, $P = 0.071$; Fig. 2 (IB)). This represents evidence for asymmetric pre-mating isolation between divergent populations. There was no significant difference between the preference of WE and CE females for WE male flour piles ($\chi^2_1 = 2.679$, $P = 0.102$), so we cannot conclude that CE females exhibit less preference for WE males.

Pre-mating isolation (II)

In Experiment IIA, WE females showed no preference when given a choice between flour scented by CE males or CE females ($\chi^2_1 = 0.114$, $P = 0.736$, $n = 79$; Fig. 2 (IIA)). The proportion of WE females that chose CE males differed from WE females in test IA ($\chi^2_1 = 6.335$, $P = 0.012$). Thus, the previous evidence that WE females prefer WE males cannot be explained by an aversion to CE males.

In Experiment IIB, WE females showed a non-significant preference for fresh flour when the alternative was flour scented by WE males ($\chi^2_1 = 3.24$, $P = 0.072$, $n = 25$; Fig. 2 (IIB)). If WE females simply failed to recognize that CE males were potential mates, we would expect that this test would give the same result as in test IA in their preference for non-WE males. Instead, WE females in this test preferred rather than avoided the non-WE male-scented flour piles ($\chi^2_1 = 3.24$, $P < 0.0001$).

WE females might also prefer WE males due to a general aversion to CE beetles regardless of sex. We performed two additional pairwise tests to eliminate this possibility. The WE females were given a choice between fresh flour and flour scented with WE females, or fresh flour and flour scented with CE females. If WE females have a general aversion to CE beetles regardless of sex, we expect a greater proportion to choose fresh flour when the alternative is a CE female than when the alternative is a WE female. This was not the case: we found a non-significant trend in the opposite direction ($\chi^2_1 = 0.771$, $P = 0.380$).

Taken together, these experiments can be used to infer that females gravitate towards flour scented with WE males because they have a greater attraction towards WE males than CE males. However, the mechanism behind this preference cannot be deduced with the given data. Two possibilities are that CE and WE females retain the ancestral attraction to

Table 2. Test for pre-zygotic isolation

Pre-zygotic isolation (GLM with quasi-poisson correction)	Estimate	<i>t</i> -value	<i>P</i> -value
F_E	0.153249	1.003	0.31659
F_R	0.773257	5.860	1.21×10^{-8}***
M_E	-0.089497	-0.577	0.56405
M_R	0.078043	0.514	0.60739
$F_E \times F_R$	0.002758	0.015	0.98839
$F_E \times M_E$	0.087913	0.395	0.69332
$F_R \times M_R$	0.115635	0.618	0.53734
$F_E \times M_E$	-0.241950	-0.979	0.32836
$F_R \times M_E$	0.354873	1.849	0.06541
$M_R \times M_E$	0.341000	1.619	0.10656
$F_E \times F_R \times M_R$	-0.248019	-0.920	0.35828
$F_E \times F_R \times M_E$	-0.007196	-0.024	0.98059
$F_E \times M_R \times M_E$	0.009568	0.030	0.97645
$F_R \times M_R \times M_E$	-0.811998	-3.099	0.00212**
$F_E \times F_R \times M_E \times M_R$	0.503127	1.286	0.19952

Note: Fecundity of factorially crossed evolved type of males (M_E) or females (F_E) and rearing type of males (M_R) or females (F_R) was assayed (see Table 1 for fecundity of all crosses). Evolved and rearing types are either wheat or corn. We found a strong negative effect of rearing females on corn, and a three-way interaction term in which local males had decreased fecundity when females were raised on corn but this was reversed when females were raised on wheat. ** $P < 0.005$, *** $P < 0.001$.

some aspect of WE males, or that females have the ability to detect the general quality of males, and thus prefer WE males over less fit CE males.

Pre-zygotic isolation

Fecundities of all crosses are shown in Table 1, with a full model analysis in Table 2. We found no evidence of pre-zygotic reproductive isolation between the WE and CE populations, in the no-choice mating and fecundity assay (no significant male \times female evolved type interaction; $\beta = -0.241$, $t = -0.979$, $P = 0.328$). In another analysis, we found no significant reduction in fecundity comparing hybrid crosses (combining the reciprocal WE*CE crosses) to pure crosses (combining WE*WE and CE*CE), overall ($\beta = 0.034$, $t = 0.554$, $P = 0.580$) or in either environment separately ($\beta = 0.095$, $t = 1.175$, $P = 0.242$ in corn; $\beta = 0.028$, $t = 0.503$, $P = 0.616$ in wheat). Dam rearing environment had a strong effect on dam fecundity ($\beta = 0.773$, $t = 5.860$, $P < 0.0001$): mean fecundity of dams raised in wheat was 2.2 times that of dams raised in corn. We observed no significant pairwise interaction between rearing environment and evolved environment, of either the dam or the sire. However, we did find a significant three-way interaction between sire evolved type, sire rearing environment, and dam rearing environment ($\beta = -0.812$, $t = -3.099$, $P = 0.002$). To interpret this three-way effect, we categorized sires as being reared on their native (WE/WR, or CE/CR) or foreign resource (WE/CR, or CE/WR). We analysed a model that includes this 'nativeness' term as well as dam rearing environment. Sires raised on their native resource had higher fecundity than sires raised on their foreign resource ($\beta = 0.168$,

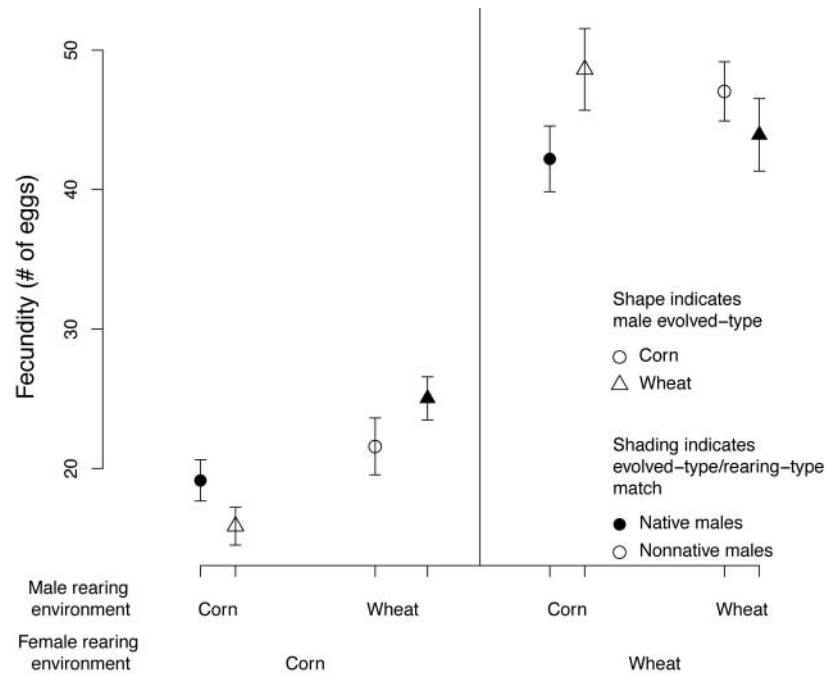


Fig. 3. We found a strong interaction effect between male rearing, male evolved, and female rearing types on fecundity. Points represent fecundity rates (# of eggs produced per female over 8 days) of combinations of crosses of evolved type and rearing environment. Bars indicate ± 1 standard error.

$t = 2.084$, $P = 0.038$), providing some evidence of local adaptation. This effect interacts with dam rearing environment ($\beta = -0.270$, $t = -2.753$, $P = 0.006$): sires raised on their native resource produced significantly more offspring when crossed with corn-reared dams; this effect was reversed (but not as strong) when native sires were mated with dams reared on wheat (Fig. 3).

Post-zygotic isolation

AIC model selection indicated that our full binomial GLM, examining egg-to-adult survival rate as a function of dam evolved type, sire evolved type, rearing environment, and all associated interactions, was over-parameterized; and that only rearing environment, sire evolved type, dam evolved type, and the interaction between sire and dam evolved type should be included. Using only these terms in the model, we found that a sire's evolved type was the largest determinant of offspring survival (Fig. 4, Table 3). CE fathers had reduced offspring survival ($\beta = 1.331$, $z = 4.903$, $P < 0.0001$). Dam evolved type did not have a significant effect on offspring survival ($\beta = -0.125$, $z = -0.611$, $P = 0.541$), but there was a marginally non-significant sire-type \times dam-type interaction ($\beta = -0.666$, $z = -1.899$, $P = 0.058$). This interaction reflects the observation that offspring survival was low for CE*CE crosses, and increased by the substitution of a WE male (CE*WE). On the other hand, WE*WE crosses had high offspring survival, and the substitution of a CE sire diminished survival. Averaging across hybrid genotype classes, survival is not significantly

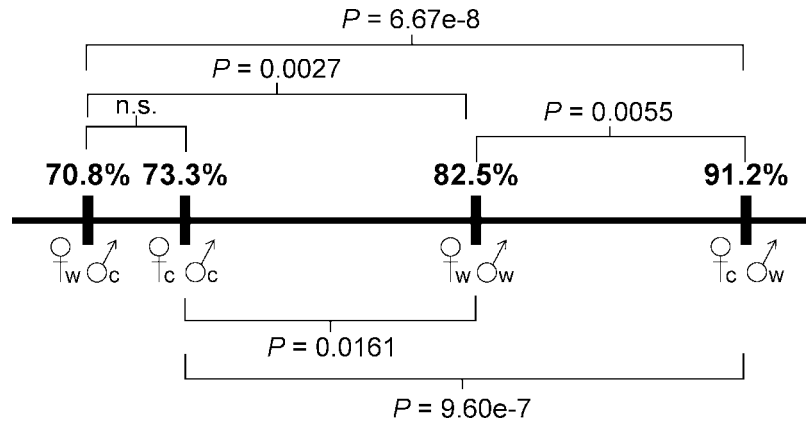


Fig. 4. Egg-to-adult survival rates of the four factorial crosses of wheat- and corn-evolved males and females. The offspring rearing environment was not a significant effect, so those treatments are pooled. The number line represents increasing offspring survival, from left to right. The cross types are indicated by female and male symbols marked with w or c to indicate the evolved environment. Statistical significance of comparisons between cross types is provided.

Table 3. Test for post-zygotic isolation

Post-zygotic isolation (binomial GLM)	Estimate	z-value	P-value
R	-0.255	-1.555	0.120
M	1.331	4.903	9.43×10^{-7}***
F	-0.125	-0.611	0.541
M \times F	-0.666	-1.899	0.058

Note: Males (M) and females (F) of wheat- or corn-evolved type were factorially crossed and their offspring were reared on either wheat or corn (R). We found a strong negative effect of corn males on offspring survival rates. *** $P < 0.001$.

different from the average of the pure types survivals, but this effect overlooks transgressive hybrid survivorship: of all cross types, CE*WE hybrids had the highest survival, and WE*CE hybrids had the lowest survival (Fig. 4), and the pure types were intermediate. Surprisingly, the medium in which offspring were raised on had no effect on survival ($\beta = -0.255$, $z = -1.555$, $P = 0.120$). We conclude that parental mating combination has a strong genetic effect on offspring survival, and that this post-mating isolation is highly asymmetric, in the same direction as the asymmetric pre-mating preferences (WE males are favoured over CE males).

Sex ratio bias

AIC model selection again indicated that our full binomial GLM was over-parameterized and the interaction between genotype and environment should be excluded. We found that CE populations were more female-biased than WE populations ($P = 0.016$). CE populations deviated from a 50% sex ratio ($44.7 \pm 2.1\%$ male; $\chi^2_1 = 6.050$, $P = 0.014$), while

WE populations did not ($51.7 \pm 2.2\%$ male; $\chi^2_1 = 0.6113$, $P = 0.434$). We also found a rearing diet effect. Beetles raised on corn were more female-biased than those raised on wheat ($P = 0.019$). Corn-fed beetles also deviated from a 50% sex ratio ($42.6 \pm 2.9\%$ male; $\chi^2_1 = 6.354$, $P = 0.012$), while wheat-fed populations did not ($50.3 \pm 1.8\%$ male; $\chi^2_1 = 0.021$, $P = 0.886$).

DISCUSSION

There is a long tradition of using laboratory experiments to test whether divergent selection and/or genetic bottlenecks induce the evolution of reproductive isolation (reviewed in Rice and Hostert, 1993; Coyne and Orr, 2004; Fry, 2009). Overall, this literature suggests that divergent selection on isolated populations can promote pre-mating isolation. Pre-mating isolation has sometimes appeared fairly quickly [e.g. 12 generations (Dodd, 1989); 5–11 generations (del Solar, 1966)] in studies that selected on non-sexual traits. Post-zygotic isolation has been tested only five times in laboratory studies of allopatric divergent selection. One such study showed extrinsic post-zygotic isolation (Dettman *et al.*, 2007) and another intrinsic post-zygotic isolation (Robertson, 1966). Reciprocal post-zygotic isolation was not found in the remaining three (Kilias *et al.*, 1980; Boake *et al.*, 2003; Kwan and Rundle, 2010). Support for founder effect speciation has been far more limited – while genetic drift can on occasion induce weak, temporary reproductive isolation, there is little evidence that drift alone can promote reproductive isolation (e.g. Powell, 1978; Rundle *et al.*, 1998; Rundle, 2003).

One limitation of previous laboratory speciation experiments is that most of them involved populations selected in opposite directions. We are aware of 21 laboratory experiments that tested for the allopatric model of speciation with divergent selection – 19 reviewed in Coyne and Orr (2004), in addition to Mooers *et al.* (1999) and Kwan and Rundle (2010). Six of these tested for pre-mating isolation between the derived and ancestral populations (Ehrman, 1969; de Oliveira and Cordeiro, 1980; Markow, 1981; Ringo *et al.*, 1985; Mooers *et al.*, 1999; Rundle, 2003), while the remainder focused on symmetrically divergent-selected populations. Divergent selection subjects both populations to roughly equally strong genetic drift and selection pressure, ending in roughly equal divergence from the ancestral phenotype on which mate preferences were previously based. In contrast, many real examples of speciation entail niche shifts by one population, e.g. when some members of an extant phytophagous insect species colonize a new host plant. In this case, there is an inherent asymmetry between the ancestral and derived population with respect to the strength of selection, the distribution of genetic substitutions, the strength of genetic drift, and similarity to the ancestral phenotype. To replicate this asymmetry, we examined reproductive isolation between a stock population that had been maintained in an essentially constant wheat flour environment for ~23 years, and a derived population subject to intense selection to persist in an unfamiliar corn flour environment for 60 months.

(Mal)adaptation in wheat- and corn-evolved populations

Our *Tribolium* populations exposed to a novel corn flour environment were subject to strong selection. All but one of the four populations maintained on the novel corn resource went extinct before the study began, and the surviving population had nearly two orders of magnitude (94%) lower population density than the otherwise similar wheat population (Agashe, 2009). Four months after the initial introduction to corn (with 120 adults), the

surviving population reached a minimum adult size of one individual, with 64 larvae. For the next 3 months, there were only two adults in the population and most larvae died. However, 20 months after initiation, this population had rebounded to 16 individuals.

There was clear evidence of adaptation to corn 2 years after population initiation, in the form of increased fecundity and egg survival relative to the start of the experiment, and increased preference for corn (Agashe *et al.*, 2011). Notably, these increases were restricted to the CE populations; WE lines exhibited no significant change in fecundity or egg survival (Agashe *et al.*, 2011). It is therefore clear that natural selection promoted adaptation in the CE population. Although there was a clear reversal of growth rate from negative to positive, this was not sustained and the population persisted at a much smaller size than its ancestral wheat-fed population.

The ancestral (WE) population exhibited reduced survival over the course of this experiment, presumably due to the fixation of maladaptive alleles. *Tribolium* populations are regulated by negative density dependence and therefore frequently undergo natural bottleneck events (Sokoloff, 1977) and variation in abundance (Agashe, 2009). However, beetles kept on wheat may have a greater ability to recover from these size reductions. After population initiation, bimonthly censuses showed that beetles kept on wheat consistently increased in population size, whereas those kept on corn immediately decreased (Agashe, 2009; Agashe *et al.*, 2011).

In contrast to our prior evidence for adaptation, 3 years later we found little evidence for corn adaptation in the same population examined by Agashe *et al.* (2011). The only result supporting local adaptation was a tendency for locally adapted males (WE/WR and CE/CR) to have higher fecundity than non-locally adapted males (WE/CR and CE/WR). Surprisingly, this effect depended on the female's rearing environment, but not the female genotype (Fig. 3). It is not immediately clear why females fed corn should lay more eggs when mated with a locally adapted male (WE fed wheat or CE fed corn). The trend is weakly reversed for wheat-fed females. Maternal effects could play a role in these fecundity measurements, especially when beetles were raised on non-native diets. This could, for instance, be reflected in an evolved-type effect in which WE beetles have higher fecundity since the positive effects of a wheat diet could be transferred to offspring. However, we do not find such an effect for either males or females, eliminating the possibility of maternal effects of diet.

The relative fecundity advantage of pure-crossed lines on their local environments – $([W_{\text{local}} - W_{\text{non-local}}]/W_{\text{total}})$ (Hereford, 2009) – was positive but small for both CE and WE types (17.8% and 5.4%, respectively). Positive mean local advantages for both types could indicate evolved fitness trade-offs, but we found no significant interaction between evolved type and rearing environment of pure crosses. The relative viability advantage was similarly small and positive for WE beetles (11.8%), but negative for CE beetles (–11.8%), indicating maladaptation to their local environment. By contrast, Hereford (2009) found an average magnitude of local adaptation of 45% in a meta-analysis of 777 estimates of local adaptation from 74 reciprocal transplant studies, and 21% of these studies reported a significant negative estimate of local adaptation. Local maladaptation is thus not uncommon in nature and could be important in understanding the evolution of mate preference.

In all other respects, we found no support for adaptation by CE beetles. The lack of evolved type \times rearing environment interaction terms suggests that CE lines were no better at using corn than WE lines, and instead CE individuals were generally less viable. Thus, the adaptation observed after 2 years of selection had been erased and even reversed (in terms

of survival). It is surprising that the final CE population should be under even greater risk of extinction in corn than it was after its initial introduction to the novel resource when the other replicates went extinct. How this population has managed to persist is not immediately clear. One possibility is that all the replicates had mean absolute fitness less than 1, but stochastic demographic variation allowed this one population to persist even as its absolute fitness declined still further. Alternatively, our measures of fitness may not be suitable to detect certain adaptations to corn, such as some forms of density- or frequency-dependent selection that were not accounted for in our fitness assays where beetles were isolated individually or in small groups.

Lower survival of the CE than WE individuals could, in principle, be explained either by the evolution of decreased survival over the final 3 years on corn, or by increased survival in the WE line. The latter explanation can be ruled out by comparing the survival rates of the founding population to the wheat- and corn-evolved lines 60 months later: both evolved lines exhibit reduced survival compared with their ancestral state. Consequently, we conclude that the lower fitness of CE lines reflects real maladaptation. We hypothesize this maladaptation reflects the effects of sustained bottlenecks in both the WE and especially the CE populations, which eroded the adaptation previously observed in the CE populations. As discussed below, it appears likely that this reduced survivorship is a result of a paternally inherited male-killing allele. In the context of the outline in Fig. 1, we suggest that the effect of natural selection was transiently beneficial (allowing the one surviving corn population to persist), but ultimately these same selective factors resulted in the fixation of maladaptive alleles through drift.

Reproductive isolation between wheat- and corn-evolved lines

Both strong selection and founder effects may play an important role in the evolution of populations during niche shifts (e.g. when insects shift to a new host plant), and laboratory experiments described above suggest that both evolutionary forces (especially selection) can promote reproductive isolation between populations. We know of two experiments that independently manipulated both selection and effective population size (through bottlenecks), to test their relative effects on reproductive isolation (Mooers *et al.*, 1999; Rundle, 2003). Neither study found assortative mating by female flies, but both studies found that both treatments reduced male mating success. No evidence of genetic change due to adaptation in the novel habitat was shown in either experiment.

Unlike these experiments, we found evidence of both adaptation (population persistence and initial increased fitness on corn) and maladaptation (reduced survival of both the wheat and especially corn populations). We therefore cannot separate the effects of drift and selection on reproductive isolation, because (as is likely true in nature) the two processes are inherently entangled. Furthermore, without replicate CE and WE populations, we are unable to contrast reproductive isolation between replicate populations between versus within habitat types. Such comparisons are necessary to statistically separate the effects of drift (which should equally generate isolation among populations of the same or different ecotype) versus selection (isolation between ecotypes). With these caveats in mind, we believe our results are consistent with a model in which asymmetric pre-mating isolation arises because of asymmetric drift-induced maladaptation. This conclusion rests on a comparison of female preferences, with the fitness of their resulting offspring (post-zygotic isolation).

Asymmetric post-zygotic isolation in nature has received considerable attention both theoretically (Turelli and Moyle, 2007) and empirically in field surveys (Tiffin *et al.*, 2001) and experiments (e.g. Coyne and Orr, 1989; Presgraves, 2002; Dettman *et al.*, 2003; Bolnick *et al.*, 2008). Unfortunately, relatively few studies of divergent-selected laboratory populations have assayed for post-zygotic isolation. Four of the 21 allopatric experiments mentioned previously tested for this isolating barrier (Robertson, 1966; Kiliyas *et al.*, 1980; Boake *et al.*, 2003; Kwan and Rundle, 2010), plus another that only tested for post-zygotic isolation (Dettman *et al.*, 2007). Of these five tests, two found post-zygotic reproductive isolation between divergently selected lines (Robertson, 1966; Dettman *et al.*, 2007). Two additional studies found reduced numbers of hybrid offspring (de Oliveira and Cordeiro, 1980), although this may have been from sexual selection rather than post-zygotic isolation (Coyne and Orr, 2004). In our experiment, we found no overall hybrid inviability (averaging across reciprocal cross directions). However, considering the reciprocal crosses separately, we found strong asymmetric reproductive isolation. The WE females suffered a 14% reduction in offspring survival when they mated with CE males, as opposed to males from their own population. In contrast, CE females gained a 24.4% increase in offspring fitness when hybridized with WE males. This asymmetry apparently occurs because WE males confer on average higher offspring survival than CE males (Fig. 4), an effect that is appreciably stronger for CE females.

We hypothesize that CE males have accumulated deleterious Y-linked substitutions, most likely as a result of strong genetic drift, which reduce their offspring survival. Deleterious X-linked and autosomal alleles would be inherited from both female and male corn parents, which is not consistent with our data. If our hypothesis were true, we would expect the deleterious Y-linked alleles to reduce the viability of male offspring, leading to populations with a female-biased sex ratio. Our test for sex ratio bias confirmed this expectation: CE populations were female-biased regardless of rearing environment. The observed female-biased sex ratio of 0.553 in the CE populations can be explained if female survival rate is equal to 1.237 times the survival of males (or, equivalently, male survival = $0.808 \times$ female survival). Averaging across both sexes, the survival rate of populations exhibiting this biased sex ratio due to male mortality should be 90.4% as large as in a population without male-biased mortality. We found that WE populations, not affected by the sex ratio bias, had a survival of 82.5%. Multiplying this baseline survival rate by 0.904 leads to the prediction that male-biased mortality in corn lines should generate an overall survival rate of 74.6%. This estimate closely matches our observed CE survival rate of $73.3 \pm 4.0\%$. It is therefore plausible that a deleterious male-killing gene in the CE population is sufficient to explain the maladaptation in the CE line. Note that the male-biased mortality may be a deleterious allele resulting from drift, but it is also possible that corn selects for a biased sex ratio (for unknown reasons). The latter possibility is suggested by our finding of a strictly environmental effect of corn on beetle sex ratio: CR populations had only 42% males regardless of which evolved type was used. If males are especially affected by a corn diet, selection may favour increased frequency of a male-killing allele to reduce competition against female kin. This is speculative, and an experiment considering density- or frequency-dependent fitness effects would be necessary to support this hypothesis, as these effects would not have been detected in our fitness assays.

Asymmetric reproductive isolation and the drift/selection dichotomy

Both pre- and post-zygotic reproductive isolation were asymmetric in our study. WE females (and to a lesser degree CE females) were more attracted to WE males, and their offspring survived better when they mated with WE males. Females thus preferred the male type that conferred higher offspring fitness. This outcome suggests that asymmetric pre-mating isolation could be a side-effect of asymmetric male quality. We therefore propose a model to explain our results: (i) strong selection to persist on corn ultimately led to low population size but at least temporarily allowed population persistence, (ii) the resulting genetic drift facilitated the spread of Y-linked deleterious allele(s), which (iii) reduced the fitness of the corn population and in particular the genetic quality of corn males, (iv) leading to asymmetric pre-mating bias for wheat males. We emphasize that although this model is entirely consistent with our data, it remains speculative and is not the only possibility. Kaneshiro (1980) proposed that populations subject to bottlenecks may stochastically lose alleles conferring male attractiveness, in which case asymmetric pre-mating isolation would be independent of male fitness. There is support for some of Kaneshiro's predictions from a meta-analysis of laboratory studies in which derived populations (with and without induced selection) were tested against their ancestral populations for pre-mating isolation (Ödeen and Florin, 2002). This study revealed that derived males tended to have lower mating success in choice tests with ancestral males. Other mechanisms to explain asymmetric isolation have been proposed, such as conserved female preferences despite male trait divergence (Schwartz *et al.*, 2010) and behavioural differences in mating strategies (Luan and Liu, 2012). However, our model specifically pertains to the common scenario in which the asymmetric advantage is tipped towards the ancestral population, and there is little or no gene flow between populations. Stronger evidence for our proposed model would require replication of the wheat and corn lines, direct measures of effective population size and the effects of drift, and experimental evidence that pre-mating isolation depends on females' assessment of males' breeding value for offspring viability (or some correlate thereof).

As in our experiment, pre- and post-zygotic reproductive isolation are often asymmetric in nature. Asymmetric hybrid inviability is apparently due to the fixation (by drift or selection) of deleterious alleles on uniparentally inherited genetic elements (Turelli and Moyle, 2007; Bolnick *et al.*, 2008). Given the frequency of asymmetric reproductive isolation in nature, we suggest that more experiments and theory are needed to understand the evolutionary and genetic basis of these asymmetries. It would be especially valuable to know whether these asymmetries are more likely to arise from selection, drift, or a complex interaction between these two processes. We propose that (i) asymmetries are more likely to arise when selection (and drift) are themselves asymmetric, as in the present study or in natural cases of speciation via host- or niche-shift, and (ii) that asymmetric fitness generates asymmetric pre-mating isolation.

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