Evolution of density-dependent movement during experimental range expansions

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Abstract

Range expansions and biological invasions are prime examples of transient processes that are likely impacted by rapid evolutionary changes. As a spatial process, range expansions are driven by dispersal and movement behaviour. Although it is widely accepted that dispersal and movement may be context-dependent, for instance density-dependent, and best represented by reaction norms, the evolution of density-dependent movement during range expansions has received little experimental attention. We therefore tested current theory predicting the evolution of increased movement at low densities at range margins using highly replicated and controlled range expansion experiments across multiple genotypes of the protist model system *Tetrahymena thermophila*. Although rare, we found evolutionary changes during range expansions even in the absence of initial standing genetic variation. Range expansions led to the evolution of negatively density-dependent movement at range margins. In addition, we report the evolution of increased intrastrain competitive ability and concurrently decreased population growth rates in range cores. Our findings highlight the importance of understanding movement and dispersal as evolving reaction norms and plastic life-history traits of central relevance for range expansions, biological invasions and the dynamics of spatially structured systems in general.

Introduction

Species’ ranges and the distribution of taxa on earth have long been studied only from an ecological point of view and put in the context of local climatic and environmental conditions. Recent theoretical work and empirical case examples, however, have expanded upon this work by including an evolutionary perspective. Such an approach is needed because rapid evolutionary changes are thought to be especially relevant under transient and nonequilibrium conditions inherent to species range dynamics and population spread (Hanski, 2012; Kubisch et al., 2014). Vice versa, range expansions can have serious consequences for the genetics of expanding populations (Excoffier et al., 2009). For instance, deleterious mutations can accumulate at range fronts creating an expansion load (Peischl et al., 2013) which may hamper further spread (Peischl et al., 2015).

The importance of an evolutionary and eco-evolutionary perspective for understanding the dynamics of species ranges becomes evident when considering the main drivers of range dynamics: dispersal and reproduction. These life-history traits not only have a genetic basis (Bonte & Dahirel, 2017; Saastamoinen et al., in press) and can be subject to evolutionary change, but ample theoretical evidence suggests that the evolution of dispersal and reproduction may also have important effects on the dynamics of species ranges (e.g. reviewed in Kubisch et al., 2014). For instance, dispersal during range expansions is predicted to evolve to higher rates at range margins, a phenomenon called ‘spatial selection’ (Phillips et al., 2010). Spatial selection relies on a simple ecological filter effect: if there is variation in dispersal ability, fast individuals will automatically be spreading more towards the range margin and colonize previously empty patches (see also Haag et al., 2005).
As a consequence, populations at the range margin have a higher proportion of more dispersive individuals that will mate with each other. In combination with fitness advantages due to low intraspecific competition at the range margin, this spatial assortment can lead to the evolution of increased dispersal and movement abilities. These evolutionary changes have important consequences for (macro)ecological patterns: range expansions and biological invasions are predicted to proceed faster if dispersal evolves (Perkins et al., 2013). Theoretical predictions regarding the evolution of reproduction have also been made, especially in the context of life-history trade-offs between dispersal, reproduction and competitive ability (Burton et al., 2010; Fronhofer & Altermatt, 2015; Fronhofer et al., 2017). As dispersal is costly (Bonte et al., 2012), models generally assume that either reproduction or competitive ability has to decrease if dispersal increases.

Although there is accumulating comparative empirical evidence of spatial selection (e.g. Thomas et al., 2001; Phillips et al., 2006; Lombaert et al., 2014), experimental confirmation is more scare. This scarcity is mainly due to the obvious challenges associated with studying how evolution impacts macroecological patterns experimentally. However, a recently increasing use of (small) model organisms has been spurring the study of experimental range expansions and population spread dynamics. As a consequence, range expansions are currently under intense scrutiny from an experimental ecological (e.g. Melbourne & Hastings, 2009; Giometto et al., 2014, in press) and evolutionary point of view (e.g. Hallatschek et al., 2007; Fronhofer & Altermatt, 2015; Grafka et al., 2016; Williams et al., 2016; Fronhofer et al., 2017; Ochocki & Miller, 2017; Wagner et al., 2017; Weiss-Lehman et al., 2017). A result of these concerted efforts is the experimental demonstration that range expansions indeed may select for increased dispersal rates and distances at the range margin (in protists: Fronhofer & Altermatt, 2015; Fronhofer et al., 2017; Ochocki & Miller, 2017; Wagner et al., 2017; Weiss-Lehman et al., 2017). A result of these concerted efforts is the experimental demonstration that range expansions indeed may select for increased dispersal rates and distances at the range margin (in protists: Fronhofer & Altermatt, 2015; Fronhofer et al., 2017; Ochocki & Miller, 2017; Wagner et al., 2017; Weiss-Lehman et al., 2017). We predicted movement to evolve towards higher values at low densities making the reaction norm less positively or even negatively density-dependent at the range margin (Travis et al., 2009; Weiss-Lehman et al., 2017). We tested this prediction using experimental evolution and a total of 48 replicate range expansions of 14 clonally reproducing Tetrahymena thermophila strains, as well as the mix of these strains. Due to the lack of initial standing genetic variation in the replicates with single clonal strains and the low population densities characteristic of expanding range margins, we expect consistent evolutionary differentiation between range core and margin populations to be rare and emerge only in a minority of our replicates. We recorded movement behaviour and changes in life-history traits, namely population growth rate and intrastrain competitive ability, to investigate potential concurrent evolutionary changes (Bonte & Dahirel, 2017).

Materials and methods

Model organism

We used the freshwater ciliate Tetrahymena thermophila (Cassidy-Hanley, 2012) as our model organism. It is, together with its sister species (Tetrahymena pyriformis), commonly and successfully employed in both ecological (e.g. Giometto et al., 2014; Fronhofer et al., 2015b) and evolutionary contexts (e.g. Fjerdingstad et al., 2007;
Friman et al., 2011; Hiltunen & Becks, 2014; Pennekamp et al., 2014; Fronhofer & Altermatt, 2015) and is known to show density-dependent movement and dispersal (Pennekamp et al., 2014; Fronhofer et al., 2015b). Tetrahymena thermophila can reproduce both sexually (if a cell from another mating type is present and mating is induced, e.g., by starvation, which was not the case in our experiments) and clonally (Cassidy-Hanley, 2012). We used a total of 14 clonally reproducing strains that were isolated as single cells shortly prior to the experiment from seven mating types originally obtained from Cornell University’s Tetrahymena Stock Center (Stock ID: MTP01). The 14 clonally reproducing strains used here differ in attributes ranging from population growth rates and equilibrium densities (Table S1) to morphology and movement characteristics (Table S2).

*Tetrahymena thermophila* was kept in filtered (cellulose paper filters, 4–7 μm; Whatman) and autoclaved protist pellet medium (0.46 g L\(^{-1}\); Protozoan pellets, Carolina Biological Supply) under constant temperature (20 °C) and light conditions (Osram Biolux 30 W). Initially, we supplemented the medium with a mix of three bacterial species (*Serratia fonticola*, *Bacillus subtilis* and *Brevibacillus brevis*; obtained from Carolina Biological Supply) at approx. 5% of their equilibrium density as a food source. During the experiment, we replaced 2 mL of 15 mL medium in each microcosm with bacterial cultures at equilibrium density (approx. 1 week old) to prevent adaptation of the bacteria and co-evolutionary dynamics from occurring. For further methodological details, see Altermatt et al., (2015).

**Experimental set-up and microcosm landscapes**

We set up microcosm landscapes to study range expansions into initially uninhabited habitat by propagating range core and range margin populations as described by Fronhofer & Altermatt (2015). In contrast to this study, Fronhofer & Altermatt (2015) investigated unconditional dispersal and used an other *Tetrahymena* species, *T. pyriformis*. The microcosm landscapes consisted of two vials (20 mL conical vials; Sarstedt) connected by silicone tubing (inside diameter: 4 mm, VWR) and a stopcock (Discofix, B.Braun) to control for dispersal (total length: 6 cm). For a graphical overview of the experimental procedure, see Fig. S1.

The first patch (start) was initially filled with the respective *T. thermophila* strain at equilibrium density, and dispersal into the empty target patch (filled with 15 mL protist pellet medium with bacteria) was allowed for 4 h. Thereafter, the populations in the start and target patches were propagated to start patches in respectively new two-patch systems. The population from the original start patch was subsequently taken as the range core. Dispersal was allowed for 4 h, and only the individuals remaining in the start patch were further propagated. By contrast, the population from the original target patch represented the range margin. Dispersal was also allowed for 4 h. Thereafter, the population in the target patch was propagated to a new two-patch system. This procedure, effectively tracking range core and expanding range margin populations, was repeated three times per week for 1 month.

During transfer to new two-patch systems, only 13 of the 15 mL of the range core was transferred and the remaining 2 mL was filled with bacterial cultures at equilibrium (approx. 1 week old) to avoid evolutionary changes in the bacterial populations. This procedure was not necessary for the range margin, as the individuals of the range margin had always just dispersed into medium with bacteria from the stock population.

We chose this experimental approach that tracks only range core and range margin for convenience and feasibility. Firstly, we can easily control resource levels and avoid loss of control and contaminations due to ageing medium in long landscapes. Secondly, we have shown that although the experimental procedure obviously prevents feedbacks between core and margin populations, our results are not altered if we indeed use long interconnected linear landscapes (Fronhofer et al., 2017). It is important to note that our approach reduces the likelihood of mutations occurring towards the range margin, as there is no possibility for dispersal, that is gene flow, from the core towards the margin, which effectively reduces the relevant population size. Our results will therefore be conservative and underestimate the potential occurrence of evolutionary change and subsequent range expansion success.

We followed a total of 48 range expansions, that is pairs of range cores and margins. We replicated the range expansion of each of the 14 strain three times and additionally included six replicates of a mix (in equal proportions) of all strains. The latter range expansions using the mix of strains thus differ from the range expansions of the single clones in that they were started with standing genetic variation.

**Recording of population densities and movement data**

During the experiment, all microcosms were sampled at every transfer, that is three times per week. We used video recording and automated analysis to collect data on population densities (number of cells) as well as movement metrics (e.g. velocity of the cells). More specifically, using a Leica M205C stereomicroscope at a 16-fold magnification and an Orca Flash 4 camera (Hamamatsu), we recorded 20 s videos (25 frames per second) of an effective volume of 34.4 μL (sample height: 0.5 mm). Videos were subsequently analysed using the *r* environment for statistical computing (version 3.2.3) and a customized version of the ‘*bemovi*’ package (available on GitHub: https://github.com/efronhofer/bemovi; originally developed by Pennekamp et al.,...
is obtained as $\hat{N} = \frac{N}{N_t}$.

The ‘removi’ package uses the image processing software IMAGEJ to locate moving particles in the videos and to calculate population densities as well as movement metrics. Data are automatically filtered to exclude artefacts such as debris that are outside the size or movement range of the study species (detailed settings available on GitHub: https://github.com/efronhofer/analysis_script_removi). As movement velocity has been shown to correlate well with dispersal rates in this system (Fronhofer & Allermatt, 2015; Fronhofer et al., 2015a) we will focus on velocity as a proxy for dispersal.

Common garden phase

To separate evolutionary from potential plastic inter-generational and environmental effects, movement behaviour of individuals from range core and range margin populations was measured not only during the evolution experiment, but also after the end of the experiment and an additional 3-day common garden phase (doubling time for the strains of T. thermophila used here: approx. 3–12 h; see Table S1). The common garden was initiated with 1.5 mL samples obtained from the respective core and margin populations from the last day of the evolution experiment that were added to 13.5 mL medium with bacteria from the stock population to homogenize environmental conditions.

In addition, we kept a subset of the individuals analysed in this first common garden for an extended period in a common environment (a total of 38 days). This allowed us to explore the long-term stability of potential evolutionary changes. Resources were not refreshed to create strong competition similar to stable range core populations.

Estimating population growth rates and intrastrain competition coefficients

Both at the beginning of the experiment and after the common garden, we used population growth experiments to estimate population growth rates ($r_0$) as well as intrastrain competition coefficients ($x_{ii}$). These experiments were always started with 1 mL of the respective strain (1 week old for the beginning from the common garden for the end) and 14 mL medium with bacteria from the stock population. We then followed population densities and movement over the course of 9 days, measuring twice in the first 2 days and then once on days 2, 3, 4, 7 and 9.

Population growth rates ($r_0$) and intrastrain competition coefficients ($x_{ii}$) were estimated by fitting the logistic growth model according to Verhulst (1838)

$$\frac{dN}{dt} = (r_0 - x_{ii}N)N$$

(1)

where $N$ is the population size, to the data obtained from the growth experiments. The equilibrium density
for in the random effects. The data are available at Dryad (https://doi.org/10.5061/dryad.36q03).

More specifically, movement velocity (log-transformed) change during the experiment was analysed using individual-based velocity data with ‘time’ as a random slope and ‘replicate’ within ‘strain ID’ as a random effect structure to account for the nonindependence of individuals measured in one sample as well as the temporal nonindependence of the repeated measures through time (see Crawley, 2013). Following (Crawley, 2013), we started with the full model including an interaction between the two explanatory variables ‘time’ and ‘range position’ and subsequently simplified the model by removing explanatory variables to obtain the minimal adequate model. We used the ‘ANOVA’ function and maximum-likelihood (ML) estimates obtained with the ‘optim’ optimizer for model simplification. Subsequently, the minimal adequate model was refit using REML to obtain model estimates.

For the analysis of movement velocity (nontransformed) after the common garden phase, the full model included ‘time’ (end of evolution experiment, common garden 1, common garden 2) and ‘range border position’ as interacting fixed effects as well as ‘time’ within ‘replicate’ within ‘strain ID’ as random effects. Model simplification was carried out as described above.

Density-dependent movement (log-transformed) was analysed analogously using ‘population density’ and ‘population history’ (range border position and starting populations) as interacting fixed effects in the full model and ‘replicate’ within ‘strain ID’ as random effects with ‘population density’ as a random slope. The analysis of population growth rates ($r_0$; log-transformed) and intraspecific competition coefficients ($s_{ji}$; inverse-transformed) included only ‘population history’ (range border position and starting populations) as a fixed effect as well as ‘strain ID’ as a random effect.

Finally, potential differences between failed and successful range expansions, that is experimental replicates in which the range margin failed to expand and went extinct versus those that kept up with our experimental treatment, were explored by relating growth rates ($r_0$; inverse-transformed), intrastrain competition coefficients ($s_{ji}$; inverse-transformed) and median and standard deviation of movement velocity of the starting populations (respectively log- and inverse-transformed) with invasion success (yes/no). As a random effect, we included ‘strain ID’.

Quantification of plasticity and evolutionary change in density-dependent movement

To quantify the different components of plasticity and evolutionary change in density-dependent movement, we used a reaction norm (RN) approach (e.g. Stoks et al., 2015; Govaert et al., 2016). Whereas the classical RN approach includes changes in the trait of interest (here, movement) only between one control setting and one treatment setting, we here expanded this idea to RNs of continuously changing environmental conditions (here, relative density). Our analysis was based on the density-dependent movement RNs estimated using the mixed models described above. To avoid artefacts due to extrapolation, we restricted our analyses to the relative density interval that includes estimated RNs for ancestor, range core and range margin. For a graphical representation of our approach, see Fig. S2.

In analogy to the original RN approach, we quantified ancestral plasticity (AP), that is the plasticity in movement exhibited by the ancestor, as the difference between the ancestor’s RN and a density-independent RN that has the same reference point as the ancestor RN. In the classical approach, the reference point is the intercept of the RN. To avoid bias due to extrapolation, we here did not use the estimated intercept, but the trait value at the lowest relative density in the relative density interval of interest. We quantified constitutive evolution (CE), that is the overall shift of the RN, as the difference between a density-independent RN at the reference point of the ancestor and a density-independent RN at the equivalent reference point of the range core, respectively range margin RN. Finally, evolution of plasticity (EP) was quantified as the difference between the ancestral RN shifted by the difference in reference points and the RN of the range core, respectively range margin. The three eco-evolutionary contributions were approximated by summing differences between the RNs.

We estimated the uncertainty of the relative contributions of AP, CE and EP using bootstrapping. We obtained a distribution of relative contributions by calculating the contributions of 10 000 randomly drawn RN sets based on estimates and standard errors extracted from the mixed model estimating the RNs. The individual RNs were first obtained in log-transformed space assuming normal distributions for the parameters as in the statistical model and were subsequently back-transformed. Subsequently, contributions were calculated in nontransformed space and normalized to sum to 1.

Results

Range expansion success

As expected, successful range expansions were rare: of 48 replicated range expansions, only four replicates belonging to three clonal strains (Fig. 1, upper panel) successfully tracked our experimental treatment and expanded their range over the entire duration of the experiment. All other replicates of both clonal strains and the genetically mixed populations only tracked the range expansion over part of the experimental duration and subsequently went extinct at the range margin.
The four successfully expanding replicates did, at the start of the experiment, not statistically differ from all other stains based on their initial properties (Fig. S3).

**Evolution of movement**

All four successfully expanding replicates showed increased movement velocity during the experimental evolution phase at the range margin (Fig. 1; LMM; minimal adequate model: time × position; intercept difference core-margin: $t_{199403} = -63.65$, $P < 0.001$; slope margin: $t_{199403} = 183.11$, $P < 0.001$; slope difference core−margin: $t_{199403} = -72.38$, $P < 0.001$). Individuals in populations at the range margin were on average 1.29 times faster than individuals in the range core (comparison of estimates; Fig. 2). Overall, these differences could be shown to be stable both after a 3-day common garden (approx. 10–20 doubling time periods based on the estimates of $r_0$ in Table S1) and after an additional month of selection for competitive ability in a common garden setting, thus indicating evolutionary change (Fig. 2; LMM; minimal adequate model: time + position; difference margin − core: $t_{199403} = -10.28$, $P < 0.001$; difference end of evolution experiment − common garden 1: $t_6 = -14.23$, $P < 0.001$; difference common garden 1 − common garden 2: $t_6 = -14.54$, $P < 0.001$).

The successfully expanding replicates did not only show differences in movement after the two common garden periods, which captures evolutionary change only under one specific environmental condition (low densities). In line with our predictions, we also found differences in movement between range core and range margin populations across a large spectrum of population densities (up to two-fold equilibrium density; Fig. 3). The slope of the density-dependent movement reaction norm for range margin populations was negative in comparison with the density-dependent movement reaction norm for range core populations which tended to be slightly positively density-dependent (Fig. 3; LMM on log-transformed data; minimal
adequate model: density x history; intercept difference core – margin: $t_{29608} = -16.39, P < 0.001$; intercept difference start – margin: $t_{29608} = 66.96, P < 0.001$; slope margin: $t_{29608} = -4.07, P < 0.001$; slope difference core – margin: $t_{29608} = 24.09, P < 0.001$; slope difference start – margin: $t_{29608} = 32.67, P < 0.001$).

Whereas the differences in intercept observed here recapture the results depicted in Fig. 2, the estimated slopes suggest that density dependence of movement only evolved at the range margin and did not change in the range core in comparison with the ancestral populations. The estimated slopes for range core and start populations both had confidence intervals that strongly overlap with zero [slope range core: 0.10 (−0.12, 0.32); slope start population: −0.0028 (−0.2166, 0.2113); values represent estimates as well as lower and upper CI for log-transformed data extracted from the minimal adequate model], suggesting no density dependence. By contrast, the slope of the density-dependent movement reaction norm for the range margin populations was clearly negative [slope range margin: −0.45 (−0.47, −0.42)]. These results were not qualitatively changed if we considered absolute instead of relative densities as an explanatory variable, except for the slope of the range core which became positive (see Fig. S4).

These results are corroborated by the analysis of eco-evolutionary contributions using the density-dependent movement reaction norms (Fig. 3, right panel). Whereas the relative contribution of ancestral plasticity (AP), that is the slope of the ancestral reaction norm, was overall small (between 2 and 4%) and its distribution overlapped strongly with zero, the contribution of constitutive evolution (CE), that is the overall shift of the reaction norms compared to the ancestor, was large (83% for range core and 73% for range margin). As in the analyses reported above, the contribution of evolution of plasticity (EP) was smaller for the range core (12%) and the distribution was wide, suggesting only a weak tendency. By contrast, the effect of EP was stronger for the range margin (25%) and the distribution did not overlap with zero.

Evolution of population growth rate and intrastrain competitive ability

Besides evolutionary changes in density-dependent movement, we could find clear differences in population growth rates after the common garden phase between range core and range margin (Fig. 4). Range margin populations exhibited population growth rates...
that were on average 3.2 times higher than range core populations (Tukey post hoc test after LMM on log-transformed data; core – margin: $z = -2.28$, $N = 22$, $P = 0.058$), whereas range margin populations did clearly not (Tukey post hoc test after LMM on inverse-transformed data; start – margin: $z = -1.20$, $N = 22$, $P = 0.45$).

Discussion

We investigated evolutionary differentiation between range core and range margin populations using highly controlled and replicated range expansions of both clonal *Tetrahymena thermophila* strains without initial standing genetic variation and a mix of all strains. Our experimental results provide evidence of rapidly emerging evolutionary differences in movement reaction norms, population growth rates and intrastrain competitive ability during range expansions.

As expected, successful range expansions (Fig. 1) occurred relatively rarely (four of 48 replicates). All other range margin populations went extinct at some point during the temporally advancing range propagation and ultimately failed to expand their range (Fig. 1). It is interesting to note that we did not observe successful range expansions in the replicates that harboured initial standing genetic variation. Furthermore, we could not identify any common attributes of the strains that successfully expanded their range, which is in good accordance with previous findings highlighting the intrinsically stochastic and variable nature of range expansions, in both an ecological (Melbourne & Hastings, 2009; Giometto et al., 2013) and evolutionary context (Ochocki & Miller, 2017; Weiss-Lehman et al., 2017). The lack of attributes that favour successful range expansions across the ancestors may also explain why replicates that started with mixes of clones did not stand out in terms of range expansion success, simply because advantageous genotypes were not present in the mix. Of course, a direct comparison in terms of expansion success between replicates with and without standing genetic variation is difficult due to the comparatively low number of mixed replicates.

**Range expansions can lead to trait evolution even in the absence of initial standing genetic variation**

Evolution of increased dispersal at range margins due to spatial selection (Phillips et al., 2010) and potentially kin competition (Kubisch et al., 2013) has been recognized to impact range expansion dynamics theoretically (Kubisch et al., 2014) as well as in empirical studies (Fronhofer & Altermatt, 2015; Williams et al., 2016; Fronhofer et al., 2017; Ochocki & Miller, 2017; Wagner et al., 2017; Weiss-Lehman et al., 2017). We here explored the relevance of spatial selection for systems that do not initially harbour high amounts of standing genetic variation. Our results may therefore be relevant.
in the context of invasions of non-native organisms, which, in contrast to expansions of species’ ranges starting from a large and potentially diverse core habitat, may often start with a colonization event involving one or very few individuals and therefore may exhibit very little standing genetic variation (e.g. Tsutsui et al., 2000; but see also Roman & Darling, 2007 and Dlugosch & Parker, 2008). Given the ecological and economical relevance of invasive species (Pimentel et al., 2000), a better understanding of the underlying evolutionary dynamics is highly valuable.

We show that differentiation between range cores and range margins in movement and other life-history traits (population growth rate and intrastrain competitive ability) is possible in a relatively short time (13 discrete dispersal and population growth phases; the growth phases correspond to approx. 10–20 doubling time periods based on the estimates of $r_0$ in Table S1) even without initial standing genetic variation and despite our experimental setting that additionally limits gene flow towards the range margin population (Figs 2–4). Nevertheless, in our study system rapid evolution in asexually reproducing lines does not seem fundamentally surprising, as mutation rates are relatively high in T. thermophila: Brito et al. (2010) performed mutation accumulation experiments and report that the observed extinction rate is two to three orders of magnitude higher than in comparable experiments with yeast and bacteria. These high rates are likely due to gain and loss of chromosomes that typically occur during the amitotic division of the macronucleus (Brito et al., 2010).

More generally, our findings fit well into the current invasion literature: for instance, Dlugosch & Parker (2008) discuss the invasive plant Hypericum canariense which shows evidence for the evolution of increased growth rates (survival and reproduction) and local adaptation in flowering time in invasive populations despite bottleneck events. Furthermore, genetic diversity does not seem to consistently predict invasion success in nature (Roman & Darling, 2007; Dlugosch & Parker, 2008), which is also consistent with our findings, especially with the failure to expand in the replicates that exhibited standing genetic variation. As Roman & Darling (2007) note, those species that do become invasive may not exhibit a general loss of diversity, which is well captured by our results, as those replicates that did expand their range overall showed diversification in life-history traits between core and margin (Figs 2 and 4). However, a direct comparison of the range expansion success in our study and natural invasion success is not readily possible, as our experimental approach is conservative and underestimates expansion success. On a more speculative note, failures of range expansions in our study may, of course, also be due to the accumulation of deleterious mutations leading to expansion load (e.g. Peischl et al., 2015).

Besides being applicable to biological invasions, our results and conclusions are potentially relevant to climate-driven or generally environmental change-driven range expansions. Our experimental procedure basically simulates a shifting window of suitable habitat for the range margin as is often done in theoretical studies (e.g. Henry et al., 2013). Figure 1 highlights that, despite often leading to extinction, evolutionary change (Figs 2 and 3) can potentially prevent these marginal populations from going extinct (evolutionary rescue: Gonzalez et al., 2013; Carlson et al., 2014). Empirical work has shown that evolutionary rescue may be promoted by dispersal (e.g. Bell & Gonzalez, 2011; Low-Décarie et al., 2015) which suggests the possibility of selection for increased dispersal in such contexts. Theoretical work indicates that, especially in the context of rapidly shifting windows of climatic suitability, evolutionary rescue can occur by selecting for increased dispersal distances which allows populations to track climate change and even cope with severely fragmented landscapes (Boeye et al., 2013). However, if one takes into account more complex scenarios and includes local adaptation (Schiffers et al., 2013), evolutionary rescue may only be partial and species ranges are predicted to be severely narrowed after climate change. Interpreting our results in the context of evolutionary rescue is of course speculative, as we cannot determine whether evolutionary change happened because of the range expansions (spatial selection) or if evolutionary change allowed for the range expansion to happen in the first place.

**Evolution during range expansions changes the density-dependent movement reaction norm**

Our work shows that movement and dispersal evolution at expanding range margins does not only impact average movement strategies and dispersal probabilities, but changes the slope of the density-dependent movement reaction norm (Fig. 3). Theory predicts that dispersal should increase even at low densities at expanding range margins, that is become less positively density-dependent (Travis et al., 2009) because conspecific density looses its value as a cue for context-dependent dispersal. As potential target patches for range margin populations tend to be empty, dispersal is advantageous already at low densities and an unconditional dispersal strategy will be less costly (Bonte et al., 2012) as energy and time do not have to be invested in information collection and processing.

We find that the starting populations showed weak or no density-dependent movement (Fig. 3). Subsequently, individuals in the range core conserved this density-independent movement strategy, whereas individuals at the range margin exhibited evolution of plasticity and evolved increased movement at low densities (Fig. 3) thereby changing the slope from slightly...
positive or neutral to negative. Our experimental results therefore substantiate the theoretical predictions of Travis et al. (2009) and are in accordance with recent empirical work that suggested a weakening of the slope of the density-dependent dispersal function in an insect system (Weiss-Lehman et al., 2017). We can only speculate what led to the overall increase in movement (intercepts; constitutive evolution) when comparing starting populations and populations after the first common garden (Figs 2 and 3). This intercept shift, which is reversed in the second common garden (Fig. 2), may be due to increased resource availability during the experimental evolution phase as a consequence of the high rates of resource replenishment we chose to avoid evolution in the resources.

Range expansions lead to the evolution of increased intrastrain competitive ability and decreased population growth rates in range cores

Whereas dispersal is a central and potentially independent life-history trait of major importance for spatial dynamics (Bonte & Dahirel, 2017), other life-history traits, such as reproduction, may evolve concurrently or even trade off with dispersal (e.g. Burton et al., 2010; Fronhofer et al., 2011; Stevens et al., 2014; Fronhofer & Altermatt, 2015). Our results show that not only movement capacities but also reproductive rates and intrastrain competitive abilities can evolve during range expansions and biological invasions (Fig. 4). Substantiating theoretical predictions (e.g. Burton et al., 2010), we experimentally find higher population growth rates at the range margin in comparison with range core populations. Whereas the slope of the movement reaction norm showed evolutionary change in comparison with the starting population only at the range margin, it is populations in the range core that exhibited lower population growth rates in comparison with populations at the range margin and the starting population and not vice versa. At the same time, we observe higher intrastrain competitive abilities in range core populations, which hints at a trade-off between intrastrain competitive ability and population growth rates.

Our results regarding the evolution of population growth and intrastrain competitive ability, as well as previous findings in a similar system (Fronhofer et al., 2017), are opposed to some recent experimental investigations reporting lower reproduction, respectively investment in population growth at the range margin (Fronhofer & Altermatt, 2015; Weiss-Lehman et al., 2017) or no effect on reproduction (Ochocki & Miller, 2017). Whereas this heterogeneity in results may be linked to differences in resource dynamics in the experiments (Fronhofer & Altermatt, 2015; Fronhofer et al., 2017), it also hints at the potential evolutionary independence of dispersal from other life-history traits (Bonte & Dahirel, 2017). Clearly, more conceptual and experimental research is needed to provide synthesis on these questions.

Conclusions

We present experimental evidence showing that range expansions can lead to the evolution of increased movement at low densities at range margins in comparison with range cores due to evolutionary changes in plasticity, specifically in the density-dependent movement reaction norm. Movement evolved to negatively density-dependent at the range margin. Additionally, we observed concurrent changes in life-history traits towards lower population growth rates and higher intrastrain competitive abilities in the range core. Importantly, these evolutionary changes emerged de novo in our experiments and did not require initial standing genetic variation. Our findings suggest that evolution can favour the spread of invasive species, even if standing genetic variation is initially low. In the context of climate change, evolution of increased movement at range margins may allow individuals to track their climatic niche by adapted dispersal and growth strategies.

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Data accessibility

The data are available at Dryad (https://doi.org/10.5061/dryad.36q03). Video analysis scripts can be found on GitHub (https://github.com/efronhofer/analysis_script_bemovi).

References


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**Supporting information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1** Experimental set-up as used here and in Fronhofer & Altermatt (2015).

**Figure S2** Eco-evolutionary partitioning using a modified reaction norm (RN) approach (Stoks et al., 2015; Govaert et al., 2016).

**Figure S3** Differences between successful and failed range expansions.

**Figure S4** Evolution of density-dependent movement in range cores and at range margins margins for the replicates that successfully expanded their range.

**Table S1** Strain characteristics: population dynamics.

**Table S2** Strain characteristics: morphology and movement.

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