Sex and gene flow modulate evolution during range expansions in the protist *Tetrahymena thermophila*

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Abstract

At species' range edges, individuals often face novel environmental conditions that may limit expansion until populations adapt. The potential to adapt depends on genetic variation upon which selection can act. However, populations at species' range edges are often genetically depauperated. One mechanism to increase genetic variation is to reshuffle existing variation through sex. During range expansions, sex can, however, act as a double-edged sword. The gene swamping hypothesis predicts that for populations expanding along an abiotic gradient, sex can hinder adaptation if asymmetric dispersal leads to numerous maladapted dispersers from the range core swamping the range edge. In this study, we experimentally tested the gene swamping hypothesis by performing replicated range expansions in landscapes with or without an abiotic pH-gradient, using the ciliate *Tetrahymena thermophila*, while simultaneously manipulating the occurrence of gene flow and sex. We show that sex accelerated evolution of the intrinsic rate of increase in absence of gene flow, but hindered it in presence of gene flow. The effect of sex, however, was independent of the pH-gradient. Conversely, sex and gene flow did not affect expansion distance, possibly due to the discrete landscape structure. Overall, our results suggest that gene swamping can affect adaptation in life-history strategies.

Introduction

Individuals living at the edge of a species' range face different conditions compared to those in the core region. For example, selection pressures differ, and often the individuals at the edge represent a small subset of a species' genetic variation [1]. The potential of a population to spread depends on its capacity to disperse, as well as on being able to grow in the local abiotic environment [2]. Consequently, when populations continue expanding, they may experience strong selection due to the range expansion itself, and are affected by concurrently changing environmental conditions.

During range expansions, populations can undergo rapid evolution, as demonstrated by recent comparative and experimental work [1], showing evolution of increased dispersal [3,4,5,6], r-selected life-history strategies [7,8], and adaptation to abiotic conditions [9,10]. Simultaneously adapting to multiple selective pressures can be challenging, but the individuals that manage to successfully establish and grow beyond the existing range typically reap massive benefits by escaping competition with conspecifics.

A major modulator for evolution is sex. Sex allows populations to reshuffle existing genetic variation, thus creating new variants that may be more fit [11,12,13,14]. Under conditions where populations face multiple abiotic stressors, or environments with heterogeneously distributed resources, sex is a favoured strategy, facilitating adaptation in populations [15,16]. This reshuffling advantage may be especially pertinent when standing genetic variation is low. This is typically the case for range edge populations, which are genetically depauperated due to repeated founder events [1,17].

The role of sex during range expansions is a double-edged sword, however. On the one hand, range expansion entails strong stochasticity due to repeated founder events, leading to neutral and maladaptive mutations becoming fixed and surfing along at the range edge [18,19]. Sex can break the linkage between adaptive genes and such maladaptive mutations [20,19, 21]. On the other hand, theory on gene swamping predicts that, during range expansion in a landscape with an abiotic gradient, sex may hinder adaptation when many dispersers move from the core population to the range edge [22,23,24,25,26]. This asymmetrical dispersal floods the range edge with individuals maladapted to the edge's abiotic conditions. If individuals

reproduce sexually, this can swamp the genepool at the range edge with maladapted genes. When strong enough, this swamping effect could prevent the population from adapting to the abiotic environment, and hence slow down and even stop further range expansion [23,24]. On the contrary, when drift strongly reduces adaptive variation, dispersal may positively affect adaptation by counteracting the effects of drift [25,26]. Gene swamping has been suggested as a mechanism leading to stable range borders. Despite the extensive theory on gene swamping, surprisingly little empirical and experimental work exists [27,28,29,30].

Here, we experimentally tested the gene swamping hypothesis using the ciliate *Tetrahymena thermophila*. We assessed how reproductive strategy (asexual or sexual reproduction) and gene flow (i.e., dispersal from the range core to the range edge) altered evolutionary adaptation during range expansions in landscapes with or without an abiotic pH-gradient. We found a distinct signal of gene swamping, where sex facilitated or hindered adaptation depending on the presence or absence of gene flow.

Material and methods

Study organism

Tetrahymena thermophila is a freshwater ciliate commonly used in ecological and evolutionary experiments [31,32,33,34,35]. We used four phenotypically divergent [36] clonal strains of *T. thermophila* obtained from the Tetrahymena Stock Center: strain B2086.2 (Research Resource Identifier TSC_SD00709), strain CU427.4 (TSC_SD00715), strain CU428.2 (TSC_SD00178) and strain SB3539 (TSC_SD00660).

Experiment

Microcosms

We performed all experimental work (experimental evolution and bioassays) in a 20 °C climate-controlled room. Following an established method [4], we used a sliding window approach to simulate the front of experimental range expansions using two-patch landscapes, which con-

sisted of two 25 mL Sarstedt tubes connected by an 8 cm long silicone tube (inner diameter 4 mm). We controlled dispersal between the patches by opening or closing plastic clamps on the silicone tubes. We measured dispersal as movement of cells from one patch (home patch) to the other patch (target patch). We subsequently exposed the dispersed part of the population to a new two-patch landscape, representing episodic dispersal across a discretized linear landscape.

We prepared 40 two-patch landscapes, and filled both patches of each landscape with 15 mL modified Neff-medium [37]. We complemented the medium (for experimental evolution and bioassays) with 10 $\mu g\,mL^{-1}$ Fungin and 100 $\mu g\,mL^{-1}$ Ampicillin to keep cultures axenic. We then inoculated one patch of each two-patch landscape with 200 µL of ancestor culture (50 µL from each of the four ancestral strains, maintained in the same medium and temperature conditions as the experiment) at the start of experimental evolution.

Treatment groups

We designed a full-factorial experiment, testing the effect of 1) abiotic pH conditions, being either constant or forming a gradient (”Const”: pH homogeneous at 6.5, ”Grad”: pH decreases by 0.5 every two to three successful dispersal events until a minimum of 4.0), 2) reproductive strategy (”Asex”: pure asexual reproduction, ”Sex”: regular sexual reproduction) and 3) gene flow (”NoGF”: no gene flow; ”GF”: regular gene flow to range edge).

Experimental evolution

We performed a range expansion experiment lasting ten weeks. During the experiment, we repeated the same procedure every 14 days. On every 1^{st}, 3^{rd}, 5^{th}, 10^{th} and 12^{th} day of each procedure cycle, we initiated dispersal by opening the clamps in the two-patch landscapes for one hour. After dispersal, we prepared 40 new two-patch landscapes. If population density was measurable ($\geq$1 cell observed during video analysis, see below) in the target patch, we transferred the content of the target patch to a new two-patch landscape. If no measurable dispersal occurred, we transferred the content of the home patch to the new two-patch landscape.

Every 8^{th} day in each 14-day cycle, we simulated additional long-distance gene flow (from

the range core to the edge, following theoretical predictions [23,24]) in the populations with a gene flow ("GF") treatment, by removing 1.5 mL of culture, and replacing it with 1.5 mL of culture with the same density and proportions of the four ancestral clones as used at the start of the experiment.

To initiate sex, we transferred all populations on each 8th day of the cycle (after gene flow) to a 10 mM Triss-solution for starvation, as *T. thermophila* only mate when starved [38] (protocol in Supplementary Material section S2) and incubated the starvation cultures on a shaker rotating at 120 rpm. After 36 hours, we placed the populations with a sexual reproduction ("Sex") treatment off the shaker, but kept populations with an asexual reproduction ("Asex") treatment on the shaker, as shaking prevents cells from conjugating/mating (tested during pilot experiments; no cell conjugation occurred when mating cultures were kept on a shaker, off the shaker almost all cells conjugated). We left cells to mate overnight, after which we transferred populations back to new two-patch landscapes. Thus, "Sex" and "Asex" treatments experienced the same nutrient availability.

Common garden

After experimental evolution, we collected 100 µL culture from all surviving populations, and transferred the cells to 25 mL Sarstedt tubes containing 15 mL Neff-medium at pH 6.5. We maintained these populations in the common garden for 72 hours before starting bioassays, to avoid epigenetic and trans-generational effects.

Bioassays

We tested performance (population growth) of ancestral and evolved populations (after common garden) under eight pH values (pH 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5 and 3.0). Specifically, we prepared for every population Sarstedt tubes containing Neff-medium with adjusted pH, and inoculating it with 100 µL of culture from the evolved/ancestral populations. We grew these populations for 12 days, sampling populations twice on the first two days, and once per day on all subsequent days. Every two days, we replaced 1 mL of culture with fresh medium to prevent population decline during bioassays.

Samping and video analysis

We measured population density and cell characteristics (morphology and movement) using an established method [32,39]. We sampled 200 µL of culture from every population, and diluted samples 10-100 fold in Neff-medium to ensure densities were similar, as excessive density prevents accurate video analysis. We then took 10 s videos (250 frames, 25 fps) using a Leica M165FC stereomicroscope and top-mounted Hamamatsu Orca Flash 4.0 camera. We analyzed videos using the BEMOVI R-package [39] (parameters in Supplementary Material section S4).

Beverton-Holt model fitting

To analyze local adaptation, we assessed growth rates by fitting a continuous-time version of the Beverton-Holt model [40], as this model is well-suited for microcosm data and facilitates biological interpretation of parameters [41,42]. The Beverton-Holt model is given by the equation:

$$\frac{dN}{dt} = \left(\frac{r_0 + d}{1 + \alpha N} - d \right) N \tag{1}$$

where the intraspecific competitive ability (α) is equal to:

$$\alpha = \frac{r_0}{\widehat{N} d} \tag{2}$$

where r_0 is the intrinsic rate of increase, N the population size, α the intraspecific competitive ability, $\widehat{N}$ the equilibrium population density and d the death rate of the population. We estimated the parameters using a Bayesian approach adapted from Rosenbaum et al. [43]. For model code see `https://zenodo.org/record/2658131`

Statistical analysis

All statistical analyses were performed with the R language for statistical computing, version 3.5.1.

We assessed how survival during range expansion was affected by reproductive strategy, abiotic pH-gradient and gene flow using Bayesian generalized linear models (binomial distri-

bution) with the 'Rethinking'-package (version 1.59). We created all possible models, and then used the Watanabe-Akaike information criterion [44] comparison to calculate relative importance of the independent factors and weighted model predictions.

We tested for local adaptation by assessing changes in the intrinsic rate of increase r_0 of the evolved populations under the pH conditions they experienced during evolution, compared to the ancestor under the same pH conditions. This was done by dividing the r_0 estimates of evolved populations by the mean r_0 of the mixed ancestral populations (populations with the initial ancestral genotype mixture), and by subsequently calculating the logarithm (base 2, for ease of interpretation) of this ratio (log-ratio response). We then fit a full interaction model ('stats'-package) containing abiotic pH-gradient, reproductive strategy and gene flow as factors, and used the dredge function ('MuMin'-package, version 1.43.6) to select the model with lowest AICc (Akaike Information Criterion, corrected for small sample size [45]) score. We calculated relative importance (RI) of independent factors as the sum of the model weights of models that included this factor, and reported statistical output of the best-fitting model.

Results

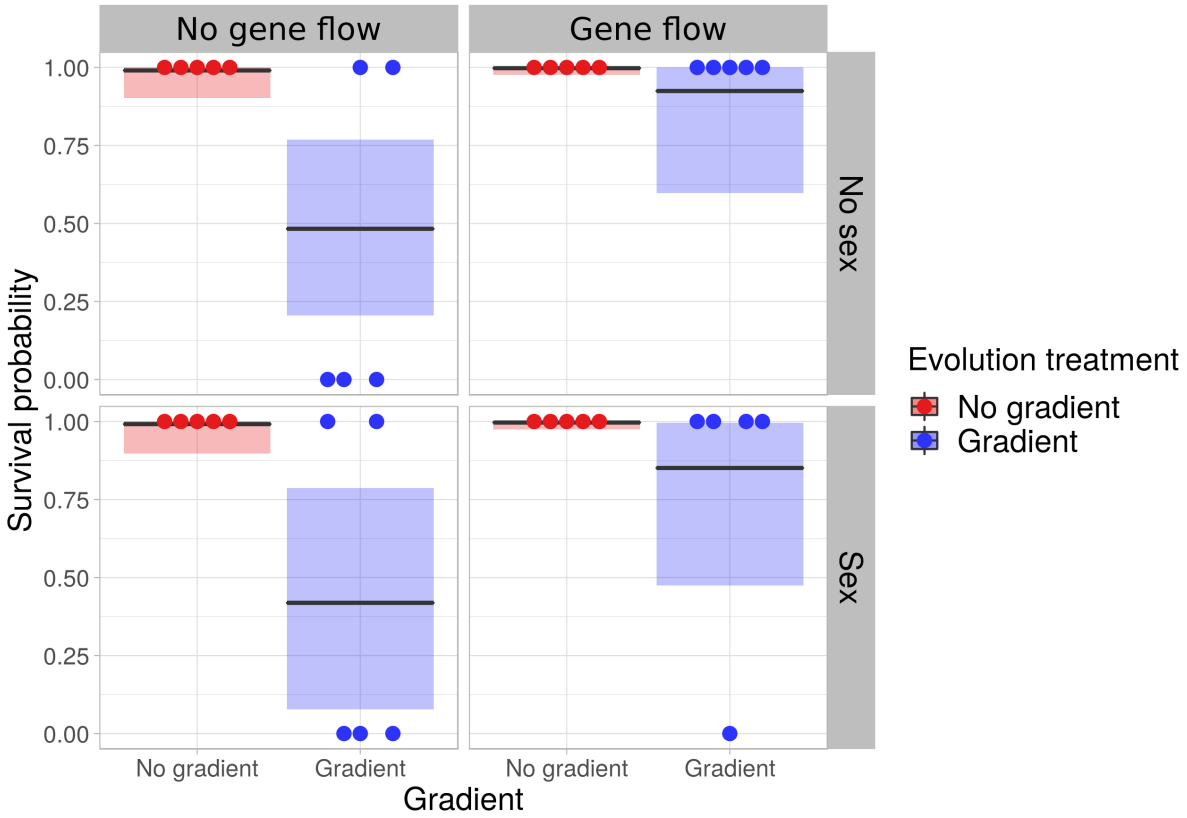

Figure 1: Survival of populations at the end of the range expansion experiment. Dots represent individual datapoints of populations that either survived (1) or went extinct (0). The boxes show the weighted predictions of survival based on the WAIC weighting on all Bayesian survival models, with the line representing the mean, and the range of the boxes the 95 % probability interval. Blue dots and boxes represent the populations expanding in a landscape without a pH gradient (”Const”) and red dots and boxes the ppulations expanding in a pH gradient (”Grad”).

Of the 40 range expansions, 33 were successful, whereas the other seven went extinct (Fig. 1). Extinctions only happened during range expansions into a gradient, but were largely counteracted by gene flow (Fig. 1; see also section S6.6 in Supplementary Material). Local adaptation (Table 1) depended on abiotic pH-gradient (RI=0.998), reproductive strategy (RI=0.880), gene flow (RI=0.898) and the interaction between reproductive strategy and gene flow (RI=0.815). Although model selection showed no absolute preference for a single model, all considered models (δAICc$<$2.0) contained these factors (model comparison table in section S6.1 of Supplementary Material). The best model (Tab. 2; Fig. 2) showed that populations expanding into a gradient (”Const”) evolved only marginally increased local adaptedness, whereas populations that expanded into a gradient (”Grad”) greatly increased local adaptation ($F_{1,29}$=128.67,

$p<0.0001$). Although the factors reproductive strategy ($F_{1,29}=0.48$,$p=0.49$) and gene flow ($F_{1,29}=2.77$, $p=0.11$) individually only marginally increased local adaptation, their interaction was significant and strongly decreased local adaptation ($F_{1,29}=10.67$, $p=0.0028$, Fig. 2, Tab. 2), with populations evolving higher intrinsic rates of increase either when there was sex without gene flow (”Sex”, ”NoGF”), or gene flow without sex (”Asex”, ”GF”).

Independent factor	**RI**
Abiotic pH-gradient	0.998
Gene flow	0.898
Reproductive strategy	0.880
Reproductive strategy * Gene flow	0.815
Abiotic pH-gradient * Gene flow	0.387
Reproductive strategy * Abiotic pH-gradient	0.284
Abiotic pH-gradient * Reproductive strategy * Gene flow	0.021

Table 1: Relative importance (RI) of the different independent variables obtained with AICc comparison of all possible models testing local adaptation as the evolution of intrinsic rate of increase r_0.

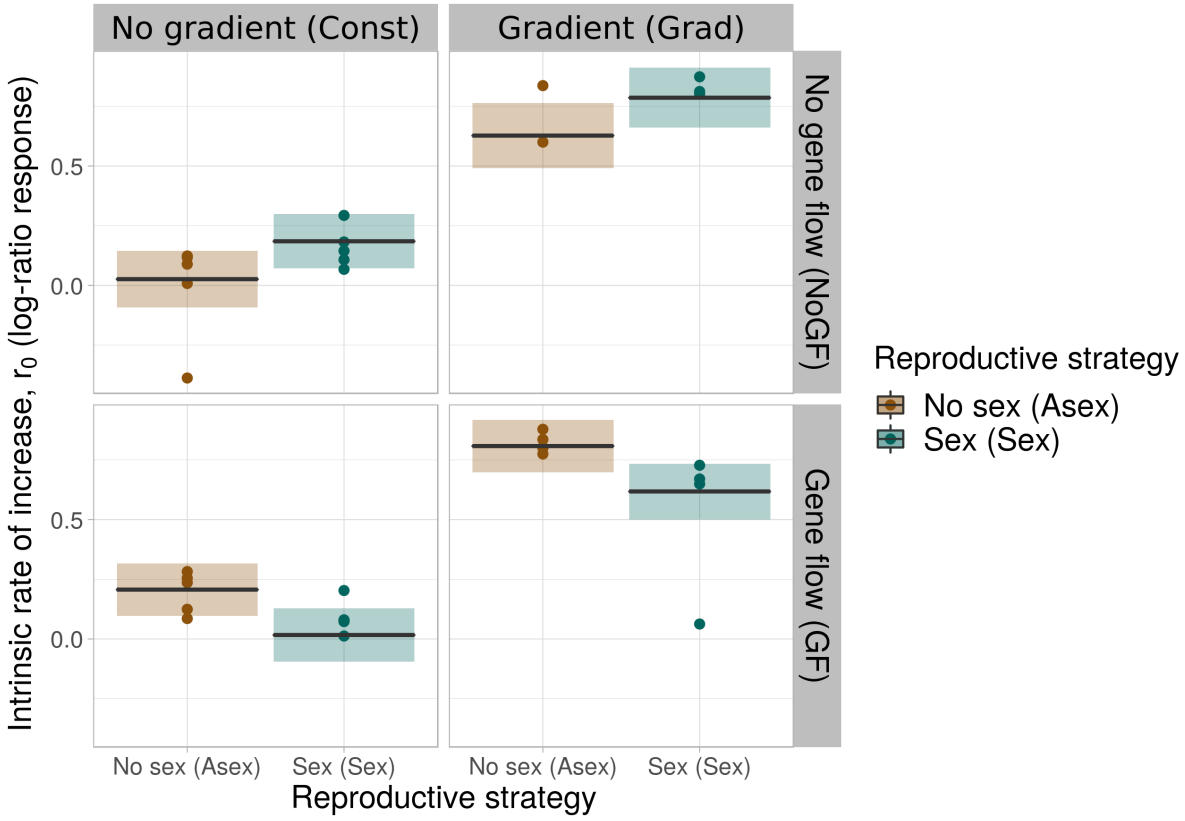

Figure 2: Local adaptation, tested as the evolution of intrinsic rate of increase r_0 under the pH-conditions experienced during range expansion. The y-axis shows the change in r_0 compared to the ancestor (log-ratio response). Dots represent individual data points, boxplots show the model predictions of the best model (mean and 95 %-confidence interval). Purple boxes and dots represent populations reproducing asexually ("Asex"), orange boxes and dots sexually reproducing populations ("Sex").

	Degrees of freedom	**F-value**	**Pr (>F)**
Reproductive strategy	1	0.482	0.493
Gene flow	1	2.768	0.107
Abiotic pH gradient	1	128.7	<0.0001
Reproductive strategy*Gene flow	1	10.67	0.003
Residuals	29		

Table 2: ANOVA table of the best model for evolution of intrinsic rate of increase r_0 according to AICc model comparison.

Discussion

We experimentally assessed the gene swamping hypothesis by performing replicated range expansions using the protist *T. thermophila*, where we experimentally manipulated abiotic pH conditions (constant versus gradient), reproductive strategy (asexual versus sexual) and gene flow (no gene flow versus gene flow). We demonstrated how sex interacts with gene flow, affecting local adaptation of organisms at the range edge (Fig. 2; Tab. 1; Tab. 2). We found that sex facilitated adaptation in the absence of gene flow, but inhibited it in presence of gene flow. However, this interaction between sex and gene flow was independent of the pH-gradient.

Populations undergoing range expansions face multiple selective pressures [1], and hence face a strong pressure to adapt. Theoretical predictions suggest sex can be advantageous or disadvantageous during range expansion, depending on the context. The theory on gene swamping predicts that during range expansions sex can hinder adaptation when populations undergo strong asymmetrical dispersal from the range core to the range edge [22,23,24]. We showed in this experiment that sex and gene flow interact during range expansions, modulating local adaptation. Despite having only four mating events, we found that sex facilitated adaptation in absence of gene flow, but hindered it when gene flow swamps the population at the expansion front with maladapted individuals. Surprisingly, while the gene swamping hypothesis predicts this pattern exclusively in the presence of abiotic gradients [22,23,24], we observed similar effects of gene swamping in presence and in absence of an abiotic pH-gradient. We argue that gene swamping in absence of an abiotic gradient could stem from evolution of life-history strategy during range expansions. Populations at the range edge undergo selection for fast reproduction [46], and thus range expansions also represent a biotic gradient in competition. Hence, gene swamping may imply that individuals maladapted in life-history strategy interbreed with the population at the range edge, and consequently gene swamping affects adaptation during range expansions without an abiotic gradient, leading to analogous changes in adaptation as in the case of range expansions into an abiotic gradients.

Although we show that gene swamping affects adaptation during range expansions, we could not detect any effects of gene swamping on range expansion rates as described in the theoretical framework, even though population growth rate is a driving force behind expansion

rate of populations [2,47]. This could stem from the experimental setup, where we used discrete landscapes connected through episodic dispersal events. This setup may be insufficiently sensitive to detect signals in expansion rate. Alternatively, this setup may lead to pushed waves rather than pulled waves (see Pachepsky and Levine [48]) which changes predictions. Furthermore, in model systems, where sexual reproduction is more frequent than in our experiment, the signature of gene swamping may be stronger, potentially leading to changes in expansion rate. Further experimental efforts may prove useful to show 1) how common gene swamping is with regards to abiotic gradients and model species, 2) quantifying the effect of gene swamping on expansion rate in more sensitive experimental setups and 3) exploring the strength of gene swamping in terms of frequency of sexual reproduction.

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Author contributions

FM, EAF, AW and FA designed the experiment. FM performed experimental work and statistical analyses. FM, FA, AW and EAF interpreted the results. FM and FA wrote the first version of the manuscript and all authors commented on the final version.

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