

LETTER

Gene-flow between niches facilitates local adaptation in sexual populations

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Abstract

In sexual populations, gene-flow between niches is predicted to have differential consequences on local adaptation contingent upon the nature of trade-offs underlying local adaptation. Sex retards local adaptation if antagonistic pleiotropy underlies trade-offs, but facilitates adaptation if mutation accumulation underlies trade-offs. We evaluate the effect of sex in heterogeneous environments by manipulating gene-flow between two niches in sexual and asexual populations using steady-state microcosm experiments with yeast. We find that only sex in the presence of gene-flow promotes simultaneous local adaptation to different niches, presumably as this exposes mutations neutrally accrued in alternate niches to selection. This finding aligns with work showing mutation accumulation underlies trade-offs to local adaptation in asexual microbes, and with inferences of divergence in the presence of gene-flow in natural sexual populations. This experiment shows that sex may be of benefit in heterogeneous environments, and thus helps explain why sex has been maintained more generally.

Keywords

Experimental ecology, gene-flow, heterogeneous environments, local adaptation, *Saccharomyces cerevisiae*, sex and recombination, trade-off.

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INTRODUCTION

The maintenance of sex is a classic conundrum in biology. Theory demonstrates that sex may aid adaptation to homogeneous environments, which exert simple directional selection, by breaking down linkage disequilibrium, which in turn increases additive genetic variance (Williams 1975; Maynard Smith 1978; Burt 2000). Experiments support this idea, and have shown that sex speeds adaptation to simple environments comprising a single niche (Colegrave 2002; Goddard *et al.* 2005; Cooper 2007; Morran *et al.* 2009). However, many sexual populations inhabit complex heterogeneous environments comprising multiple niches, where divergent rather than directional selection prevails. It is not necessarily clear how sexual species persist under these arguably more realistic conditions (Felsenstein 1981; Butlin 2005; Smadja & Butlin 2011). This is an important issue not only in its own right, but also because it underlies processes that help explain why sex is maintained more generally, and potentially how sexual populations go on to form species in sympatry (Felsenstein 1981; Butlin 2005; Smadja & Butlin 2011).

In line with theory (Rundle & Nosil 2005; Schluter 2009), microcosm experiments with asexual microbial populations (Rainey & Travisano 1998; Dettman *et al.* 2007; Jasmin & Kassen 2007; Kassen 2009) have shown that divergent selection may drive simultaneous adaptation to divergent niches. The resulting local adaptation supposedly establishes linkage disequilibrium between sub-populations. In sexual species recombination and assortment act to eliminate linkage disequilibrium and constantly homogenise populations and form hybrids. However, the degree to which sexual populations may achieve local adaptation to alternate niches in heterogeneous environments is theoretically contingent on the nature of trade-off underlying costs to local adaptation

(Felsenstein 1981; MacLean 2005; Martin *et al.* 2006; Smadja & Butlin 2011).

Work building on a classic strand of theory suggests that sex retards local adaptation, and this prediction critically hinges around trade-offs being manifest in the form of ‘functional interference’ or exhibiting ‘antagonistic pleiotropy’: here the actions of loci that confer adeptness in one niche actively confer a regress in another (Bell 2008). In this case hybrids of parents adapted to alternate niches are predicted to be maladapted (Felsenstein 1981). Here, when there is gene-flow between niches, sex is classically predicted to retard local adaptation, and ultimately prevent adaptive radiation and sympatric speciation (Coyne & Orr 2004; Butlin 2005; Smadja & Butlin 2011). In line with these ideas, it appears there are common and widespread inverse relationships between trait divergence and gene-flow in sexual populations (see Nosil & Crespi 2004 and refs within). Recent work reinforcing the potential negative effects of gene-flow on divergence in sexual taxa estimated that the transition to asexuality in *Onagreae* sp (Marigolds) resulted in diversification rates approximately eight-times faster than diversification rates in sexual counterparts, presumably as the asexuals are free of the homogenising effects of sex and recombination (Johnson *et al.* 2011). Under this classic model, the homogenising consequences of sex become unimportant if there is no gene-flow between niches, and thus barriers to gene-flow have been implemented to theoretically explain how sexual populations become locally adapted (Rundle & Nosil 2005; Schluter 2009; van Doorn *et al.* 2009). The process of divergence (and speciation) in sexual populations once mechanisms to prevent gene-flow have evolved is well characterised (Coyne & Orr 2004; Rundle & Nosil 2005; Smadja & Butlin 2011), and divergence by postzygotic isolation has been shown to occur in isolated experimental asexual yeast populations exposed to divergent selection with no gene-flow (Dettman *et al.* 2007). Conceptually

gene-flow *per se* will not necessarily retard local adaptation if trade-offs are due to antagonistic pleiotropy. Low-level gene-flow may be of little consequence, especially if divergent selection is strong. As the strength of gene-flow increases so will the degree to which adaptation is retarded due to homogenisation in sexual populations. However, the effect of varying strengths of gene-flow on local adaptation in sexual populations is not well understood (Smadja & Butlin 2011). Using a neutral model of bacterial divergence, which included recombination, Fraser *et al.* (2007) show that diversification is prevented when recombination rates are between one-fourth and twice as much as the mutation rate; however, how this translates to situations where divergent selection is operating to promote local adaptation is uncertain. Doebeli & Dieckmann (2003) use spatial simulations to examine the effect of gene-flow on diversification (evolutionary branching) along an environmental gradient and show that complete gene-flow (random mating) prevents diversification, but if the movement of individuals is constrained to allow various degrees of localised non-random mating, diversification occurs. However, Doebeli & Dieckmann (2003) did not explicitly analyse how divergence differs as a function of gene-flow. Recent comparative work by Kisel & Barraclough (2010) involving 471 putative speciated genera on oceanic islands show that the strength of gene-flow explained 76% of variance in the spatial scale of speciation, again demonstrating the relative effect of gene-flow on diversification in sexual species.

However, trade-offs to local adaptation may also arise because of 'mutation accumulation', which suggests those mutations resulting in a regress in one niche are not those that confer adaptation to an alternate niche, but due to different mutations that neutrally accrued as a by-product of adaptation. Experimental work with asexual microbial populations tends to support the idea that trade-offs to local adaptation are due to mutation accumulation (MacLean 2005), but examples of antagonistic pleiotropy may also be found (Cooper & Lenski 2000). If trade-offs are due to mutation accumulation, then in asexual populations, and in sexual populations with no gene-flow between niches, linkage disequilibrium is predicted to build-up between subpopulations due to drift. In this case sex may theoretically be favoured in heterogeneous environments as it breaks down this disequilibrium, and allows selection to more effectively purge these accumulated mutations across niches (Martin *et al.* 2006). Additional theory also finds conditions under which sex may be of benefit in heterogeneous environments: if locally adapted alleles are common (i.e. gene-flow is weak), sex may be advantageous as it breaks down maladapted genotypes formed as a result of gene-flow (Lenormand & Otto 2000; Becks & Agrawal 2010). These ideas possibly align with recent experimental work with rotifers showing that higher rates of sex evolve in heterogeneous environments, though the reasons for this observation are not clear (Becks & Agrawal 2010). Thus, the effects of sex on local adaptation are theoretically contingent upon the nature of trade-offs that underlie costs to local adaptation.

There is evidence to suggest that diversification with gene-flow may occur in natural populations (Nosil 2008; Schluter 2009; Papadopoulos *et al.* 2011), but we are aware of no previous experimental work to directly examine the effects of sex, with varying amounts of gene-flow between niches, in heterogeneous environments. Here we experimentally focus on processes that occur before barriers to gene-flow have arisen in sexual populations inhabiting heterogeneous environments. What is the fate of sexual species in complex

environments prior to and despite the appearance of barriers to gene-flow? Is it an essential prerequisite that barriers to gene-flow arise to allow sexual populations to undergo local adaptation? The prediction arising from classic theory, which assumes trade-offs to adaptation are due to antagonistic pleiotropy, is that increasing levels of gene-flow increasingly retard simultaneous adaptation to multiple niches in sexual but not asexual populations. However, if trade-offs to local adaptation arise because of mutation accumulation, then sex with gene-flow should aid local adaptation. We evaluate the effects of sex in a heterogeneous environment using steady-state microcosm experiments with yeast. We are able to hold all factors constant and manipulate only sexual status and levels of gene-flow between otherwise equivalent diploid populations of *Saccharomyces cerevisiae* exposed to two niches intended to promote diversification: 0.2 M NaCl with N-limitation at 30 °C (osmotic-N) and 0 M NaCl with C-limitation at 37 °C (hot-C). We constructed these niches based on previous work showing local adaptation, and trade-offs to adaptation, to carbon and nitrogen limitation in asexual *S. paradoxus* populations (note that the mechanisms underlying this trade-off are not known), and that increased temperature and salinity were stressful to *S. cerevisiae* populations (Goddard & Bradford 2003; Goddard *et al.* 2005). We controlled the extent of gene-flow between niches by varying rates of reciprocal population transplantation, ranging from none to panmictic, with three orders of magnitude in-between. We show that sex with gene-flow did not retard but promoted the extent of simultaneous local adaptation of populations to multiple niches.

MATERIAL AND METHODS

Experimental organisms

Yeast divides mitotically when supplied with sufficient nutrients, but starvation induces meiosis (sporulation) in diploids resulting in four haploid recombined spores. Each spore may be one of two mating types (a or α), and spores of the opposite type may mate once germinated. Meiosis in yeast is experimentally manipulated by starvation, meaning that asexual and sexual treatments experience different selection regimes. Moreover, starvation in *S. cerevisiae* is known to increase mutation rates (Marini *et al.* 1999), which will tend to increase genetic variation. Thus, starvation does not just have effects on the mode of reproduction but also on aspects that may alter the extent of adaptation independent of sexual reproduction. Here we employ a *S. cerevisiae* system that circumvents this issue as sexual and asexual populations experience identical conditions and differ only in their ability to engage in recombination, random assortment and syngamy (Goddard *et al.* 2005; Goddard 2007). Two genes required for normal recombination and meiosis were deleted to create the asexual but sporulating strains used here. *SPO11* controls chiasmata during meiosis by making double strand breaks in chromosomes: in its absence meiotic recombination does not occur (Klapholz *et al.* 1985). *SPO13* determines whether a cell goes through one or two meiotic divisions by altering the sister chromatid cohesion process (Shonn *et al.* 2002); in its absence only the second non-reductive meiotic division is achieved, resulting in the production of two diploid, as opposed to four haploid, spores. Chiasmata are required to stabilise chromosome segregation, and so nonfunctional mutations of *SPO11* would normally lead to aberrant chromosomal segregation; however, this phenotype is rescued if

SPO13 is nonfunctional as well and leaves the diploid double mutant fully fertile, producing diploid un-recombined asexual spores that are genetically identical to the parent (Goddard *et al.* 2005). Thus, both sexual and asexual populations may experience identical ecological regimes. *SPO13* was replaced with a G418 resistance gene (kanMX4) to allow easy differentiation between sexual and asexual individuals. The mitotic fitness effects of deleting these genes appear insignificant, and sporulation rates of sexual and asexual strains are equivalent (Goddard *et al.* 2005).

Microcosm experiments

The base media for the two niches comprised all essential nutrients for growth apart from a source of carbon and nitrogen (1.7 g Yeast Nitrogen Base without amino acids; Formedium, Hunstanton, UK). One niche was limited for carbon and had a thermal stress (hot-C): 5 g L⁻¹ ammonium sulphate and 0.08 g glucose g L⁻¹ at 37 °C. The other niche was limited for nitrogen and had an osmotic stress (osmotic-N): 0.05 g ammonium sulphate g L⁻¹ and 20 g glucose g L⁻¹ with 0.2 M NaCl at 30 °C. Populations were paired between niches and propagated in triplicate for each sexual status by rate of

gene-flow treatment (Fig. 1). Each population experienced 12 rounds of approximately 25 generations of mitotic growth, where mutation and selection may occur, followed by meiosis. Populations were mitotically propagated under continuous culture, which meant they remained approximately at a steady-state with regard to environmental parameters, nutrient concentrations, and carrying capacity. Flow rates were set to 8 mL per hour in a 40 mL culture volume, resulting in one mitotic generation approximately every 3 h, and thus 25 generations over 3 days. Mean effective population sizes were $1.26 \times 10^8 \pm 2.6 \times 10^7$ (SD) across all treatments. After 25 mitotic generations 1.5×10^7 individuals were put through meiosis (sporulation) at each cycle for each population, which was induced by starvation with 5 g L⁻¹ potassium acetate. *S. cerevisiae* has a tendency to inbreed as spores derived from the same meiotic event are co-located in asci and thus are likely to mate once they germinate (Reuter *et al.* 2007). High levels of inbreeding in sexual populations will restrict gene-flow and confound this experiment. Therefore, asci were disrupted and spores randomised using 80 units mL⁻¹ Lyticase and 50 mM Dithiothreitol, followed by 1% SDS (to lyse and kill all unsporulated cells) and 3 300 units of β -Glucuronidase (to break apart asci). Following Goddard *et al.* (2005)

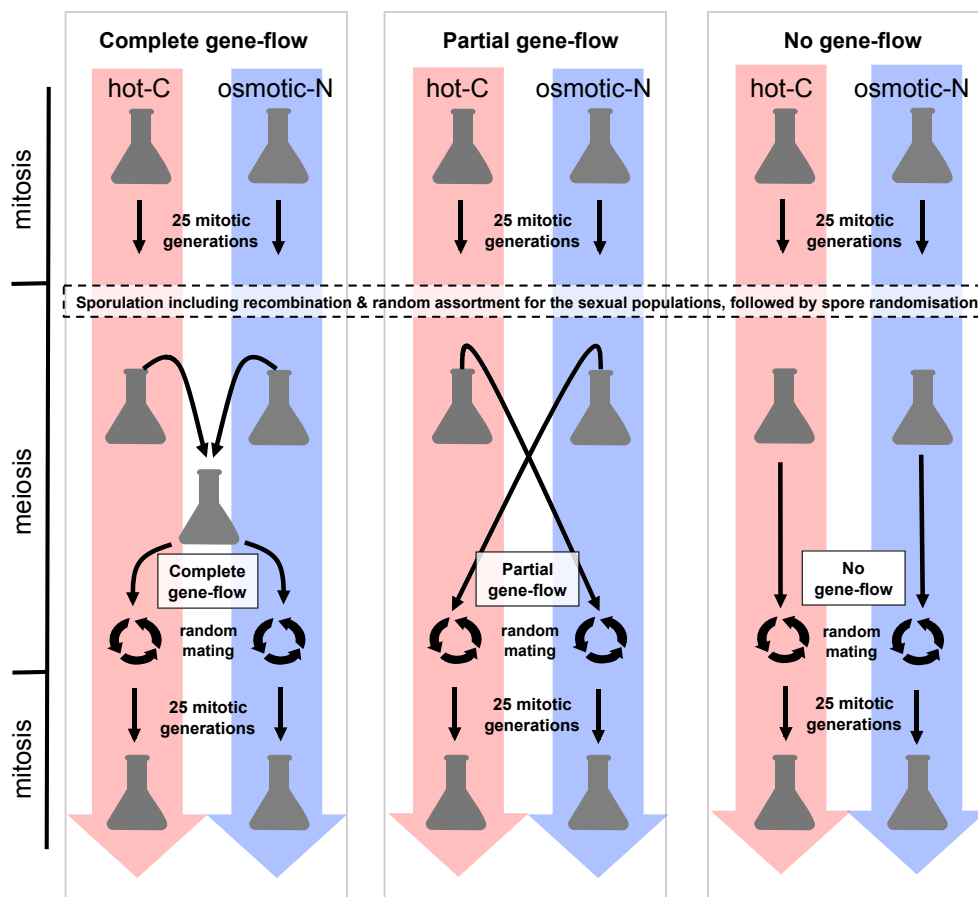


Figure 1 Schematic representation of the experimental design. Triplicate sexual and asexual populations were initiated for each niche by strength of gene-flow treatment for a total of 60 populations. The construction of the asexual sporulating strains meant that all populations were treated identically throughout the experiment. Gene-flow ranged from none to panmictic with intermediates of 0.0001, 0.01 and 1%, and was manipulated by the reciprocal transfer of appropriate proportions of gametes (spores) between paired populations in alternate niches. Mating between spores occurred after gene-flow, and the resulting populations were propagated at steady state for approximately 25 mitotic generations before meiosis was induced again. All populations underwent approximately 300 mitotic generations interspersed with 12 rounds of meiosis including sex and recombination for the sexual treatments.

we assayed the efficacy of this procedure to promote outcrossing and found it resulted in a mean inbreeding coefficient of 0.22 (95% CI \pm 0.06; n = 300); thus sexual populations were highly outcrossed. Both sexual and asexual populations were exposed to this treatment. Gene-flow was conducted on randomised spores at every episode of meiosis, before spore germination and mating. 0, 0.0001, 0.01, 1, and 50% of spores from each population were reciprocally transplanted between alternate niches for each pair of populations. Thus, even at the lowest rate of gene-flow (0.0001%), the gametes from approximately 200 individuals were randomly transplanted between alternate niches. For the complete (50%) gene-flow treatment, gametes from both populations were completely homogenised and then redistributed: thus on average 50% of the gametes were dispersed to the alternate niche from which their parents were derived. After gene-flow, spores germinated in their new niche, and mated randomly in the sexual treatments, prior to population growth. Since unsporulated cells were selected against, there were little to no diploids present in the sexual populations prior to mating. Mating and germination takes around 5 h, but populations were left overnight before continuous culture was resumed to mitigate the time cost associated with this in sexuals, as well as any differing rates of germination and mating (syngamy). Asexual populations were treated identically, and thus effective population sizes were likely very similar across treatments. The 60 populations were propagated for a total of 300 mitotic generations under continuous culture interspersed with 12 episodes of sex and gene-flow where appropriate; see Fig. 1. Adaptation was estimated by assaying the relative fitness of a population compared to the ancestor. Malthusian fitness (m) [this is the log of % Darwinian (w) fitness] was estimated by the change in ratio of ancestral and derived populations during head-to-head competitions in the appropriate niche. This was possible since one of the two competitors was marked with G418 resistance and thus could be distinguished.

Data analyses

Final competitive abilities were analysed with various ANOVAs; sexual status, niche and gene-flow rate were treated as fixed effects. The dynamics of specialisation were analysed by comparisons of mixed-effect linear models that did and did not include sexual status and gene-flow treatments; main model parameters were treated as fixed effects, and line specific variation was a random effect. These analyses were conducted using the nlme package in R 2.11.1 (Pinheiro & Bates 2000; R Development Core Team 2011).

RESULTS

There was no initial difference in extent of adaptation (Malthusian fitness) of ancestral sexual and asexual populations to either niche (hot-C: mean 0.00054 ± 0.0077 (SD), n = 14, t -ratio = 0.264, P = 0.7950; osmotic-N: mean 0.00205 ± 0.01956 (SD), n = 13, t -ratio = 0.378, P = 0.7121). To generally examine the effects of ecological niche, sexual status and strength of gene-flow on adaptation after 300 mitotic generations interspersed with 12 episodes of sex or asex with varying levels of gene-flow we conducted a three-way ANOVA. Fitness for each population was measured in the niche of propagation only, and thus there was only one end point fitness measurement for each population. First, this analysis revealed the niche of selection had a significant effect on the extent of adapta-

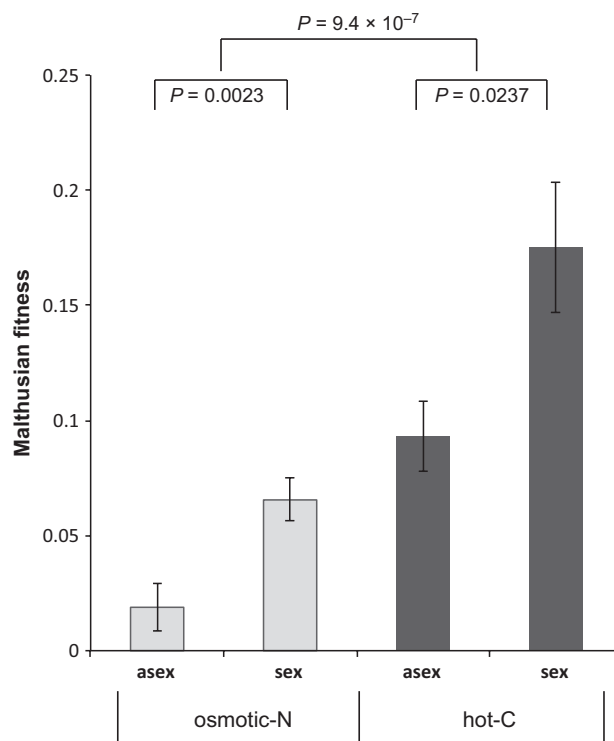


Figure 2 The mean (\pm SE, n = 15) Malthusian fitness of sexual and asexual yeast populations after 300 mitotic generations, interspersed with 12 episodes of sex where appropriate, with varying levels of gene-flow between the osmotic-N and hot-C niches. Selective environment had a significant effect on final fitness ($F_{1,45} = 32.204$; $P = 9.4 \times 10^{-7}$), and sexual populations had significantly greater fitness in each niche ($F_{1,24} = 5.830$, $P = 0.0237$ and $F_{1,28} = 11.298$, $P = 0.0023$ for hot-C and osmotic-N respectively).

tion ($F_{1,45} = 32.204$; $P = 9.4 \times 10^{-7}$): populations evolved in the hot-C niche increased in fitness by an average of 15% ($m = 0.14$), and those in the osmotic-N environment increased by only 4% ($m = 0.04$). However, populations in both niches demonstrated a significant increase in adaptation (hot-C, n = 26, t -ratio = 7.438, $P = 8.63 \times 10^{-8}$; osmotic-N, n = 30, t -ratio = 5.221, $P = 1.37 \times 10^{-5}$). Second, sexual status had a significant effect on adaptation generally ($F_{1,45} = 14.756$; $P = 0.0004$), with the sexual populations achieving an average of 14% greater fitness than the asexuals overall. Sex also allowed populations to achieve greater fitness within each niche. Sexual populations were an average of 9% ($F_{1,24} = 5.830$, $P = 0.0237$) and 5% ($F_{1,28} = 11.298$, $P = 0.0023$) greater in fitness than asexuals in the hot-C and osmotic-N niches respectively; Fig. 2. Third, there was no significant effect of differing strengths of gene-flow between niches on fitness generally ($F_{4,51} = 1.010$; $P = 0.4112$), nor did adaptation significantly differ within each niche as a result of varying gene-flow treatments, regardless of sexual status (hot-C, $F_{4,21} = 1.544$, $P = 0.226$; osmotic-N, $F_{4,25} = 0.731$, $P = 0.579$).

The initial question of interest is not just the effects of gene-flow *per se*, but whether differing strengths of gene-flow differentially effect the degree to which sexual and asexual populations are able simultaneously adapt to different niches. The fitness data broken down by sexual status, strength of gene-flow and niche are shown in Fig. 3. An ANOVA reveals no significant interaction between sexual status and gene-flow ($F_{4,45} = 0.905$; $P = 0.4691$), indicating that

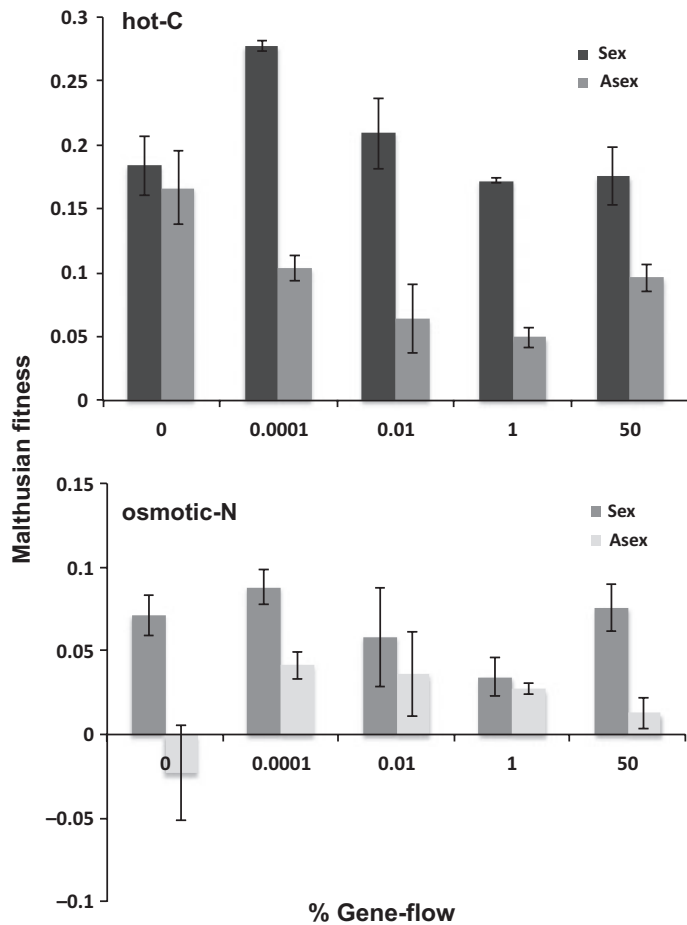


Figure 3 The mean (\pm SE, $n = 3$) final Malthusian fitness of sexual and asexual yeast populations after 300 mitotic generations, interspersed with 12 episodes of sex where appropriate, by strength of gene-flow between the osmotic-N and hot-C niches. There is no significant effect of strength of gene-flow on extent of adaptation overall ($F_{4,51} = 1.010$; $P = 0.4112$) or within each niche by sexual status (sexual populations: $F_{4,9} = 1.060$, $P = 0.2545$ and $F_{4,10} = 0.961$, $P = 0.4698$; hot-C and osmotic-N respectively; asexual: $F_{4,7} = 2.251$, $P = 0.1643$ and $F_{4,10} = 1.4061$, $P = 0.3007$; hot-C and osmotic-N respectively).

the strength of gene-flow does not differentially effect the extent of adaptation in sexual and asexual populations. Within neither niche is there an effect of strength of gene-flow on adaptation in the sexual ($F_{4,9} = 1.060$, $P = 0.2545$ and $F_{4,10} = 0.961$, $P = 0.4698$; hot-C and osmotic-N respectively) nor asexual ($F_{4,7} = 2.251$, $P = 0.1643$ and $F_{4,10} = 1.406$, $P = 0.3007$; hot-C and osmotic-N respectively) populations. In sum, sexual status affected the extent of adaptation, with sex conferring an average of 7% greater fitness across both niches, but the strength of gene-flow did not alter the rate of adaptation of either sexual or asexual populations in either niche. Thus, in populations that have become significantly simultaneously adapted to two niches, we can provide no support for the hypothesis that increasing levels of gene-flow increasingly retard local adaptation in sexual but not asexual populations.

Analysis of final fitness is the most relevant snap-shot in time, but comparisons of adaptive trajectories provide another way to evaluate the effects of sex and gene-flow; see Fig. 4. Populations exposed to both niches appeared to follow approximately linear fitness increases. The fitness gains in the osmotic-N niche were rela-

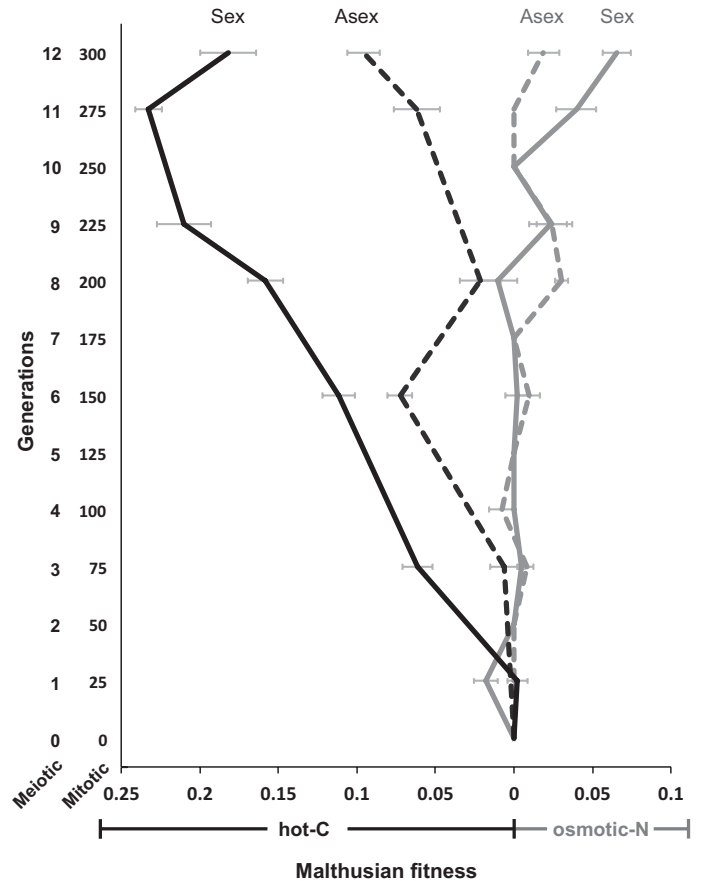


Figure 4 The mean (\pm SE, $n = 15$) adaptive trajectories of sexual and asexual populations, with varying strengths of gene-flow, in the hot-C and osmotic-N niches over 300 mitotic, interspersed with 12 meiotic, generations. Sex had a significant effect on adaptation to the hot-C ($P < 0.0001$), but not osmotic-N niche ($P = 0.1116$). There is not a significant effect of gene-flow within each niche generally (osmotic-N $P = 0.6352$; hot-C $P = 0.5039$) nor on adaptation in either sexual (osmotic-N $P = 0.8966$; hot-C $P = 0.8470$) or asexual (osmotic-N $P = 0.3110$; $P = 3094$) populations independently in either niche.

tively small, and a model that accounted for sexual status was not different from one that did not (Likelihood ratio = 4.386, $P = 0.1116$). We also tested for an overall effect of gene-flow by removing this treatment, which revealed no differential effect of gene-flow on adaptation to this niche generally (Likelihood ratio = 6.107, $P = 0.6352$) nor on adaptation in either sexual (Likelihood ratio = 3.532, $P = 0.8966$) or asexual (Likelihood ratio = 9.383, $P = 0.3110$) populations independently. In line with previous observations for these populations in similar niches (Goddard *et al.* 2005), the extent of fitness gain in the hot-C environment was greater. Comparison of linear models fit with and without sexual status revealed a significant effect of sex on adaptation overall (Likelihood ratio = 32.888, $P < 0.0001$): sexual populations adapted significantly more rapidly than asexual populations. However, a model incorporating differing amounts of gene-flow was not a significantly better fit than one not accounting for gene-flow (Likelihood ratio = 1.371, $P = 0.5039$). Again, there was no significant effect of differing strengths of gene-flow on adaptation in either the sexual (Likelihood ratio = 0.331, $P = 0.847$), or asexual (Likelihood ratio = 2.346, $P = 0.3094$) populations in the hot-C niche. Overall, where there was a marked increase in adaptation, sex conferred a

more rapid and greater degree of adaptation, but we can provide no support for the hypothesis that sex retards local adaptation in heterogeneous environments by any of our analyses.

An immediate explanation for this result might be that these experimental niches were imposing similar selection pressures, such that adaptation to one niche was correlated with adaptation to the other. This situation essentially collapses to a single niche imposing directional selection where sex has been shown to enhance adaptation. We used reciprocal transplant experiments and compared the extent of local adaptation to each niche (in the absence of gene-flow) to the change in adeptness in the alternate niche (to which populations had not been exposed). In short, we tested for a trade-off to adaptation in the absence of gene-flow. Fitness was significantly negatively correlated between the two niches (Pearson's product-moment correlation = -0.54 ; $P = 0.043$, $n = 11$), with a mean fitness increase of 10% in one niche correlated with a mean regress of 6% in the other. This correlates with previous observations of trade-offs to adaptation between similar niches with experimental asexual yeast populations (Goddard & Bradford 2003). Secondly, if selection was very strong in this system it is possible that in treatments with gene-flow that the majority of 'maladapted hybrids' created by sex were quickly removed from populations, and thus sexual populations did not suffer this load. We estimated this by assaying a sub-sample of random individuals' fitness from sexual populations in the last experimental cycle in the hot-C niche that had just experienced complete gene-flow between niches. The mean and variance of fitness did not significantly change after 25 mitotic generations of selection (t -test, $P = 0.65$; F -test, $P = 0.30$; $n = 29$) suggesting that selection is not removing less fit migrants and/or maladapted hybrids at a significantly greater rate than they are intro-

duced. Thirdly, if hybrids are maladapted, then selection is predicted to act strongly on any mechanism that prevents their formation. Reproductive isolation has been shown to evolve in experimental yeast populations under extreme selective conditions (Leu & Murray 2006) and in asexual yeast populations under divergent selection in the absence of gene-flow (Dettman *et al.* 2007). It is unlikely, although conceivable, that reproductive isolation may have evolved here, thus circumventing any potential cost of sex in heterogeneous environments. We have no evidence that reproductive isolation has arisen in any of the sexual populations as the between-population mating rates of all derived sexual populations, across the different degrees of gene-flow, were not significantly different from one another, or from the ancestor ($P = 0.1$, $F_{5,11} = 2.474$, $n = 17$ populations with ≈ 300 individuals assayed in each).

In asexuals, after 300 mitotic generations, an average increase of 7% fitness in the hot-C niche correlated with a regress of 5% in the osmotic-N, and in sexual populations in the absence of gene-flow an increase of 12% in the hot-C niche correlated with a regress of 8% in the osmotic-N. Given this trade-off to local adaptation, how have the sexual populations increased in fitness in the presence of gene-flow between these two niches? We investigated the nature of adaptation in sexual populations subject to complete gene-flow. In contrast to populations where gene-flow was absent, five of the six sexual populations exposed to complete gene-flow between niches did not demonstrate a fitness trade-off between these niches after 300 generations; see Fig. 5. A Fisher's exact test shows the distribution of trade-offs is significantly different between sexual populations that were and were not exposed to gene-flow ($P = 0.015$). The same was not true for the asexual treatments ($P = 0.5$) where trade-offs were apparent regardless of the presence of gene-flow. In sexual populations exposed to complete gene-flow between niches, an average increase of 13% in fitness the hot-C niche correlated with an increase of 9% in the osmotic-N niche.

DISCUSSION

Sexual status had a significant effect on fitness generally ($F_{1,45} = 14.7$; $P = 0.0004$), with the sexual populations achieving an average of 14% greater fitness than the asexuals overall. When zero gene-flow populations are excluded, the fitness of sexual populations remained greater than asexual populations in both niches (hot-C, $F_{1,19} = 5.6750$, $P = 0.0278$; osmotic-N, $F_{1,22} = 6.3118$, $P = 0.0198$). These data show that in sexual populations gene-flow between niches does not constrain but facilitates local adaptation to differing niches within a heterogeneous environment. In these experimental populations, where sexual status and strength of gene-flow between divergent niches was tightly controlled, sex in the presence of gene-flow appears to have enabled simultaneous local adaptation by circumventing trade-offs to adaptation that arose in all asexual populations, and sexual populations in the absence of gene-flow. Sex with gene-flow appears to have facilitated the emergence of superior-generalists (generalists that are superior across multiple niches).

We have examined and discounted a number of experimental aspects that may have produced this result. The immediate explanation for the observation that sex with gene-flow facilitates the emergence of superior-generalists is that trade-offs to adaptation seen in the absence of gene-flow resulted from mutation accumulation and not functional interference/antagonistic pleiotropy (Bell 2008).

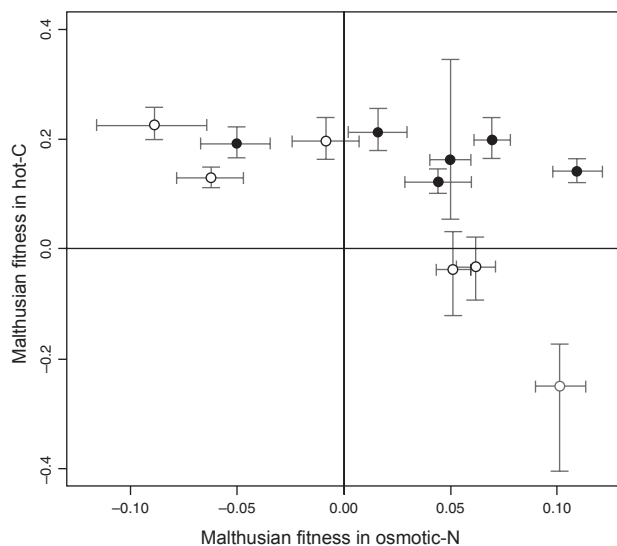


Figure 5 Adaptation of sexual populations in the absence (open circles) or presence (closed circles) of complete gene-flow between the hot-C and osmotic-N niches, after 300 mitotic generations interspersed with 12 episodes of sex. Each fitness estimate is bounded by 2-log likelihood support limits based on the binomial distribution ($n \approx 300$). The distribution of fitness between those populations adapting in the presence or absence of gene-flow is significantly different ($P = 0.015$): it is apparent that all but one of the populations exposed to gene-flow has increased in fitness in both niches, but that a trade-off to adaptation exists in the absence of gene-flow.

Trade-offs to adaptation due to mutation accumulation have been shown with experimental asexual microbial populations (MacLean 2005). If mutation accumulation underlies the trade-off to local adaptation seen here in asexuals and sexuals in the absence of gene-flow, then this cost would be greatly reduced by sex with gene-flow as it would expose these neutrally accumulated mutations to selection and they would be removed, which is in line with theory (Martin *et al.* 2006). The data presented here are also conceptually in line with the idea that sex provides a benefit as it serves to liberate locally adapted alleles that have become entangled in detrimental genetic backgrounds that arose in alternate niches due to the accumulation of mutations, by the mating of locally adapted to migrant genotypes. Selection would be blind to these accrued mutations in asexual populations and sexual populations in the absence of gene-flow. A second idea to explain these observations focuses more on alleles involved in adaptation, and not those that confer maladaptation: Livnat *et al.* (2008) proposed that the advantage of sex lay in its ability to select for alleles that perform well across different background combinations, or for 'mixability'. This idea might be extended to suggest that in heterogeneous environments, sex in the presence of gene-flow enables selection for combinations of alleles that are optimal across multiple environments, rather than simply those best in one. Here, trade-offs to adaptation in the absence of sex with gene-flow may be due to the fact that mixable alleles are only a small sub-set of possible alleles that confer an advantage in any one environment. Both these possibilities may be realised as the more rapid development of phenotypic plasticity (Auld *et al.* 2010) by sexual populations exposed to gene-flow. Of course, it is conceivable that sex confers a benefit since it both aids selection to eliminate mutations neutrally accrued in alternate niches and incorporate alleles that confer adeptness across multiple niches. This idea is in line with inferences regarding the function of sex in a single niche, where sex unlinks beneficial from detrimental mutations, and allows selection to act on both more efficiently (Gray & Goddard 2012).

This work shows that barriers to gene-flow are not a necessary pre-requisite for sexual populations to undergo local adaptation in heterogeneous environments. These data support ideas about the roles sex might play in facilitating local adaptation in complex environments, where trade-offs to local adaptation are likely due to mutation accumulation and not antagonistic pleiotropy. These findings fit with work from asexual microbial populations showing mutation accumulation largely explains trade-offs to local adaptation, and they also fit with findings from natural sexual populations of macro-organisms inferred to have undergone local adaptation in the face of gene-flow (Nosil 2008; Schluter 2009; Papadopoulos *et al.* 2011). Together this suggests, as a first approximation, that trade-offs to local adaptation in these higher taxa might also be due to mutation accumulation and perhaps not generally due to antagonistic pleiotropy. This work demonstrates that sexual populations may not only persist with but outcompete asexual taxa in heterogeneous environments, and thus provides another step towards understanding why sex has been maintained since it evolved. In the short term this might delay the process of reproductive isolation in sexuals. However, if sexual populations are unable to persist, the potential for subsequent between niche genetic divergence and successive sympatric speciation, by whatever mechanism, is denied, as sexual populations will be displaced by other taxa that undergo local adaptation more rapidly and effectively.

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AUTHORSHIP

MRG conceived and designed the study, JCG conducted the experimental work. Both authors analysed the data and wrote the manuscript.

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