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ROLE OF SEX AND MIGRATION IN ADAPTATION TO SINK ENVIRONMENTS

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Understanding the effects of sex and migration on adaptation to novel environments remains a key problem in evolutionary biology. Using a single-cell alga *Chlamydomonas reinhardtii*, we investigated how sex and migration affected rates of evolutionary rescue in a sink environment, and subsequent changes in fitness following evolutionary rescue. We show that sex and migration affect both the rate of evolutionary rescue and subsequent adaptation. However, their combined effects change as the populations adapt to a sink habitat. Both sex and migration independently increased rates of evolutionary rescue, but the effect of sex on subsequent fitness improvements, following initial rescue, changed with migration, as sex was beneficial in the absence of migration but constraining adaptation when combined with migration. These results suggest that sex and migration are beneficial during the initial stages of adaptation, but can become detrimental as the population adapts to its environment.

KEY WORDS: **Evolutionary rescue, experimental evolution, migration, sex, source-sink dynamics.**

A species' range is a reflection of its ecological niche (Sexton et al. 2010), defined by the range of environments where its birth rate is higher than its death rate and where a viable population can be sustained. At the edges of its range, a species is exposed to environments where its fitness is reduced, even to the point where its survival is threatened (Sexton et al. 2010; Geber 2011). Well-established species ranges are becoming increasingly threatened by climate and other anthropogenic changes (Geber 2011). Studying how a population adapts to marginal environments is of key interest to further understand how biodiversity loss might be slowed (Lavergne et al. 2010). Although populations in marginal habitats might have a limited ecological role due to their relatively low sizes (Kawecki 2008), they can be crucial from an evolutionary perspective as phenotypes selected under those conditions often drive adaptation to other novel environmental extremes (Ackerly 2003).

The evolutionary events occurring at species margins have often been studied using source-sink models (Pulliam 1988; Holt and Gomulkiewicz 2004). In a source-sink scenario, source environments are those to which a species is well adapted, such that the population growth rate is enough to maintain a viable population. Individuals inhabiting source environments experience relatively high fitness, which is maintained by stabilizing selection. In contrast, sink environments are those to which the species is not currently well adapted, and the rate of population growth does not exceed the rate of death. Individuals in sink populations are subject to directional selection and in the absence of other forces will go extinct. Populations can, nevertheless, be maintained in sink environments if immigration of individuals from source populations is enough to counterbalance the loss of individuals from the sink environment (Kawecki 2008). However, incorporating the sink environment into a species' niche requires evolutionary change. Initially the population must adapt to the level where it can maintain a viable population in the sink environment in the absence of immigration, a phenomenon called evolutionary rescue (Gonzalez et al. 2012). This viable population may then become further adapted, under continued selection. Determining the factors that favor and limit the ability of species

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to expand into sink environments is central to our understanding of the evolution of a species niche breadth and range expansion, as well as having implications for how species will cope with environmental change.

Although patterns of migration and dispersal play a crucial role in keeping species' ranges fluid and constantly changing (Brown et al. 1996; Gaston 2003), they can have contrasting effects on the rates of evolutionary rescue (Bell and Gonzalez 2011). On one hand, migration load can mean that immigration from a source into a sink population reduces the absolute fitness of the sink population, by swamping it with alleles that are beneficial in the source environment, but deleterious in the sink (LoFaro and Gomulkiewicz 1999; Kawecki and Holt 2002; Kawecki and Ebert 2004). On the other hand, immigration can facilitate adaptation to a sink environment by increasing beneficial mutation supply (Holt 2003; Sexton et al. 2010). The increased supply of beneficial mutations can arise either because immigration will maintain a larger population in the sink environment increasing the amount of beneficial mutations that arise in situ, or because immigrants may bring in beneficial mutations from the source population. The latter effect will depend on the pattern of genotype-by-environment interaction in the mutations involved in adaptation to the sink environment (MacLean et al. 2010). For example, if mutations beneficial in the sink environment are associated with a pleiotropic fitness cost in the source environment, as often observed for antibiotic (Andersson and Hughes 2010) and pesticide (Vila-Aiub et al. 2009) resistance mutations, then these will be at a low frequency in the source population, and the flow of beneficial mutations from the source population is likely to be minimal.

Another factor, which may have important consequences for the rate of evolutionary rescue, is recombination. The effect of sex on the rate of adaptation to a novel environment has been explored both theoretically (Hadany and Comeron 2008; Otto 2009; Hartfield and Keightley 2012) and experimentally (Colegrave 2002; Kaltz and Bell 2002; Goddard et al. 2005; Cooper 2007; Becks and Agrawal 2010, 2012). In situations where adaptation depends on beneficial mutations at multiple loci, sex can increase the rate at which individuals carrying multiple beneficial mutations first appear in a population, by bringing together beneficial mutations that have occurred in different lineages (mutation assembly; Fisher 1930; Muller 1932). Sex can also enhance rates of evolutionary rescue by separating a beneficial mutation from a "bad" genetic background (Peck 1994). Finally, sex can affect the rate at which these mutations fix in the population, but the effect depends on the patterns of linkage disequilibrium between mutations involved in adaptation (Otto and Lenormand 2002). When linkage disequilibrium is negative, recombination increases the genetic variance for fitness and the response to selection (Eshel and Feldman 1970). In an asexual population, negative disequilibrium will be generated by selection if beneficial mutations are very rare, such that adaptation is proceeding through competition among lineages carrying different beneficial alleles. Even if beneficial mutations are common, such that individuals carrying multiple beneficial mutations are present in the population, selection can also generate negative linkage equilibrium if there is negative epistasis among these mutations in their effects on fitness (Otto and Gernstein 2006). In contrast, when there is positive epistasis among mutations, selection will generate positive linkage disequilibrium. In this situation, sex has the opposite effect, reducing the genetic variance for fitness as it brings the population closer to linkage equilibrium, slowing the response to selection as a consequence (Eshel and Feldman 1970).

Migration and sex may also interact to affect the rate of rescue of a population in a sink environment (Pylkov et al. 1998; Lenormand and Otto 2000). Benefits of sex for rates of adaptation require multiple beneficial mutations to be segregating within a population (Fisher 1930; Muller 1932). As a result of this, immigration might enhance the effects of sex, at least in the early stages of adaptation, by increasing the supply of beneficial mutations. However, as populations become better adapted to the sink environment, immigration will tend to generate positive linkage disequilibrium for fitness loci, with combinations of beneficial mutations being present in individuals from the adapting population but not among the individuals from the source. Recombination will reduce this linkage disequilibrium, breaking up the complexes of beneficial mutations that have been assembled by selection while simultaneously breaking up maladapted genotypes from the source population (Lenormand and Otto 2000; Otto and Lenormand 2002). The effect of this will be to reduce genetic variance for fitness and consequently the efficiency with which selection can fix the beneficial mutations and purge the deleterious ones (Otto 2009).

Many experimental studies have demonstrated the positive effects of migration on the ability of a population to adapt to a sink habitat (Morgan et al. 2007; Perron et al. 2007; Bell and Gonzalez 2011; Ching et al. 2012). Similarly, the benefit of sex in increasing the rate of adaptation to novel environments has been experimentally demonstrated (Colegrave 2002; Goddard et al. 2005; Becks and Agrawal 2012). However, the interaction between sex and migration on the evolutionary rescue of populations to sink environments has not, to our knowledge, been tested experimentally. Given that the majority of eukaryotes are sexual, examining the interaction between these factors is critical for our understanding of adaptation to sink environments.

We addressed whether sex and migration interact and affect the ability of populations to adapt to sink environments, using the single-celled, facultatively sexual chlorophyte *Chlamydomonas reinhardtii* as a model organism. Under standard laboratory culture conditions, *C. reinhardtii* reproduces asexually,

however, when starved of nitrogen the two mating types (+ and −) differentiate into gametes which pair and fuse to produce a diploid zygote. The zygotes undergo meiosis and hence recombination, producing four haploid daughter cells (Harris 2008). These facultatively sexual characteristics make *C. reinhardtii* a useful model to study the evolutionary implications of sex (Colegrave 2002; Colegrave et al. 2002; Bell 2005). We created source-sink dynamics by exposing populations of *C. reinhardtii* to three growth-inhibiting herbicides that acted as sinks, and controlled the level of immigration from a wild-type source population into these sinks. We also manipulated the rate of sexual reproduction. This allowed us to observe the short- and long-term effects of migration and sexual reproduction, and to explore whether the two factors interacted to affect rates of evolutionary rescue and fitness.

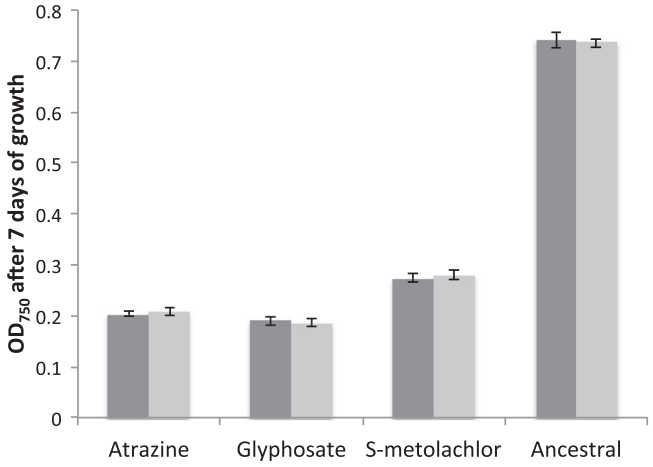

Figure 1. OD_{750} of source (ancestral) populations after seven days of growth in atrazine, glyphosate, S-metolachlor, and the ancestral environment (BM). Darker bars are measurements taken prior to selection procedure, when the dose of herbicides to be used had been determined. The lighter bars are measurements taken after the selection procedure, when evolved populations were assayed for their fitness in the presence of herbicide. Error bars are standard errors of the mean.

Materials and Methods

FOUNDING POPULATIONS

Base populations were created by extracting 12 spores (six mating type plus and six mating type minus) from a genetically diverse ancestral population and using these spores to found 12 genetically homogeneous lines. The ancestral population was originally initiated by mixing 14 standard laboratory strains of *C. reinhardtii* CC-1373, CC-1952, CC-2342, CC-2344, CC-2931, CC-1690, CC-1691, CC-2343, CC-2932, CC-2935, CC-2936, CC-2938, obtained from the *Chlamydomonas* Resource Center; along with Sammlung von Algenkulturen (SAG) 32a and SAG 32b from the SAG culture collection. Prior to extracting the spores, the ancestral population was put through two cycles of sexual reproduction.

Six "sexual populations" were then founded by mixing pairs of these lines of opposite mating type, with different lines being used for each population. Two lines were used to minimize the amount of genetic variation present in these base populations, within the constraint that sexual reproduction in *C. reinhardtii* requires two genotypes of opposite mating types (Harris 2008). By limiting starting diversity, we aimed to ensure that the majority of any adaptive response was based on novel mutations arising during the experiment, rather than being strongly influenced by the particular sample of standing variation present at the beginning of the experiment. Six "asexual populations" were founded in the same way, but with the pairs of genotypes having the same mating type. *Chlamydomonas reinhardtii* cells cannot switch mating type, and both mating types are required for sexual reproduction to occur, so these asexual populations are unable to reproduce sexually. One hundred microliters of each population was inoculated into 20 ml of fresh media and allowed to grow for seven days prior to the start of selection procedures. These populations were used as source populations for immigration during selection procedure.

CULTURE CONDITIONS AND HERBICIDES

All experiments were conducted in 20 ml of modified Bold's medium (subsequently BM), and the growth conditions were the same as in Lagator et al. (2012). Cultures were propagated every seven days (see below), by which time an ancestral (source) population growing in the absence of herbicides would have reached stationary phase (3.1×10^7 cells/20 ml). Three herbicides were used: atrazine (photosystem II inhibitor), glyphosate (EPSP synthase inhibitor) and S-metolachlor (inhibitor of very long chain fatty acids). These herbicides are known to inhibit growth of *C. reinhardtii*. Prior to the start of the selection procedure, the growth rate of all founding populations was tested at a range of concentrations of each herbicide. The herbicide dose for sink environments (0.09, 65, and 0.75 mg/l for atrazine, glyphosate, and S-metolachlor, respectively) was determined such that ancestral populations grew at only 70% of the rate that they could achieve in Bold's media (Fig. 1). Under our weekly serial transfer regime, this growth rate is not enough to maintain a constant population size and should lead to extinction within five to eight weeks in the absence of either migration or evolutionary rescue. The point of evolutionary rescue was defined as the first week when the population growth became positive (as further explained in the statistical analyses section). We also determined the minimum inhibitory concentration (MIC) of each herbicide (0.125, 95, and 1.1 mg/l for atrazine, glyphosate, and S-metolachlor, respectively), this

being the concentration that inhibited measurable growth after four days following inoculation with 125,000 cells into fresh media.

SELECTION PROCEDURE

Twelve experimental populations (six sexual and six asexual) were selected in three herbicides at three different rates of immigration—no, low, and high immigration (total of 108 evolving populations). All 12 founding populations were also propagated in BM in the absence of herbicides and transferred into fresh media every seven days. These unselected populations acted as "source populations" during the study as well as providing control populations to account for potential adaptation to laboratory culture conditions without the imposed selection pressure during the course of the selection experiments. During periods of asexual growth, transfers into fresh media containing appropriate herbicides were carried out every seven days, at the end of which the number of cells in each population was estimated by measuring optical density at 750 nm (OD_{750}).

In the populations without any immigration (nomig), 200 μl of each evolving population was transferred into fresh media at each transfer. In low immigration (lowmig) populations, 150 μl was transferred from the evolving population and 50 μl from the corresponding source population, whereas in high immigration (highmig) populations, 100 μl came from the evolving and 100 μl from the corresponding source population. At the first transfer, immigrants from the source were responsible for approximately 55% and 80% for lowmig and highmig regimes, respectively, of the cells transferred into fresh media. Populations experienced a rotation consisting of two cycles of asexual reproduction followed by a sexual cycle over the course of the selection experiments.

To initiate the sexual cycle, 7 ml of each of the evolving populations was transferred into separate 15 ml falcon tubes and centrifuged for 6 min at 4000 rpm. The BM supernatant was then removed and the pellets were re-suspended in 3 ml of ddH_2O and maintained under lights without shaking for 24 h to induce starvation that elicits sexual mating. At this point, mats of zygotes were clearly visible on the surface of the H_2O in the sexual populations, and were removed and placed on solid BM plates containing 1.5% agar. The asexual lines were exposed to the same mating protocol as the sexual lines, to account for potential effects that starvation could have on mutation rates (Goho and Bell 2000), but 300 μl of the H_2O containing a suspension of unmated cells was placed on solid BM. All plates (for sexual and asexual populations) were incubated at 25°C in the dark for five days so that the zygotes in the sexual populations would mature, and were then placed in light for two days so that zygotes would germinate. The number of zygotes present was very large, meaning there was no significant bottleneck in population size. Cells from each plate were placed back into liquid BM using a sterile loop and allowed to grow for six days to increase population size, at which time their OD_{750} was estimated prior to transfer into BM containing the appropriate herbicide. We transferred a number of evolving and immigrant cells that was approximate to the number of cells that would have been transferred if the sexual cycle had not been carried out, so that the sexual cycle had no direct effect on the demographics of the evolving populations. The experiment was carried out for 26 asexual (12 sexual) cycles. After every sexual cycle, the populations were put onto agar slopes containing Bold's media with 1.5% agar and left under dim lighting for long-term storage. Under such conditions, minimal growth occurs and the potential for evolutionary change is limited over the timescales of our study (Collins and De Meaux 2009). By week 14 (after the seventh sexual cycle), any populations that had not been rescued by adaptation would have gone extinct. We call the populations preserved on 1.5% agar slopes at week 14 of the selection procedure "early" resistant populations. The fitness of these "early" resistance populations was compared to fitness of populations at week 26 at the end of the experiment (see below).

FITNESS OF SOURCE POPULATIONS

To determine that any adaptation we see in the selected environments was due to adaptation to that environment rather than general laboratory adaptation, we measured the growth rates of source populations at the end of selection procedure in the ancestral environment (BM) and in the presence of each of the herbicides. These growth rates were compared to the growth rates of source populations at the beginning of the selection procedure, when herbicide doses to be used were determined.

FITNESS ASSAYS

When selection experiments were completed (after 26 weeks), we measured fitness in the presence of the appropriate herbicide of populations that had been maintained on agar slopes since week 14 ("early" populations) and the "final" population (week 26). Populations after asexual week 14 (seventh sexual cycle) were used for assessing fitness, as this was the first time point when all populations would have gone extinct without evolutionary rescue. The experiment ran for 26 asexual weeks, which is approximately 100 generations after week 14. Fitness was only measured for those populations that had undergone evolutionary rescue (i.e., populations that exhibited positive growth rates and did not go extinct). Prior to conducting these fitness assays, all rescued populations were transferred into fresh culture containing only BM and were grown for seven days. To measure fitness, the populations stored on agar slopes after asexual week 14 were inoculated into 20 ml of liquid Bold's media and grown for seven days. A total of 125,000 cells of each population (both early and final) were transferred into fresh media containing MIC of the appropriate herbicide, and the fitness in the presence of herbicide

was estimated as the OD_{750}, a measure of cell density, after seven days of growth. Each assay was repeated twice.

STATISTICAL ANALYSES

Rates of evolutionary rescue

To analyze the dynamics of evolutionary rescue (rates of resistance evolution), we carried out proportional hazard nonparametric survival analysis (rphfit function in Genstat, 15th edition). We modeled the weeks to rescue (first week when rescue was observed) for each population as a response, with sex (two levels—presence or absence), migration (three levels), and the interaction between the two terms, as fixed factors. We included the source population (which source population the evolving population has been inoculated with) as a random factor nested within sex. Due to the U-shaped nature of population growth over the course of the experiment arising from an initial decrease in population size prior to evolutionary rescue (emergence of resistance), determining the week to rescue was not straightforward. To determine this, we used a series of linear contrasts assessing the slope of the linear regression over three consecutive weekly OD_{750} measurements (the first for weeks 1–3, second for weeks 2–4, and so on) for each population, similar to Lagator et al. (2013). The middle point of the linear contrast (week 2 for contrast of weeks 1–3, for example) with the first significantly positive slope was used as the estimate of the "week to rescue." To explore whether sex and migration had the same effect in all three environments, the interaction between each of the two factors and the herbicide environment was also included as a fixed factor. As we identified an interaction between herbicide environment and sex, a follow-up analysis consisted of separately analyzing the rates of evolutionary rescue for the populations selected in each herbicide in the manner described above.

Fitness of source populations

We also tested if the fitness of the source populations in both the ancestral environment and in the presence of herbicides at MIC changed through the course of the selection procedure. To do this, we conducted a pairwise *t*-test between the measurement of the number of cell divisions in the ancestral environment of the source populations at the beginning and at the end of the selection procedure. The same test for the source populations was performed for the number of cell divisions in the presence of each of the herbicides.

Fitness

We analyzed the effects of "sex," "migration," and "time" on fitness in the presence of the MIC dose of each herbicide by modeling "OD750 at transfer (day 7)" as our response variable using the aov function in "R" 2.15.0 statistical package. The model fitted sex, migration, and time ("time" is a factor differentiating between the "early" and "final" evolved populations of the same regime) as fixed factors. The model also tested for the effects of a three-way interaction between them, as well as two-way interactions between sex and time, between migration and time, and between sex and migration. The interactions between sex and time, and between migration and time, tested whether the effects of sex or migration, respectively, differed between early and final evolved populations. The model included two random factors, source and line. "Source" describes which source population the evolving population has been inoculated with (and in the case of migration regimes, which source population is used for immigration). It was nested within the factor "sex." "Line" accounts for each population being measured at two time points (after week 14 and week 26). It was nested within base × migration, with base nested within sex.

Results

EVOLUTIONARY RESCUE

Evolutionary rescue was eventually observed in the majority of experimental populations (Table 1; Fig. 2). It was observed in all populations experiencing immigration, and in all but two populations with no immigration exposed to atrazine (one sexual and one asexual), one asexual population in glyphosate, and four populations in S-metolachlor (two sexual and two asexual). We identified a significant interaction between sex and herbicide environment ($z = 2.95$, $P < 0.05$), indicating that the effects of sex varied across environments, which can be attributed to the lack of effect of sex on rates of rescue in glyphosate ($z = 1.45$, $P = 0.15$). We identified a significant effect of sex on rates of rescue for populations selected in atrazine ($z = 2.48$, $P < 0.05$) and S-metolachlor ($z = 3.34$, $P < 0.001$; Fig. 2A). Migration also had a positive effect on rates of rescue (Fig. 2B), as populations not experiencing immigration were rescued through resistance significantly more slowly than those experiencing low ($z = 3.29$, $P < 0.001$) and high ($z = 4.47$, $P < 0.001$) levels of immigration. There were no significant differences in the rates of rescue between the two immigration regimes ($z = 1.47$, $P = 0.14$). We did not identify a significant interaction between sex and migration on the rates of rescue ($z = 1.19$, $P = 0.27$).

FITNESS OF SOURCE POPULATIONS

We did not observe any changes in growth rates in the ancestral environment of the source populations for the duration of the selection procedure ($T_{23} = 0.23$, $P = 0.82$; Fig. 1). The fitness of source populations in the presence of herbicides did not change either, as the growth rates were not significantly different between the measurements at the beginning of the experiment and during the assays that followed the selection procedure (for atrazine: $T_{23} = 0.37$, $P = 0.71$; S-metolachlor: $T_{23} = 0.48$, $P = 0.64$;

Table 1. Time to evolutionary rescue for each experimental regime. Six regimes were created for each selected (herbicide) environment, with six replicates per regime. The table provides information on the mean time to evolutionary rescue per regime (in weeks), and the standard error of the mean. Only the populations that got rescued were included in the calculation of the mean time to rescue, and the number of replicate populations that got rescued is also provided. The survival analysis presented in Fig. 2 takes into account both the mean time to rescue as well as the number of replicates that got rescued.

Herbicide	Mode of reproduction	Immigration	Time to rescue (weeks)	Standard error	No. of populations that got rescued
Atrazine	Sexual	No	4.00	0.74	5/6
	Sexual	Minimum	2.67	0.33	6/6
	Sexual	Maximum	3.33	0.42	6/6
	Asexual	No	4.75	0.25	4/6
	Asexual	Minimum	6.50	1.02	6/6
	Asexual	Maximum	4.50	0.71	6/6
Glyphosate	Sexual	No	4.50	0.73	5/6
	Sexual	Minimum	2.67	0.21	6/6
	Sexual	Maximum	3.17	0.30	6/6
	Asexual	No	6.40	0.40	4/6
	Asexual	Minimum	5.67	0.49	6/6
	Asexual	Maximum	4.83	0.47	6/6
S-metolachlor	Sexual	No	7.00	0.79	5/6
	Sexual	Minimum	4.17	0.30	6/6
	Sexual	Maximum	4.17	0.16	6/6
	Asexual	No	7.00	0.40	4/6
	Asexual	Minimum	7.17	0.16	6/6
	Asexual	Maximum	5.83	0.65	6/6

glyphosate: $T_{23} = 0.45$, $P = 0.66$; Fig. 1). These results suggest that no general adaptation to laboratory conditions or to herbicides occurred in the source populations propagated in the ancestral environment for the duration of the selection procedure.

FITNESS

We identified an effect of sex and migration on the fitness of the final evolved populations. The change in fitness between weeks 14 and 26 for the populations selected in atrazine was significantly affected by the interaction between sex and time ($F_{1,28} = 16.736$, $P < 0.001$), as well as an interaction between sexual reproduction and migration (and the factor "time"; $F_{2,28} = 4.032$, $P = 0.029$), as growth improved in all but the populations undergoing sex and migration (Fig. 3A). We also identified a significant effect of "time" in atrazine, as the growth rates between populations at weeks 14 and 26 were significantly different ($F_{1,28} = 36.395$, $P < 0.001$). In S-metolachlor, the combined effect of sexual reproduction and time ($F_{1,26} = 17.568$, $P < 0.001$) and of migration and time ($F_{2,26} = 9.882$, $P < 0.001$) was significant. The three-way interaction between sex, migration, and "time" on the change in fitness between weeks 14 and 26 was also significant ($F_{2,26} = 8.054$, $P < 0.005$), arising from lack of fitness improvement in the populations undergoing sexual reproduction with migration (Fig. 3B). We also observed a significant effect of "time" ($F_{1,26} = 55.707$, $P < 0.001$). No effects of sex and migration were observed in the presence of glyphosate, with the overall fitness in the presence of herbicides (effect of "time") increasing over time ($F_{1,29} = 26.454$, $P < 0.001$; Fig. 3C).

Discussion

Our results show the role that sex and migration can have on adaptation to a novel sink environment and on population fitness. In particular, we demonstrate that both sexual reproduction and migration act to accelerate rates of evolutionary rescue in a novel marginal habitat, but subsequently interact to slow down the rate at which population fitness is further improved.

We first explored the rates of evolutionary rescue by observing the fate of populations early in exposure to a novel sink environment, when fitness was severely reduced and populations were in decline (Fig. 2). We observed a positive effect of sex on the rate of evolutionary rescue (Fig. 2A). When a population first encounters a novel stressful environment, the beneficial mutations that will ultimately form the basis of any adaptation will be very rare. As a consequence, the probability of an individual carrying multiple beneficial mutations is small and such individuals are unlikely to be present in a finite population (Maynard Smith 1978). In an asexual population, adaptation will proceed through

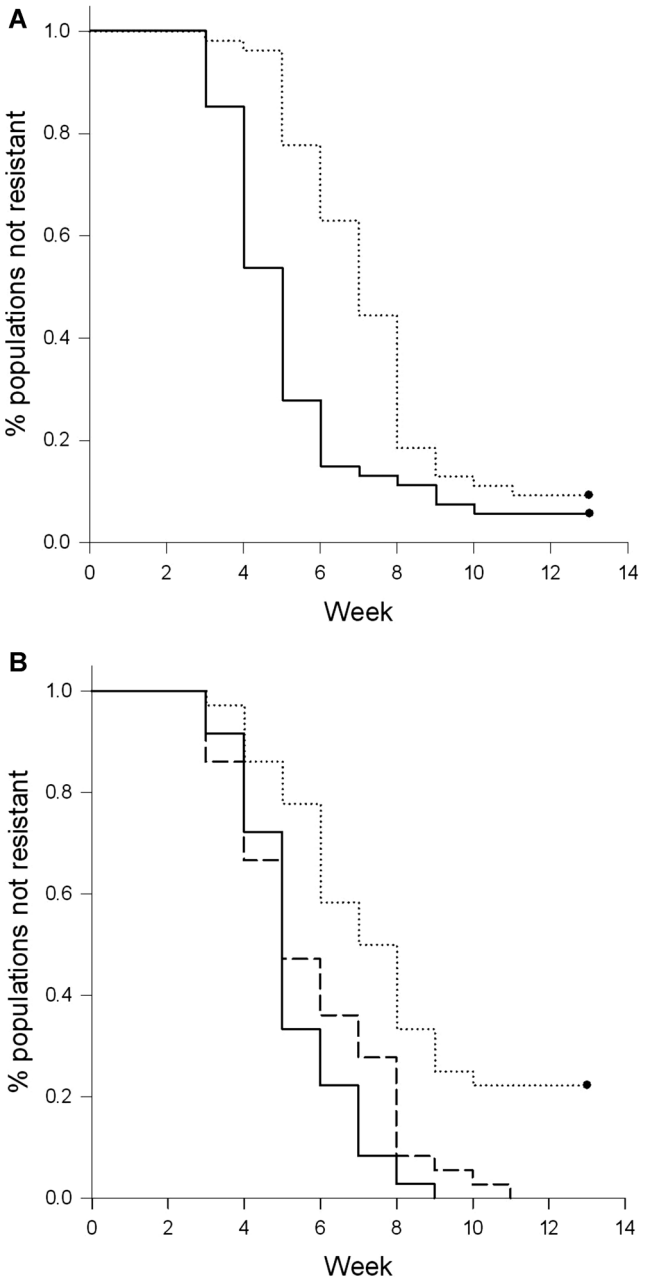

Figure 2. Dynamics of evolutionary rescue. Kaplan–Meyer survivorship, showing the percentage of the populations that have not been rescued by a given time point (experimental transfer period). (A) Rates of evolutionary rescue in sexual (solid line) versus asexual (dotted line) populations. (B) Rates of evolutionary rescue in populations under high (solid), low (dash), and no (dotted) immigration regimes. Large dot indicates the existence of populations that have gone extinct.

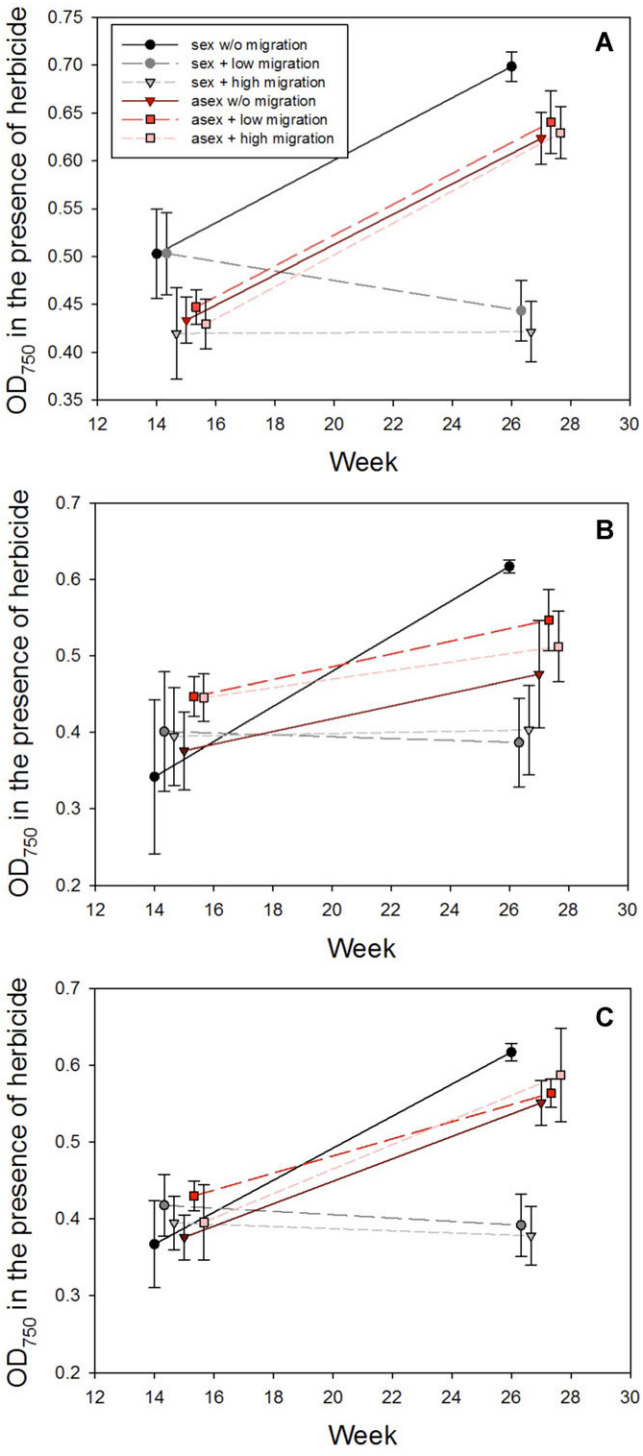

Figure 3. Fitness in the presence of herbicide. Fitness was measured as OD_{750} after seven days of growth, in populations after 14 and 26 asexual transfers (week). The mean fitness of each of the six regimes is presented independently, and the mean fitness of the same regime at two time points are connected with a line. Sexual (sex) populations are presented in gray, asexual (asex) populations in red. (A) Fitness in atrazine; (B) S-metolachlor; (C) glyphosate. Bars are standard errors of the mean. Figure in color available on the web.

competition among lineages carrying different beneficial mutations. Under this scenario, recombination can bring together beneficial mutations from different lineages, reducing the negative linkage disequilibrium that is built up by selection and increasing the rate of adaptation (Fisher 1930; Muller 1932; Maynard Smith

1978). This effect is likely to have led to the increased rate of rescue observed in our sexual populations. Similar effects of sex on rates of adaptation have been seen in other studies of *C. reinhardtii* (Colegrave 2002) and yeast (Goddard et al. 2005), suggesting that a major evolutionary benefit of sex may occur when species enter new habitats or the environment changes.

Rates of evolutionary rescue in a sink habitat depend on the standing genetic variation and supply of novel beneficial mutations (Barrett and Schluter 2008). In a sink habitat, where populations are in decline, migration acts to sustain the population by increasing the effective population size. The increase in the rates of evolutionary rescue accompanying immigration (Perron et al. 2007), which we observed (Fig. 2B), can be attributed to immigrants introducing novel beneficial mutations or increasing population size and hence generation of novel mutations (Kawecki 2000; Holt et al. 2003). Sexual reproduction and migration appeared to show independent effects on the rates of evolutionary rescue, so that an increase in population size that accompanied immigration into the sink did not add to the benefits of mutation assembly provided by sex.

Following the initial gain in fitness through evolutionary rescue, fitness subsequently improves by the beneficial complex moving toward fixation and/or through fixation of subsequent beneficial mutations. In an asexual population without immigration, the beneficial gene complex conferring herbicide resistance is likely to get fixed rapidly due to the large fitness benefit it provides compared to nonresistant individuals (Elena and Lenski 2003; Palmer and Kishony 2013), and subsequent improvements in fitness depend on the frequency of novel beneficial mutants (Barrick et al. 2009). Although immigration from the source into a well-adapted asexual population would reduce population fitness (LoFaro and Gomulkiewicz 1999), further fitness gains would still depend on the frequency of novel mutants within the individuals containing the beneficial gene complex. In line with previous findings of frequent improvements in fitness through adoption of further resistance (Palmer and Kishony 2013) or compensatory mutations (Wiesch et al. 2010; Andersson and Hughes 2010), we observed increases in fitness in all asexual populations following evolutionary rescue (Fig. 3).

Following initial evolutionary rescue, the subsequent adaptation observed in the absence of migration was comparable for sexual and asexual populations. The lack of an effect of sex may reflect the fact that, as a population becomes better adapted the supply of beneficial mutations will decline as there are fewer ways in which fitness can be improved. If beneficial mutations are rare, such that one can get to high frequency before the next appears, then effects of recombination will be limited (Fisher 1930; Muller 1932).

We also provide evidence that, as the populations become adapted to the novel conditions, the continuing influx of maladapted individuals from the source population can act to reverse the effects of sex, presumably by generating positive linkage disequilibrium for fitness loci. In two of our selection environments, sex slows the subsequent rate of adaptation when coupled with migration. To our knowledge, this is the first time that this effect has been shown experimentally. The effect is not universal, as we did not observe this effect in glyphosate where all populations achieved comparable levels of fitness by the end of the experiment (Fig. 3B). We also did not observe a positive effect of sex on the rates of rescue in glyphosate. If the phenotype giving rise to evolutionary rescue arises from a single point mutation, recombination is predicted not to have an effect, resulting in similar evolutionary trajectory between sexual and asexual populations (Otto 2009).

There are two aspects of our experimental system that will not apply to many natural systems. First our sexual lines only went through intermittent sexual cycles, with periods of asexual growth in between. However, theoretical models show that the genetic consequences of occasional and obligate sexual reproduction are very similar (D'Souza and Michiels 2010), and so we would expect our conclusions to apply to obligatory sexual organisms. Second, the fact that *C. reinhardtii* requires genotypes of opposite mating type if mating is to occur led us to initiate all of our lines with pairs of genotypes. This could lead to high levels of linkage disequilibrium being present in the initial populations, which would enhance or diminish effects of sex depending on its overall sign. Without details of the genetic basis of adaptation in this study, it is impossible to say with certainty what affects this had on our results. However, there are reasons to expect that any effect would have been small. The initial genotypes used to found all populations were not resistant to the herbicide treatments, and so the adaptation observed is most likely due to novel mutations arising during the experiment. Patterns of linkage disequilibrium among such novel beneficial mutations should be unaffected by the initial conditions. Furthermore, the fact that similar patterns were seen for populations founded by different pairs of genotypes suggests that the initial conditions are not having large effects on the outcome of selection in this study.

Our results show that sex and migration can interact in complex ways that change as a population becomes better adapted to a marginal habitat. Most importantly, we demonstrated that the adaptive benefits of sexual reproduction are reversed in the presence of migration. We observed populations soon after evolutionary rescue and 100 generations later, allowing us to assess the potentially different impact that recombination and migration might have prior to and after initial adaptation to a novel environment. Our findings are in line with previous demonstrations of the positive effects of sexual reproduction (Colegrave 2002; Colegrave et al. 2002; Becks and Agrawal 2012) and migration (Perron et al. 2007, 2008) on rates of adaptation. Over the

period of 100 generations, we demonstrated that sex and migration interact to slow down the rates of further fitness improvements, potentially explaining why asexual variants are often found at the ranges of species distribution (Kawecki 2008). This finding brings into question whether the previous reports of benefits of sex in simple environments could be extrapolated to more complex, natural environments.

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DATA ARCHIVING

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