Gene flow accelerates adaptation to a parasite

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ABSTRACT

Gene flow into populations can increase levels of additive genetic variation and introduce novel beneficial alleles, thus facilitating adaptation. However, gene flow may also impede adaptation by disrupting beneficial genotypes, introducing deleterious alleles, or creating novel dominant negative interactions. While theory and fieldwork have provided insight as to the effects of gene flow, direct experimental tests are rare. Here, we evaluated the effects of gene flow on adaptation in the host nematode *Caenorhabditis elegans* during exposure to the bacterial parasite *Serratia marcescens*. We evolved hosts against non-evolving parasites for ten passages while controlling host gene flow and source population. We used source nematode populations with three different genetic backgrounds (one similar to the sink population and two different) and two evolutionary histories (previously adapted to *S. marcescens* or naïve). We found that nematode populations with gene flow exhibited greater increases in parasite resistance than those without gene flow. Additionally, gene flow from adapted nematode populations resulted in greater increases in resistance than gene flow from naïve populations, particularly with gene flow from novel genetic backgrounds. Overall, this work demonstrates that gene flow can facilitate adaptation, and suggests that the genetic architecture and evolutionary history of source populations can alter the sink population's response to selection.

INTRODUCTION

Gene flow, the movement and establishment of alleles into a novel population (Endler, 1977), is a fundamental evolutionary force. Gene flow is predicted to have a multitude of effects on the evolutionary trajectories of sink populations (Garant, Forde and Hendry, 2007). Depending on the quantity and effect sizes of the specific alleles introduced, gene flow has the potential to either facilitate or impede adaptation. At the extremes, when migrants comes from a genetically similar population, gene flow can be functionally understood as a simple increase in effective population size (Wright, 1931), which largely facilitates adaptation. At the other extreme, when gene flow comes from a different species, it generally results in dramatic fitness reductions, often via the introduction of dominant negative interactions (Turelli and Orr, 2000 ; Turelli, Barton and Coyne, 2001). In between these extremes, projected outcomes often depend on modeling assumptions that are rarely tested by experimental studies.

Generally, theory predicts that gene flow will constrain adaptation when selection is not strong enough to maintain high frequencies of advantageous alleles (Haldane, 1930). Models investigating the impact of gene flow on adaptation have often focused on the disruptive effects of gene flow in preventing local adaptation within populations, and adaptive divergence between populations (Wright, 1931; Slatkin, 1987). Gene flow is predicted to impede these processes by

reducing the genetic differences between populations, reducing the frequency of locally advantageous alleles, and by disrupting beneficial associations between genes for adaptation or reproductive isolation (Coyne and Orr, 2004; Garant, Forde and Hendry, 2007). This is especially true in models of symmetrical gene flow, where alleles move between populations that are concurrently adapting. Empirical studies have provided support for these ideas. For instance, experiments in insects (Ross and Keller, 1995; Nosil and Crespi, 2004; Nosil, 2009), spiders (Riechert, 1993), birds (Blondel *et al.*, 2006), mammals (Hoekstra, Krenz and Nachman, 2005; Sullivan *et al.*, 2014), fishes (Lu and Bernatchez, 1999; Ferchaud and Hansen, 2016), reptiles (King and Lawson, 1995; Calsbeek and Smith, 2003), and plants (Santon and Galen, 1997; Sambatti and Rice, 2006; Papadopulos *et al.*, 2011), have shown an inverse relationship between divergence and gene flow. Some illustrative examples are studies investigating adaptive divergence in sticklebacks (*Gasterosteus aculeatus*), where researchers found that populations in environments connected via gene flow showed less morphological divergence than those living in isolated environments (Hendry, Taylor and McPhail, 2002; Hendry and Taylor, 2004; Ferchaud and Hansen, 2016). Other studies have supported the ability of gene flow to impede the process of local adaptation (Storfer, 1999; Fedorka *et al.*, 2012). For example, research examining phenotype mismatching in the parsnip webworm *Depressaria pastinacella* determined that gene flow in worm populations led to increased trait mismatch frequency when grazing on allopatric wild parsnips (*Pastinaca sativa*) (Zangerl and Berenbaum, 2003). Similarly, research in the spider *Agelenopsis aperta* provided evidence that gene flow between woodland and desert habitats was associated with maladaptive behavioral traits (Riechert, 1993).

Despite the potential for gene flow to disrupt adaptive evolution, research also suggests a more complex and multifaceted role of gene flow with regards to adaptation. Theory suggests (Haldane 1930 ;Pinho and Hey, 2010), and experiments support, strong selection maintaining divergence and adaptive traits despite gene flow (Danley *et al.*, 2000; de Leon *et al.*, 2010; Sullivan *et al.*, 2014; Fitzpatrick *et al.*, 2015; Dennenmoser *et al.*, 2017; Kolora *et al.*, 2021). For example, work in water snakes (*Nerodia* spp.)(Rautsaw *et al.*, 2021), yeast (Tusso *et al.*, 2021) and crickets (Zhang *et al.*, 2021), has shown that significant adaptive divergence is possible despite gene flow. Further, some studies indicate that gene flow has the potential to facilitate adaptation, but the outcome is dependent on the strength and direction of selection over time and space. Several mechanisms may explain the potential benefits of gene flow for adaptative change (Garant, Forde and Hendry, 2007; Tigano and Friesen, 2016). First, gene flow increases the standing genetic variation of a sink population, thus giving selection additional material on which to act (Ingvarsson and Whitlock, 2000). This is important, as adaptation from standing variation has various advantages to adaptation from new mutations, and some studies indicate that standing variation is the primary driver of adaptation in many contexts (Barrett and Schluter, 2008; Karasov, Messer and Petrov, 2010). One recent example of this is the genetic rescue of inbreeding *Drosophila* populations during experimental evolution, in which gene flow alleviated deleterious behavioral traits and decreased fecundity (Jørgensen, Ørsted and Kristensen, 2022). Additionally, gene flow can lead to adaptive introgression by facilitating the spread of beneficial

alleles (Hedrick, 2013; Hawkins *et al.*, 2019; Taylor and Larson, 2019). Advantageous alleles may be introduced to the sink population at relatively high frequencies, increasing their probability of fixation relative to standing genetic variation or novel mutations. For instance, work investigating the spread of pesticide resistance in two-spotted spider mite (*Tetranychus urticae*) populations suggests that introgression through gene flow is likely responsible for the spread of a major resistance mutation (Shi *et al.*, 2019).

Ultimately, the fate of incoming alleles may be determined by the genetic architectures of both the migrants and the sink population (Tigano and Friesen, 2016). Allele effect size (Griswold, 2006; Yeaman and Otto, 2011; Yeaman and Whitlock, 2011), linkage between alleles (Bürger and Akerman, 2011; Feder *et al.*, 2012), recombination rates (Samuk *et al.*, 2017), and the number of loci involved in conferring an adaptive trait (Mackay, 2001) all contribute to the allele frequencies within a population. Epistatic interactions may determine the cost or benefit of incorporating novel alleles acquired via gene flow, rather than the additive benefit of an individual allele itself. These dynamics are most apparent in studies that observe outbreeding depression between diverged populations (Dolgin et al., 2007). Therefore, the outcome of selection in the presence of gene flow likely depends on the evolutionary history, and ultimately genetic architecture, of both the sink and source populations. Given the many differing predictions on the effects of gene flow on a population's evolutionary trajectory and the challenges of isolating the effects of gene flow in natural population, we set out to directly test the effects of gene flow on adaptation via experimental evolution. Here, we use the *Caenorhabditis elegans* - *Serratia marcescens* host – parasite system to test the effects of gene flow on adaptive evolution, as the system permits control of both gene flow and a population's evolutionary history.

In a previous experiment, Morran *et al*., 2011 divided a population of obligately outcrossing *C. elegans* into isolated groups and independently mutagenized them, thus creating genetically differentiated host populations. Each population was then split into two treatment groups, one with hosts exposed to live *S. marcescens* strain Sm2170 and a control with hosts exposed to heat-killed Sm2170 and evolved for 30 generations (Figure 1). Hosts passaged against heat-killed parasites showed no improvement in their ability to defend against Sm2170, while those passaged against live Sm2170 adapted to the parasites, exhibiting lower morality rates over time (Figure 2; Morran *et al.*, 2011; Penley, Ha and Morran, 2017). This knowledge of the genetic background and evolutionary history for each population allowed us to use experimental evolution to test the effects of gene flow, source population genetic background, and source population parasite exposure history (naïve or adapted), on the evolution of host defense within the sink population.

In this experiment, we utilized the beforementioned obligately outcrossing *C. elegans* host populations to evaluate the effects of one-way gene flow on host adaption to a non-evolving parasite. Beginning with a common sink population, we exposed host populations to either live or heat killed *S. marcescens* SM2170. After several exposures, host populations received gene

flow from one of several source populations (Figure 3 a&b). These source populations varied in whether they had previously adapted to SM2170 and in their genetic background relative to the sink population. Following several subsequent exposures to either live or heat killed Sm2170, we evaluated the effects of each treatment on mean mortality in the host populations. Thus, we determined how host defense evolution was influenced by gene flow, and we assessed effects of the source population on the evolutionary trajectory of the sink population. By investigating one-way gene flow, rather than symmetric sustained flow between populations, we sought to directly test the impact of alleles entering the population and their impact on host defense. We hypothesized that, 1) gene flow would facilitate adaptation to live Sm2170, relative to exposure without gene flow, and that 2) the benefit associated with gene flow would be stronger when gene flow originated from populations that had previously adapted to Sm2170. Further, we expected these results to be dependent on the migrant's genetic background. We predicted that, 3) shared backgrounds between the source and sink populations would provide the greatest benefits from gene flow. We hypothesized that alleles from shared backgrounds would maintain beneficial epistatic interactions and thus provide either the greatest benefit or least disruption (Griswold, 2006; Hansen, 2006).

MATERIALS AND METHODS

Host & Parasite Populations

C. elegans host populations were derived from the highly inbred and obligately outcrossing PX386 strain. Briefly, this strain was derived from the CB4856 strain (Morran, Parmenter and Phillips, 2009) and carries the fog-2 (q71) mutant allele, which prevents hermaphrodites from self-fertilizing (Schedl and Kimble, 1988). In a previous experiment, a population of PX386 nematodes was divided into multiple populations and each was independently mutagenized with 40 mM ethyl-methanesulfonate (EMS) to generate genetically variable populations prior to selection (Morran *et al.*, 2011). Populations were exposed to mutagenesis for four hours during three consecutive generations, inducing ~1,000 point mutations per lineage in each isolated population (Anderson, 1995). Following this process, populations were kept under standard laboratory conditions for four generations in order to purge the most deleterious mutations. This sequence created three populations, founded from one inbred population, with separate mutational backgrounds. These populations were maintained on 10cm Petri dishes filled with NGM Lite (Nematode Growth Medium-Lite, US Biological, Swampscott, MA, USA) and seeded with 30µL of *Escherichia coli* OP50 stored at 20°C.

The independently mutagenized populations were subsequently divided into different treatment groups, with each unique population represented within each treatment group. One treatment exposed host *C. elegans* to heat-killed Sm2170 as a control, while another treatment

exposed host populations to live Sm2170 (one-sided evolution) (Figure 1). Following 30 generations of experimental evolution, these host populations were frozen and stored at -80 °C. After experimental evolution, mortality rates were measured for each of the populations by assessing their ability to resist infection from SM2170. Additionally, fecundity and competitive fitness measurements were taken for each population (Morran *et al.*, 2011; Penley, Ha and Morran, 2017). Briefly, populations that were passaged with live SM2170 adapted to their parasites while those passaged with heat killed SM2170 did not, which is indicated by the comparatively low mortality rates recorded by the live evolution groups (Penley, Ha and Morran, 2017; Figure 2). Further, adaptation was driven by decreased mortality rates in host populations exposed to Sm2170, as opposed to changes in fecundity. In this study, we utilized the previously evolved Sm2170 naïve and Sm2170 adapted populations to investigate how gene flow, and source population evolutionary history, impact host adaptation to parasites. The populations we chose represent three independently mutagenized backgrounds (Groups A, B, and C in Figure 1). Each of the three independent backgrounds have one population which has undergone experimental evolution with live Sm2170 (adapted A, adapted B, and adapted C), and one which has been passaged with heat-killed SM2170 (naïve A, naïve B, and naïve C).

The bacterial parasite *S. marcescens* Sm2170 is known to be highly virulent toward *C. elegans* hosts (Schulenburg and Ewbank, 2004). Hosts become infected via feeding on *S. marcescens*-inoculated Petri dishes, and susceptible hosts often die within 48 hours. The Sm2170 used here was acquired from S. Katz at Rogers State University (OK, USA). *E. coli* strain OP50 is the primary laboratory food source of *C. elegans* and was acquired from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota. Both OP50 and Sm2170 were transferred from frozen stock to Luria Broth (LB) and grown overnight at 28°C; they were then used to seed 10cm Petri dishes filled with NGM-Lite and grown at 28°C overnight. Prior to each round of selection, colonies were selected from these Petri dishes and grown in 5 mL test tubes of LB for 24 hours at 28°C. Importantly, Sm2170 was not permitted to coevolve with host populations during experimental evolution. The Sm2170 in the one-sided treatments is in evolutionary stasis, as parasites cannot evolve a counter adaptive response to the host population (Brockhurst and Koskella, 2013).

Experimental Evolution of Host Populations

Experimental evolution was conducted using *Serratia* Selection Plates (SSP) as previously described (Penley, Ha and Morran, 2017). Briefly, SSPs consist of a 10cm Petri dish filled with autoclaved NGM Lite. One side of the plate was seeded with 35µl of E. *coli* while the other side was seeded with 35µl of either live (one-sided) or heat-killed (control) *S. marcescens*. 20 µl of ampicillin (100 mg/mL) was streaked across the plate between the bacterial lawns to prevent the spread of *S. marcescens* during the experiment. During experimental evolution, *C. elegans* were placed directly into the parasite bacterial lawn (alive or heat-killed) and required to

crawl through it to safely reach their food source (Morran, Parmenter and Phillips, 2009). After 48 hours, living individuals were transferred from the *E. coli* food source to a standard Petri dish seeded with *E. coli*. These plates also contained streptomycin to control the spread of *S. marcescens* and were seeded with the streptomycin resistant *E. coli* strain OP50-1 as a food source for the worms. Following three days on the dish, approximately 1000 individuals were moved from OP50-1 to the next round of selection on SSPs. These methods were used for each of the host populations in this experiment for 10 consecutive rounds of selection (Figure 3b). Importantly, these methods also select against *C. elegans* leaving the plate or any *Serratia* avoidance behaviors, as positive fitness within our experiment is dependent on being able to successfully navigate the parasite lawn and make it to the *E. coli* food source.

In total, the experiment consisted of 70 *C. elegans* populations (2 bacterial treatments × 7 gene flow treatments × 5 replicate populations per treatment). All host populations, except those that did not receive gene flow, received their migrants as they began their 5th passage on SSPs (Figure 3a). During this step, only 950 individuals were moved from the last round of selection instead of the normal count of 1000 individuals, and each population received approximately 50 migrants. The number of migrating individuals was chosen to enable sufficient gene flow into sink populations to reduce the strength of genetic drift relative to selection (Hartl and Clark, 2006). Gene flow came from the six host populations described in figure 1 (Overview in Figure 3a). In each of our treatments, sink populations were founded from Group A-naïve hosts. Each of these treatments, except the no migration treatment, then received immigrants from one of the previously evolved populations (Backgrounds A, B, and C, either adapted or naïve) (Figure 3). This allowed us to directly examine how adaptation proceeds in the sink population under gene flow from source populations with different mutational backgrounds and/or adaptive histories.

Mortality Assays & Statistical Analysis

Mortality assays were conducted following experimental evolution to determine the change in host resistance over time. Mortality assays were conducted on SSPs with methods similar to passaging methods used during experimental evolution. Additionally, these same methods were used to collect mortality rates in the previous work (Morran *et al.*, 2011; Penley, Ha and Morran, 2017). We use population level mortality as a measure of host defense. Within the context of our experiment, host fitness is primarily determined by survival following Sm2170 exposure and subsequent reproduction. To calculate mortality rates, approximately 200 individuals were placed onto the *S. marcescens* lawn and exposed for 48 hours (Figure 3). Following 48 hours, living individuals were counted and the mortality rate was determined using the formula $1 - (\frac{number\ of\ living\ worms}{number\ of\ worms\ plated})$. When performing mortality assays, each of the five replicate populations within each treatment had four technical replicates, totaling 280 assay plates. Importantly, our mortality assays did not differentiate between dead and unaccounted (lawn leaving or escape) hosts. However, within the context of our experimental evolution,

escape prevents reproduction and thus is functionally equivalent to host death. Further, we did not directly observe lawn leaving behavior in our assays. Mean mortality rates were analyzed using generalized linear models (GLM) fitted with a normal distribution and identity link function. We did not detect overdispersion using a Pearson test. Then, we tested for the effects of bacterial treatment (live or heatkilled), gene flow (no gene flow, adapted gene flow with a shared background, adapted gene flow with a novel background, naïve gene flow with a shared background, or naïve gene flow with a novel background), and the interaction between the two. We then performed post hoc contrast tests to compare differences within the model. Additionally, we analyzed our mortality data as a binomial distribution (scoring each individual as alive/unaccounted for) using a GLM fitted with a binomial distribution and logit link function. The results of the binomial vs normal GLM were qualitatively similar, but the normal GLM served as a more conservative measure by allowing us to analyze population means. Thus, we report the results of the normal GLM. All statistical analyses were performed in JMP Pro (v.16) (SAS Institute, Cary, North Carolina).

RESULTS

First, we sought to investigate how passaging *C. elegans* hosts on live Sm2170 versus heat-killed Sm2170 in the previous experiment (Morran *et al.*, 2011) impacted the *C. elegans* populations used in this study. This served two purposes. One, observing differences between mortality rates in the live versus heat-killed treatments supports our use of populations as "naïve" and "adapted" sources of gene flow (Figure 3a). Second, observing different mortality rates between groups indicates differences in their overall innate resistance to Sm2170. This provides evidence for relevant genetic differences between the populations, indicating that the initial EMS mutagenesis, and subsequent 30 generations of evolution, created differentiation between the populations (Figure 1). Using data from Penley, Ha, and Morran (2017), we found that hosts that had been passaged with live parasites, as compared to hosts that had been passaged on heat-killed parasites, exhibited significantly lower mortality rates when exposed to live Sm2170 (x_1^2= 21.327, P = x < .0001; Table 1; Figure 2). We also found different levels of parasite resistance across the differently mutagenized groups or backgrounds, (x_1^2= 21.709, P = x < .0001; Table 1; Figure 2). Together, these results enabled us to use these populations to examine the impact of one-way gene flow and evolutionary history on adaptation (Figure 3a).

To investigate the results of experimental evolution conducted within this study (Figure 3), we first tested for the evolution of elevated defense in host populations exposed to live Sm2170 relative to those passaged with heat-killed Sm2170. We found that host populations passaged with live Sm2170 exhibited significantly lower mortality rates when exposed to Sm2170 than did host populations which had been passaged with heat-killed Sm2170 (x_1^2= 7.022, P = 0.008; Table 2; Figure 4). This is indicative of adaptation to the parasite in our live

treatments, and a lack of such adaptation in our heat-killed treatments. Next, we tested the effect of gene flow on host mortality during exposure to Sm2170. We found that, across all treatments, there was no statistical difference between groups that received gene flow and those that did not when controlling for whether Sm2170 was alive or heat-killed (x_6^2= 1.488, *P* =0.9603; Table 2; Figure 4). However, the interaction of bacterial treatment and gene flow status was statistically significant (x_6^2= 22.278, *P* =0.0011; Table 2; Figure 4), indicating that the effect of gene flow on host defense was context dependent. We then ran contrast tests to further contextualize the relationship between gene flow status, gene flow source, and bacterial treatment.

To begin, we tested the impact of gene flow on host mortality in populations that had been passaged with live Sm2170. We found that populations that received gene flow during exposure to live Sm2170 exhibited significantly lower mortality rates when compared to populations which did not receive gene flow while being passaged on live Sm2170 (x_1^2= 14.345, *P* = x < 0.001; Table 2; Figure 4). This demonstrates the ability of gene flow to facilitate host adaptation to parasites. Next, we examined the impact of gene flow source resistance on host mortality rates for sink populations passaged in the presence of live Sm2170. We found that populations that received gene flow from previously adapted populations exhibited significantly lower mortality rates when compared to host populations that received gene flow from naïve populations (x_1^2= 20.798, *P* = x < 0.0001; Table 2; Figure 4). This is potentially indicative of beneficial alleles being transferred from previously adapted populations, and further supports the idea that these populations evolved elevated resistance to Sm2170 during the previous experiment (Morran et al. 2011). We then evaluated the impact of source population genetic background on the resulting adaptation of the sink population. We found that populations that received gene flow from sources that did not share their genetic background (novel populations) exhibited greater resistance against Sm2170 than those that received gene flow from the shared background (x_1^2= 11.505, *P* = x < 0.001; Table 2; Figure 4). We further tested for differences between host populations that received gene flow from adapted populations with shared genetic backgrounds versus those that received migrants from adapted novel genetic backgrounds. Here, we found that sink populations adapted at greater rates when receiving gene flow from previously adapted source populations with novel backgrounds (x_1^2= 8.500, *P* = x < 0.0035; Table 2; Figure 4). Lastly, we found that populations that received gene flow from naïve populations with novel backgrounds exhibited lower mortality rates than those which received naïve gene flow from shared backgrounds (x_1^2= 3.860, P =0.04817; Table 2; Figure 4). This is likely indicative of the benefits of additive genetic variation during the adaptive process in host populations.

DISCUSSION

In this study, we investigated the impact of gene flow and source population on host adaptation to non-adapting parasites. We predicted that (1) gene flow would facilitate increased host defense relative to populations that did not receive gene flow. Further, we hypothesized that the benefits of gene flow during adaptation would be most pronounced when gene flow came from (2) adapted populations, and those with a (3) shared genetic background. Overall, we found that (1) gene flow facilitated host adaptation to the parasite *S. marcescens* via the evolution of elevated host defense, whereas gene flow provided no benefit in the absence of parasite exposure (Table 2; Figure 4). Further, the benefit of gene flow was dependent on both the parasite exposure and the evolutionary history of the source population. As predicted, (2) gene flow from previously adapted populations resulted in the greatest increase in host defense. However, contrary to our predictions, we observed that (3) gene flow from novel backgrounds facilitated greater reductions in host mortality than gene flow from populations with shared genetic backgrounds (Table 2; Figure 4). Therefore, we found that gene flow can facilitate adaptation, but the effects of gene flow can be context-dependent and influenced by the evolutionary history of the sink and source populations.

Notably, gene flow had the most beneficial effect on adaptation in host populations receiving gene flow from parasite-adapted novel backgrounds (Figure 4). This demonstrates that the fitness effects of beneficial alleles evolved in the source populations were at least somewhat independent of the genetic background in which they evolved. Presumably, gene flow permitted the introduction of novel alleles conferring greater host resistance, which facilitated an increased rate of adaptation. Importantly, we did not observe any detriment to gene flow from novel backgrounds that would indicate strong epistatic effects underlying increased host resistance. This overall benefit of gene flow from novel backgrounds may be the result of overall genetic similarity between all of our host populations. Indeed, while host populations differed in their evolutionary histories and overall resistance (Figures (1 & 2), host populations used in this experiment were all derived from a CB4856 background (Morran, Parmenter and Phillips, 2009). While EMS mutagenesis infused the populations with genetic variation and experimental evolution permitted divergence, the groups started with a relatively uniform background. However, gene flow from populations with more divergent backgrounds could cause a greater impediment for adaptation. Consistent with this idea, various studies provide evidence that natural populations of *C. elegans* may commonly suffer from outbreeding depression (Dolgin *et al.*, 2007; Anderson, Morran and Phillips, 2010; Gimond *et al.*, 2013; Snoek *et al.*, 2014, but see Crombie *et al.*, 2019), suggesting that differing populations of *C. elegans* in nature may be diverged to the point that gene flow impairs adaptation.

While models are far more common than experiments in assessing the impact of gene flow, our results are consistent with the results of other experiments assessing one-way gene flow into sink populations. A previous experiment evaluating the role of gene flow in increasing adaptive potential found that populations of *Drosophila* that received gene flow showed a 30-40% increase in trait response during laboratory evolution (Swindell and Bouzat, 2006). Our

results further demonstrate that gene flow can facilitate adaptation to directional selection, but also indicate that the response to selection can depend upon the source population. We observed a benefit to gene flow from adapted populations compared to those that were Sm2170 naïve (Figure 3), suggesting that the alleles conferring increased resistance carried by the migrants were responsible for the increased rate of adaptation in the source population. It is important to note that while each treatment group received an equal number of migrants (opportunity for gene flow), the level of actual gene flow between treatments may have varied depending upon the source population. Just as in natural populations, migrants within our experiment must survive in the environment and successfully mate to integrate their alleles into the sink population. Thus, compared to adapted populations, it is likely that naive populations contributed less gene flow to their sink population. Further individual migrants carrying alleles that conferred greater resistance likely disproportionately contributed to gene flow. Therefore, the influx of beneficial alleles likely drove the increase in host defense within our populations.

One limitation of this study is our use of one-directional gene flow as opposed to two-way gene flow between adapting populations. In terms of its impact on variation, gene flow generally works to increase variation within populations while decreasing between population variation (Slatkin, 1987; Hendry, Day and Taylor, 2001; Lenormand, 2002; Garant, Forde and Hendry, 2007). Many of the presumed deleterious effects of gene flow on adaptation, like the breakdown of local adaptation, are dependent on the exchange of alleles between populations and a degree of environmental antagonism in their fitness effects (Dias, 1996). As such, one-directional gene flow may be biased toward positive effects during adaptation. Another limitation is that gene flow only occurred once during our experiment. Populations were allowed to adapt to their parasites, received gene flow, and were subsequently exposed again. This may have allowed selection to limit the spread of maladapted alleles more effectively, thus allowing for greater fitness benefits. Under repeated unidirectional gene flow, following the classic Island-mainland model, maladapted alleles may persist longer in the sink population, leading to less adaptation in the hosts (Lenormand, 2002). However, continuous gene flow may have also added adaptation depending on the primary adaptive mechanism working in the sink population. For example, in populations receiving previously adapted migrants, a continuous flow of preadapted alleles may have caused greater proliferation of those alleles and more rapid adaptation.

Conceptually, the one-way gene flow utilized here is perhaps most analogous to assisted gene flow (ASG), or the purposeful movement of gametes already adapted to an environment to populations currently undergoing adaptation to a changing environment (Aitken and Whitlock 2013). ASG has most notably been used to restore populations of the Florida panther (*Puma concolor*)(Johnson *et al.*, 2010; Hostetler *et al.*, 2013) and has been suggested as a potential technique to combat species loss due to anthropogenic environmental change in a range of organisms (Aitken and Whitlock, 2013). These include, but are not limited to, salmon (Pregler *et al.*, 2022), koalas (Seddon and Schultz, 2020), sea corals (Hagedorn *et al.*, 2021), and various species of amphibians (Byrne and Silla, 2022). For certain species, like long-lived forest trees,

this may present the most effective strategy to mitigate species loss (Aitken and Bemmels, 2016). Resistance alleles may also be able to spread this way; however, their impact on the population will also depend on the nature of the evolutionary interaction that the population is engaged in. For example, in antagonistically coevolving systems where populations are chasing moving peaks across the fitness landscape (Thompson, 2009), interactions between genes are also important, and so genetic architecture will impact the fate of an immigrating allele (Hansen, 2006; Bürger and Akerman, 2011; Akerman and Bürger, 2014). This adds an additional layer of complexity and has been reflected in studies of gene flow in coevolving systems, as they show a multitude of effects ranging from positive to negative (Garant, Forde and Hendry, 2007).

In this experiment, we found that gene flow into populations facilitated adaptation to a fixed bacterial parasite. Further, we found that the benefit of gene flow was contingent upon the evolutionary history of the source population and the environment of the sink population. This result aligns with past research that has indicated potential advantages to gene flow during adaptation. Further, despite the breadth of work investigating the role of gene flow in evolutionary biology, this work highlights the complexity of predicting the effects of gene flow on sink populations. Overall, gene flow is relatively common, yet intricate, evolutionary force that merits much further theoretical and empirical investigation.

COMPETING INTERESTS

The authors declare no competing interests.

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FIGURE & TABLE LEGENDS

Figure 1. Migrant Evolutionary History
Experimental evolution history of each background (Adapted from Morran *et al.*, 2011). A population of C. elegans was divided into three groups and then mutagenized to infuse standing variation (creating three distinct genetic backgrounds). Each group (A, B, & C) was then split into two treatments and exposed to either heat-killed, or live *S. marcescens* Sm2170, for 30 generations. The resulting parasite naïve hosts served as naïve gene flow sources in this experiment, while the live parasite exposed hosts served as adapted gene flow sources. Created with BioRender.com.

Figure 2. Host Mortality Rates from Previous Experiment
Average mortality rate for each population following their previous experimental evolution (Adapted from (Penley, Ha and Morran, 2017). Each open circle represents a single mortality assay replicate and black circles represent the average mortality rate for the treatment population. Each error bar is constructed using one standard error from the mean. Groups A, B, & C refer to different mutational backgrounds, as described in figure 1. Control populations were passaged with heat killed parasite, while live treatments were passaged with normal Sm2170. Asterisks

designate statistically significant differences between groups (< 0.001). Full statistics summarized in Table 1.

Figure 3. Experimental Overview

a. Overview of Treatments & Significance. Group A Naïve worms (Figure 1) were divided into seven treatment groups before the start of the experiment. Each treatment group consisted of 10 replicate populations, with five passaged against heat-killed Sm2170, and five against live Sm2170. Each treatment group, outside of the no migration control, received gene flow during the 5th passage. Gene flow varied in whether it came from Sm2170 naïve or Sm2170 adapted sources, and whether populations had a shared background with the sink population (Group A) or different background (Groups B&C). **b. Passaging Methodology.** Each host sink population began with ~1000 individuals from a previous experiment where they had been exposed to heat-killed Sm2170 for 30 generations (Group A Naïve worms; Figure 1) (Morran et al. 2011). Populations were then passaged on *Serratia* Selection Plates with either live or heat-killed Sm2170 for 4 passages. In each round of passaging ~1000 individuals were moved randomly. On passage 5, experimental populations received 50 migrants from one of 7 source populations. Groups receiving migrants received 950 individuals from their previous round of passaging, while control groups received the usual 1000. After passage 5, populations were passaged for 5 additional generations. Created with BioRender.com.

Figure 4. Host Mortality Rates

For each mortality assay 200 worms were exposed to Sm2170 for a period of 48 hours using *Serratia* Selection Plates. Surviving worms were counted and the mortality is expressed as ((worms plated – worms counted)/ worms plated). Each open circle represents the average mortality rate of 3 replicate assays for a given replicate population. Black circles represent the average mortality rate of all host populations within a given treatment. Each error bar is constructed using one standard error from the mean. For reference, shared background refers to migration from populations which have a shared mutagenized background group (Refer to Figure 1). Novel backgrounds do not share this origin with the sink population. All sink populations started from Group A naïve worms. Novel background I refers to populations from Group B, while novel background II refers to populations with a Group C background. Adapted populations have previously been passaged with live Sm2170, while naïve populations have not. Full statistics in Table 1. Each treatment column has received a designator from a-n to allow for ease of comparison with the statistics table.

Table 1. Statistical Values for Previous Migrant Adaptation

Table 2. Statistical Values for Gene Flow & *Serratia*

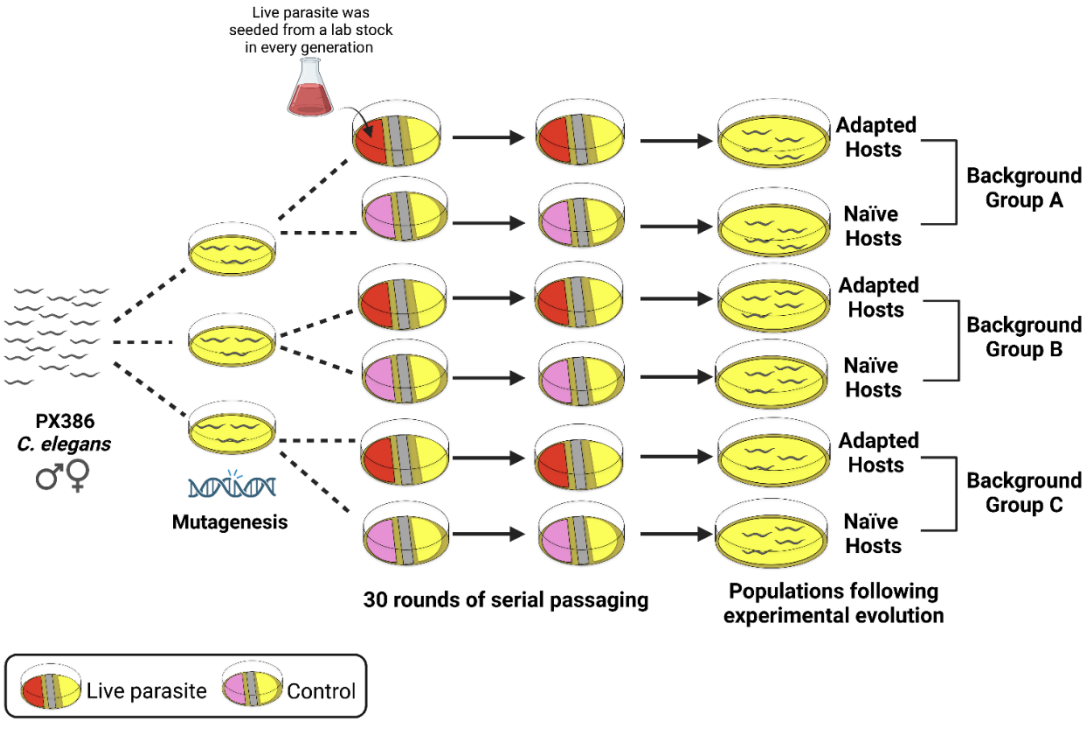
Table 1. Statistical Values for Previous Migrant Adaptation

Data from Penley, Ha and Morran, 2017. Shows the difference in mortality rates for treatments after experimental evolution described in Morran *et al.*, 2011. Treatment refers to whether populations were exposed to heat-killed or living Sm2170. Background refers to whether the populations came from mutagenized background group A, B, or C. Mean mortality rates were analyzed using generalized linear models (GLM) fitted with a normal distribution and identity link function.

Table 2. Statistical Values for Host Gene Flow, *Serratia* status, and Contrast Tests

Difference in mortality rates for treatments after exposure to heat-killed, or living, *Serratia marcescens* Sm2170. *Serratia* refers to population exposure to living or heat killed parasite, while gene flow refers to presence or absence of gene flow. Contrast tests compare the treatments passaged on live Sm2170. Mean mortality rates were analyzed using generalized linear models (GLM) fitted with a normal distribution and identity link function. Letters correspond to the columns being compared in Figure 4.

FIGURES



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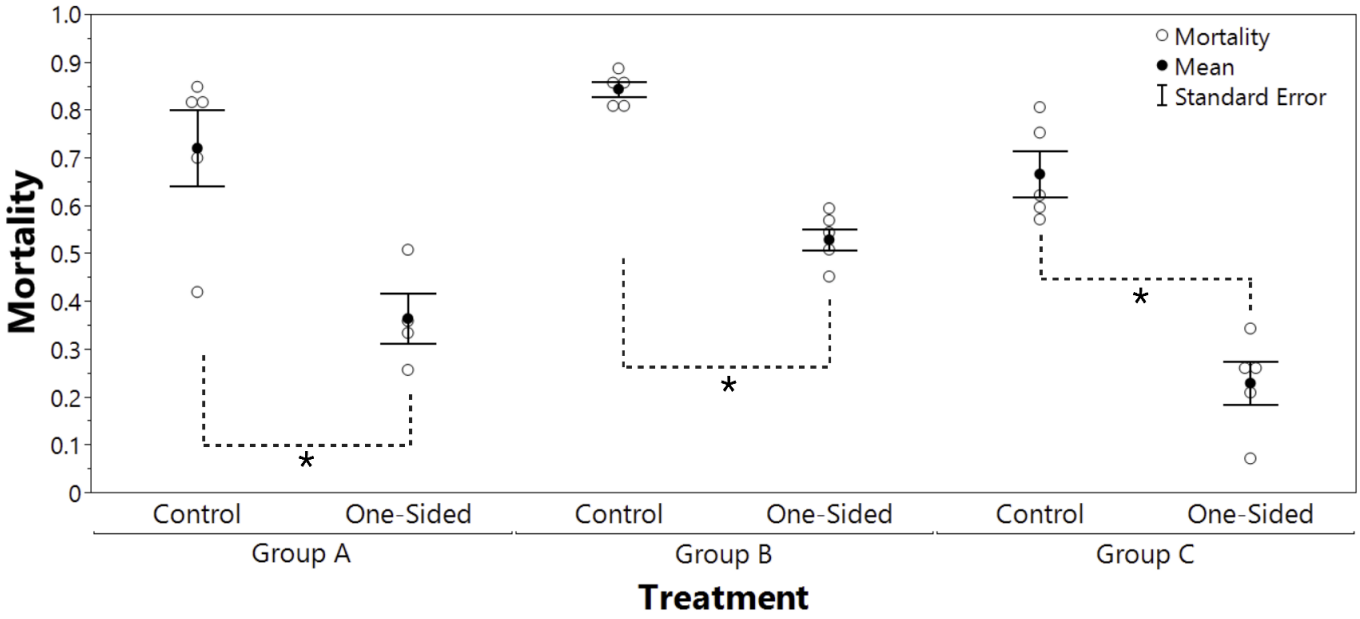

Each error bar is constructed using 1 standard error from the mean.

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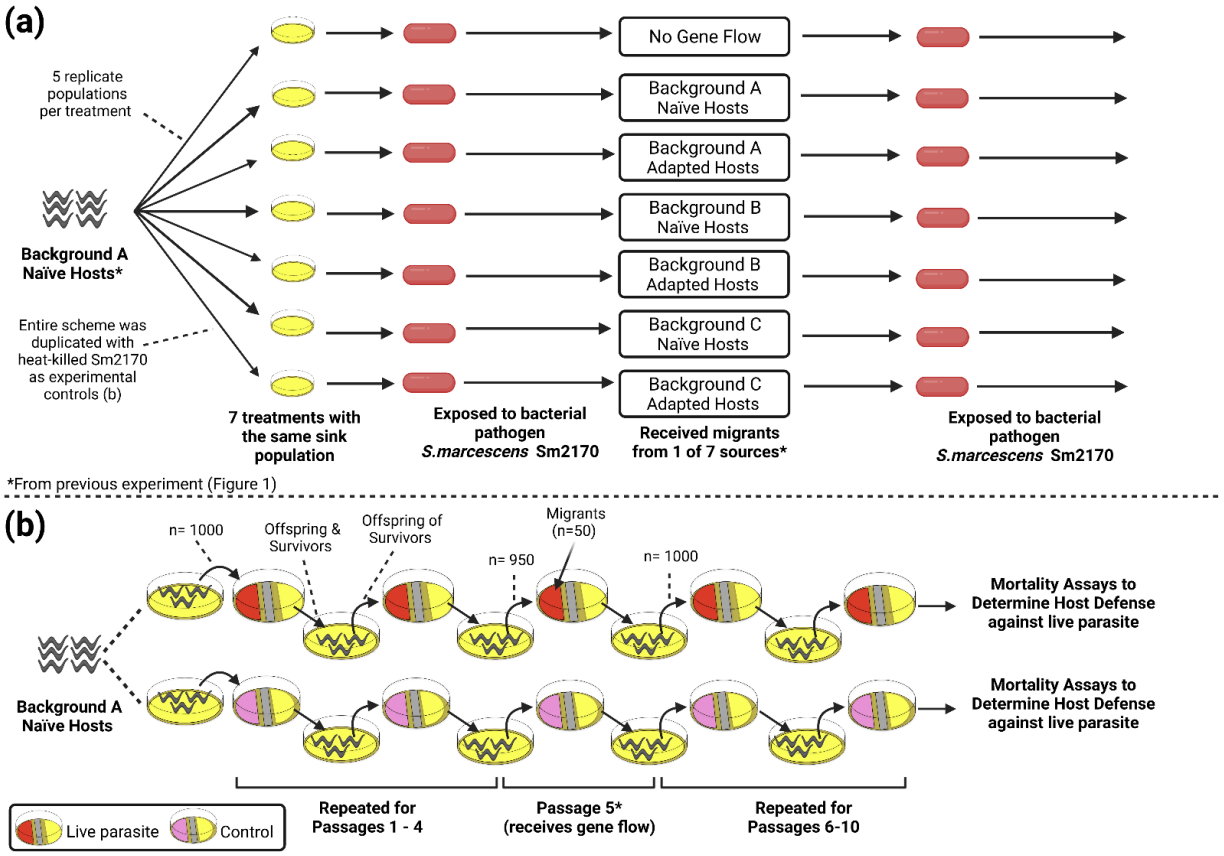
(a)
5 replicate populations per treatment
Background A Naïve Hosts*
Entire scheme was duplicated with heat-killed Sm2170 as experimental controls (b)
7 treatments with the same sink population
Exposed to bacterial pathogen S.marcescens Sm2170
No Gene Flow
Background A Naïve Hosts
Background A Adapted Hosts
Background B Naïve Hosts
Background B Adapted Hosts
Background C Naïve Hosts
Background C Adapted Hosts
Received migrants from 1 of 7 sources*
Exposed to bacterial pathogen S.marcescens Sm2170
*From previous experiment (Figure 1)
(b)
n= 1000
Offspring & Survivors
Offspring of Survivors
n= 950
Migrants (n=50)
n= 1000
Background A Naïve Hosts
Mortality Assays to Determine Host Defense against live parasite
Mortality Assays to Determine Host Defense against live parasite
Live parasite
Control
Repeated for Passages 1 - 4
Passage 5* (receives gene flow)
Repeated for Passages 6-10

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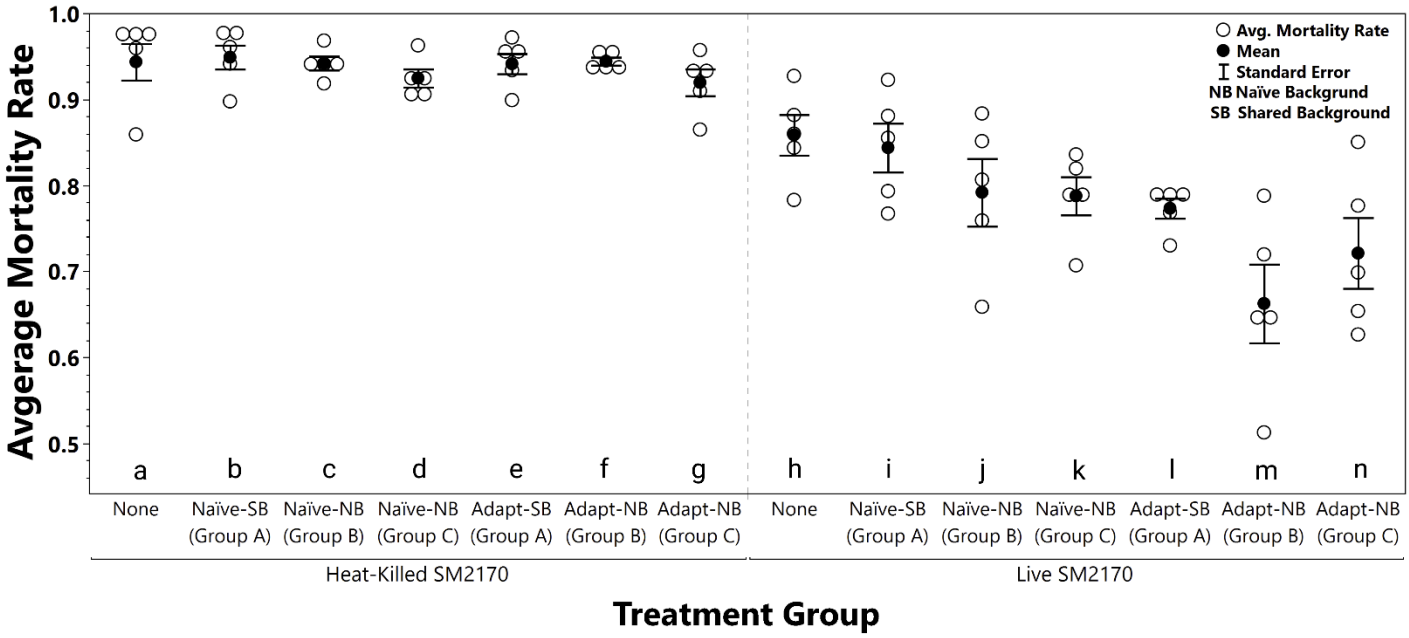

Each error bar is constructed using 1 standard error from the mean.

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755 **Table 1.**

Effect Tested	Degrees of Freedom	Chi-square	Prob> Chi-square
Treatment	1	21.327	*P*= x < 0.001
Background Group	2	21.709	*P*= x < 0.001
Treatment * Background Group	2	2.077	*P*= 0.3540

756 **Table 2.**

Effect Tested	Degrees of Freedom	Chi-square	Prob> Chi-square
Serratia (live or heat-killed) (a-g vs. h-n)	1	7.022	*P*= 0.0081
Gene Flow (Received or did not) (a&h vs. b-g&i-n)	6	1.488	*P*= 0.9603
***Serratia* * Gene Flow interaction**	6	22.278	*P*= 0.0011
Contrasts Tests			
Effect Tested	**Degrees of Freedom**	**Chi-square**	**Prob> Chi-square**
No Gene Flow vs. Gene Flow (live *Serratia*; h vs i-n)	1	14.345	*P*= 0.0001
Adapted vs. Naïve Gene flow (live *Serratia*; i-k vs. l-n)	1	20.798	*P*= x < 0.001
Novel vs. Shared Background Gene Flow (live *Serratia*; jkmn vs. il)	1	11.505	*P*= 0.0007
Naïve Shared background vs. Naïve Novel Background (live *Serratia*; i vs. jk)	1	3.860	*P*= 0.04817
Adapted Shared background vs. Adapted Novel Background (live *Serratia*; l vs. mn)	1	8.500	*P*= 0.0035

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