

Evolution of life history and dispersal traits during the range expansion of a biological control agent

Eliza Clark¹, Ellyn Bitume², Dan Bean³, Amanda Stahlke⁴, Paul Hohenlohe⁴, and Ruth Hufbauer¹

¹Colorado State University

²USDA Forest Service Institute of Pacific Islands Forestry

³State of Colorado Department of Agriculture

⁴University of Idaho

July 29, 2021

Abstract

Evolutionary theory predicts that the process of range expansion will lead to differences between core and edge population in life history and dispersal traits. Selection and genetic drift can influence reproductive ability while spatial sorting by dispersal ability can increase dispersal at the edge. However, the context of individuals (e.g., population density and mating status) also impacts dispersal behavior. We evaluated theoretical predictions for evolution of reproductive life history and dispersal traits using the range expansion of a biological control agent, *Diorhabda carinulata*, or northern tamarisk beetle. We found divergence of fecundity, age at first reproduction, and female body size between core and edge populations. We also show that density and mating status influence dispersal and that dispersal increases at the edge of the range. We demonstrate that theory of evolution during range expansions applies to the range expansion of a biocontrol agent, especially when the ecological context is considered.

1. Introduction

Understanding the ecological and evolutionary processes at play during range expansion is key to predicting the spread of invasive species (Hastings *et al.* 2005), potential success of biological control agents (Szűcs *et al.* 2019), and the ability of threatened species to track recent climate change (Mustin *et al.* 2009). During range expansion, the landscapes encountered by expanding populations represent novel selective environments (Brown *et al.* 2013; Van Petegem *et al.* 2016), and simultaneously, the expansion itself can be a catalyst for evolution through spatial sorting and genetic bottlenecks (Phillips *et al.* 2010b; Shine *et al.* 2011). Thus, range expansion can result in evolved differences in reproductive life history and dispersal traits between populations at the core of the range and the edge of the expansion front (Simmons & Thomas 2004; Peischle *et al.* 2013; Phillips 2015). Population density can also change across range expansions, and associated differences in competitive environment and mate availability can immediately affect dispersal traits (Brown *et al.* 2013; Pettit *et al.* 2016). Little is known about how evolutionary and plastic responses will interact in natural range expansions across heterogeneous environments. Here, we combine predictions regarding the evolution of life history and dispersal traits across range expansions with conceptual understanding of the ecology of dispersal to place range expansion theory in ecological context and develop refined predictions. We test these new predictions using the range expansion of an introduced biological control (hereafter, biocontrol) agent to improve our understanding and ability to predict the speed and extent of range expansions.

1.1 Life history evolution during range expansions

Life history traits, such as fecundity and age at first reproduction, are predicted to either increase or decrease during range expansions, depending upon whether selection or non-adaptive evolutionary processes are dominant at the expansion front (Phillips *et al.* 2010b). Selection at the expansion front is hypothesized to differ from the core due to differences in density between edge and core (Burton *et al.* 2010; Fronhofer & Altermatt 2015). Species undergoing range expansion generally have lower population densities at the edge, where individuals are dispersing to uncolonized territory, and higher densities at the core (Burton *et al.* 2010; Altwegg *et al.* 2013). This means that individuals at the edge will experience exponential, or density-independent, population growth, while those at the core experience logistic, or density-dependent, population growth (Phillips *et al.* 2010b). Thus, selection at the low density edge will favor increased fecundity and younger age at first reproduction relative to the core (Fig. 1A) (Phillips *et al.* 2010b).

In contrast, when non-adaptive processes are dominant, reduced fitness, or expansion load, is predicted at the edge relative to the core due to accumulation of deleterious alleles (Fig. 1B) (Travis *et al.* 2007; Peischl *et al.* 2013; Peischl & Excoffier 2015). This can occur when edge populations are so small that they experience strong genetic drift and deleterious alleles ‘surf’ the wave of expansion during repeated founder events (Klopfstein *et al.* 2006). Expansion load has been predicted using models (Peischl *et al.* 2013) and demonstrated in one plant (González-Martínez *et al.* 2017). This pattern of reduced fecundity at the edge relative to core might also be generated through trade-offs between dispersal, reproduction, and competitive ability (Burton *et al.* 2010; Phillips *et al.* 2010b; Fronhofer & Altermatt 2015).

1.2 Dispersal evolution during range expansions

Here, we consider traits affecting dispersal separately from reproductive life history traits (Bonte & Doherty 2017). Range expansion theory predicts that a population at the expanding edge will evolve increased dispersal ability relative to a population at the core through the process of spatial sorting (Shine *et al.* 2011). During range expansion, individuals with greater dispersal ability are more likely to arrive at the range edge and disperse to new territory, resulting in populations at the expanding edge being a non-random selection of better dispersers. Since dispersal ability is a heritable trait in many species (Dällenbach *et al.* 2018), this gradient is further reinforced by assortative mating between individuals on the edge. Spatial sorting has been predicted using mathematical models (Fisher 1937; Kot 1996; Travis & Dytham 2002; Bénichou *et al.* 2012) and demonstrated using model organisms (Simmons & Thomas 2004; Fronhofer & Altermatt 2015; Van Petegem *et al.* 2016; Ochocki & Miller 2017; Szücs *et al.* 2017; Weiss-Lehman *et al.* 2017). The evolution of increased dispersal at range edges has also been documented empirically, both in species whose ranges are shifting due to climate change (Cwynar & MacDonald 1987; Thomas *et al.* 2001; Simmons & Thomas 2004; reviewed in Hill *et al.* 2011), and in invasive species (Phillips *et al.* 2006, 2010a; Monty & Mahy 2010; Berthouly-Salazar *et al.* 2012; Lombaert *et al.* 2014; Merwin 2019; but see Ashenden *et al.* 2017).

External conditions, such as population density, can be important signals to individuals about the potential costs and benefits of emigration (Clobert *et al.* 2009; Endriss *et al.* 2019) and can influence dispersal evolution along expansion fronts (Travis *et al.* 2009). Species that exhibit positive density-dependent dispersal (increased dispersal at high densities) may be less likely to evolve increased dispersal ability at the edge of the range expansion where population density can be low (Travis & Dytham 2002; Fronhofer *et al.* 2017), while species with negative density-dependent dispersal (increased dispersal at low densities) may be more likely to evolve increased dispersal abilities and generate accelerating expansion fronts (Altwegg *et al.* 2013). For many species, high population density can signal strong intraspecific competition, which may increase emigration. Alternatively, high population density can signal high mate availability, which may decrease emigration.

How an individual incorporates external conditions into dispersal behavior also depends on internal state, such as whether an individual has previously mated (Clobert *et al.* 2009). A mated individual may increase its fitness by dispersing from high density environments to reduce competition and inbreeding (Clobert *et al.* 2009), while an unmated individual may increase its fitness by dispersing from low density environments to increase the chances of finding a mate. Here, we combine the predictions from spatial sorting theory (Shine *et al.* 2011) with those from informed dispersal theory (Clobert *et al.* 2009) to develop the refined predictions

shown in Fig. 1C. We predict edge populations will disperse more often or further than core populations, but dispersal will also depend upon mating status and density (Fig. 1C), thus we can only be confident in our dispersal comparisons across a range when controlling for those contexts. Since we seek to apply evolutionary theory to natural range expansions, these refined predictions will enable us to evaluate the evolution of dispersal during range expansion and how expression of such evolutionary shifts might depend upon both external and internal factors that organisms experience.

1.3 Study system

While evolutionary theory and research on model systems predict evolution during range expansions, there are few tests of the theory on non-model organisms from natural populations (but see Phillips *et al.* 2006, 2010a; Wolz *et al.* 2020) and no studies on introduced biocontrol agents (Szűcs *et al.* 2019), or species imported from the native range of an exotic pest for long-term control (Heimpel & Mills 2017). Evolution of biocontrol agents post-release is of interest to the biocontrol community (Wright & Bennett 2018). Evolutionary changes in host preference are a particular concern (Van Klinken & Edwards 2002), but understanding the evolution of fecundity and dispersal are crucial to improving efficacy of the agent across the range of a target pest species (Szűcs *et al.* 2019).

Diorhabda carinulata, the northern tamarisk beetle, was collected from two central Asian sites and released in 2001 into the western United States for the biological control of invasive riparian shrubs in the genus *Tamarix*, also known as saltcedars or tamarisk (DeLoach *et al.* 2003). Beetles have dispersed southward from a few initial release sites (Bean *et al.* 2012, Fig. 2) following remote riparian corridors, which likely represent independent dispersal pathways. Success of the program has provided impetus for research, including this study, focused on the evolutionary and ecological processes enabling beetles to suppress a major invasive plant across western North America (Bean *et al.* 2012). In addition to practical implications for invasive plant control on a vast geographic scale, the *D. carinulata* range expansion provides an excellent study system within which to test the predictions of range expansion theory because original release sites are known, and the range expansion has been monitored (Bean & Dudley 2018).

In this study, we used a common garden approach to compare life history and dispersal traits of eight populations collected from the core and edge of the *D. carinulata* range in the western US. We evaluated the reproductive life-history traits of fecundity and age at first reproduction, and body mass, which is often linked to fecundity. We predicted either increased reproductive capacity if *selection at low densities* is the dominant process at the edge of the range expansion (Fig. 1A) or decreased reproductive capacity if *expansion load or trade-offs* are the dominant processes at the edge (Fig. 1B). We evaluated dispersal by measuring traits linked to both emigration propensity and flight ability using tethered flight mills. To better contextualize flight behaviors, we quantified dispersal in mated and unmated individuals reared at high and low densities. We predicted an interaction between density and mating status and an additive increase in dispersal ability in beetles from edge populations (Fig. 1C).

2. Materials and methods

2.1 *D. carinulata* populations

Eight collections of *D. carinulata* were made from across the introduced range of the species. Four collections were from well-established original introduction sites in the north of the range and represent the range core. Another four collections were from the newly established southern edge of the range (Fig. 2, Table S1). The La Joya, New Mexico population (H in Fig. 2 and Table S1) was collected slightly behind the probable edge in 2017 in order to avoid collecting other *Diorhabda* species and their hybrids that were moving northward and overlapping the *D. carinulata* range (Ozsoy *et al.* 2019). Two populations (one core and one edge) were collected Fall 2017 and the first lab generations were put into reproductive diapause to reduce the number of lab generations before the start of the experiment. All other populations were collected in Summer 2018 and cultured in the lab for one generation to standardize maternal environment effects prior to starting experiments (Table S1). All insects were reared in growth chambers under reproductive conditions, with a 16h/8h light/dark cycle and 25°C/20°C day/night temperatures and were fed fresh tamarisk 3 times per

week or as needed.

2.2 Life history traits

Newly emerged adult females of the first lab generation were weighed before feeding and reared individually thereafter. Three days after eclosion, each female was paired with a male from the same population of about the same age and allowed to mate for 24 hours before the male was removed. Each 0.24 L container with a female was checked daily for oviposition. All eggs that were laid on the first day of oviposition were counted to provide a measure of early fecundity and the age at first reproduction in days was recorded. If an individual had not oviposited within 10 days of emergence, they were counted as non-layers (Lewis *et al.* 2003).

2.3 Dispersal traits

Dispersal ability was measured for male *D. carinulata* since, in the field, they have been observed dispersing first and using pheromones to attract mixed-sex aggregations of reproductive adults (Cossé *et al.* 2005). After emergence as adults, males were randomly assigned to a density treatment and a mating treatment. Males assigned to the mated treatment were paired with a female from the same population for 24 hours. The males were thereafter reared in 0.24 L containers in groups of five (high density) or alone (low density). All males in each high-density container were of the same mating treatment. All containers received the same surplus amount of fresh tamarisk, regardless of how many beetles were in the container. Male beetles were weighed on the day of the dispersal trial.

We assessed dispersal of male beetles using tethered flight mills (Maes *et al.* 2014; Jones *et al.* 2016; Tanaka & Murata 2017; Dällenbach *et al.* 2018; Minter *et al.* 2018), similar in design to Maes *et al.* (2014) (Appendix S1). Between 6 and 23 days after eclosion, each beetle was given a one-hour flight trial to take any number of flights. Data from each trial was converted into four dispersal parameters: presence of at least one flight, number of flights, total flight distance, and average flight speed (Appendix S2).

2.4 Statistical Analyses

Weight of female beetles at adult emergence was analyzed with a linear mixed model, with range as a fixed effect and population as a random effect. Weight of male beetles at the time of the dispersal trial was analyzed with a linear mixed model, with range (core or edge), rearing density (low or high), mating status (unmated or mated), and all interactions as fixed effects, age as a fixed covariate, and population as a random effect.

Since some females did not lay eggs within 10 days, the 24-hour egg count data were split into two datasets, one including the egg count for laying individuals, and the other including the binary response (laying or non-laying) for all individuals, in order to assess whether probability of laying and fecundity differed between core and edge populations. To account for overdispersion in the dataset, a negative-binomial mixed model was fit to the count data (excluding non-laying individuals) using the `glmmTMB` package version 1.0.2.1 (Brooks *et al.* 2017) with range, weight, and age as fixed effects and population as a random effect. A logistic mixed model was fit to the binary dataset with range and weight as fixed effects and population as a random effect. Since we were primarily interested in differences between the core and edge of the range and to avoid correlations among predictor variables, we fit the same models as above, but excluded weight as a predictor given our findings of significant differences in weight between core and edge (see Results).

Age at first reproduction was analyzed using a Conway-Maxwell Poisson mixed model, which correctly accounts for under-dispersion in this dataset (Brooks *et al.* 2019), with range and weight as fixed effects and population as a random effect. Because range and weight were correlated, we fit the same model again excluding weight as a predictor.

For each of the four dispersal measurements (presence of flight, number of flights, total distance, and average speed), the same factors were included in the full models. Density, mating status, range, and all interactions were fixed effects. Weight at the time of dispersal trial, age, mill friction, and temperature were fixed covariates. Population of origin and trial date were random effects. Unlike in the life history data, range and

weight of male beetles at the time of dispersal trial were not correlated, so weight was always included as a predictor variable in the models.

The presence of at least one flight was analyzed with a binomial model with the packages lme4 version 1.1-26 (Bates *et al.* 2015) and lmerTest version 3.1-3 (Kuznetsova *et al.* 2017). The number of flights during the 1-hour trail was analyzed using negative binomial and Poisson mixed models in the glmmTMB package and a final model was chosen based on residual diagnostics from the DHARMA package version 0.3.3.0 (Hartig 2020). Total distance was a count of revolutions of the flight mill and thus a discrete variable. Six models were fit to the total distance variable: a linear mixed model on log-transformed distance, a negative binomial mixed model, a generalized Poisson model (commonly used for highly right skewed data with a high frequency of low counts (Joe & Zhu 2005; Brooks *et al.* 2019)), and a zero-inflated version of each of those, using the glmmTMB package. A final model was chosen based on residual diagnostics from the DHARMA package. Average speed during the 1-hr trial included only trials in which the beetle took at least one flight and was analyzed with a linear mixed model using lme4 and lmerTest.

In all dispersal models, the three-way interaction was dropped from the model if it was not significant. Significance of random effects was determined by comparing models with a χ^2 test. Post hoc comparison of means was done with the emmeans package version 1.5.4 (Lenth 2020). All analyses for both life history and dispersal traits were done in R version 3.6.2 (R Core Team 2018).

3. Results

3.1 Life History Traits

We collected weight and egg count data from 130 core and 140 edge female *D. carinulata*, and age at first reproduction from 104 core and 118 edge females. We weighed 279 core and 311 edge males on the day of their dispersal trials.

The weight of females at adult emergence ranged from 6.5 to 14.7 mg. Females from the edge of the range were larger than females from the core (core mass=9.65 mg, edge mass=10.30 mg; $F=8.04$, $p=0.030$; Fig. 3A). The population random effect was not significant (variance=0.06, $\chi^2=2.53$, $p=0.112$).

The weight of males at the time of the dispersal trial (thus, after feeding *ad libitum*) ranged from 6.9 to 19.3 mg. There was no difference in weight of males between core and edge populations ($F=0.67$, $p=0.445$). Older beetles were larger than younger beetles ($F=46.55$, $p<0.001$) and males reared at high density were larger than those reared at low density (high mass=12.2 mg, low mass=11.8 mg; $F=15.09$, $p<0.001$). The population random effect was significant (variance=0.10, $\chi^2=18.11$, $p<0.001$).

The number of eggs from the first day of reproduction ranged from 0 to 30. The proportion of beetles laying eggs during the study did not differ between the core and edge of the range (core proportion=0.813, edge proportion=0.845, $\chi^2=0.46$, $p=0.499$). Of those that oviposited during the study, edge beetles were slightly more fecund (core eggs=10.9, SE=0.40; edge eggs=12.0, SE=0.40; $\chi^2=3.39$, $p=0.067$) (Fig. 3B). Age at first reproduction was not a significant predictor of the number of eggs laid ($\chi^2=0.01$, $p=0.916$), but larger females laid more eggs ($\chi^2=5.79$, $p=0.016$). The population random effect was not significant (variance=0.001, $\chi^2=0.11$, $p=0.738$). When weight was excluded as a predictor due to its correlation with range, range became a significant predictor of fecundity (core eggs=10.8, SE=0.45; edge eggs=12.1, SE=0.46; $\chi^2=4.45$, $p=0.035$).

Age at first reproduction ranged from three to seven days after adult emergence. Edge beetles reproduced slightly earlier than core beetles (core age=5.51 days, SE=0.11; edge age=5.24 days, SE=0.10; $\chi^2=3.64$, $p=0.058$; Fig. 3C). Weight at emergence was not a significant predictor of age at first reproduction ($\chi^2=1.92$, $p=0.166$). When weight was excluded from the model due to correlation with range, range became a significant predictor of age at first reproduction (core age=5.54 days, SE=0.11; edge age=5.22 days, SE=0.10; $\chi^2=5.01$, $p=0.025$). The population random effect was not significant (variance=0.00, $\chi^2=0$, $p=1$).

3.2 Dispersal

We collected dispersal data from 279 males from the core and 311 males from the edge, with 65 to 81 males in each density-mating treatment combination and at least 15 from each population. Average speed was calculated for 231 core and 266 edge males that took at least one flight, with 56 to 71 males in each density-mating treatment combination. Results for covariates and random effects are in Table S2. The three-way interaction between range, density, and mate status was not significant in any model, so it was removed.

During the dispersal trials, 84.2% of all beetles took at least one flight. For presence of flight, the interaction between density and mate status was marginally significant ($\chi^2=3.01$, $p=0.083$), such that unmated beetles were more likely to fly than mated beetles at low density, but there was no difference between mated and unmated at high density (Fig. 4A). The interaction between mate status and range was significant ($\chi^2=4.14$, $p\text{-value}=0.042$), such that unmated edge beetles had significantly higher probability of flight than unmated core beetles (core probability=0.84, edge probability=0.93; $p=0.021$), but there was no difference for mated beetles (core probability=0.86, edge probability=0.84; $p=0.722$; Fig. 4B).

The number of flights during the 1-hr trial ranged from 0 to 13 (median=2). Based on residual diagnostic plots, the negative binomial mixed model best met assumptions of normality of residuals and was chosen as the final model. The interaction between density and mate status was marginally significant ($\chi^2=3.48$, $p=0.062$), such that unmated beetles took more flights than mated beetles at low density and the opposite at high density (Fig. 5A). The interaction between density and range was significant ($\chi^2=4.93$, $p\text{-value}=0.026$), such that at low density, beetles at the edge took more flights than core beetles (core flights=1.68, SE=0.15; edge flights=2.13, SE=0.17; $p=0.040$), but there was no difference at high density (core flights=2.03, SE=0.16; edge flights=1.93, SE=0.15; $p=0.625$; Fig. 5B).

Individuals flew up to 2.3 km (median=8 m), during the 1-hr trial. Based on residual diagnostic plots, the zero-inflated generalized Poisson model best met assumptions of normality of residuals and was chosen as the final model. The interaction between density and mate status was significant, such that unmated beetles flew further than mated beetles at low density, but the opposite at high density ($\chi^2=6.13$, $p=0.013$; Fig. 6A). The range main effect was also marginally significant, such that edge beetles flew further than core beetles (core distance=59.6 m, SE=10.01; edge distance=69.5, SE=11.45; $\chi^2=2.998$, $p=0.083$).

Average flight speed ranged from 0.138 m/s to 0.914 m/s. None of the main effects or two-way interactions between range, density, and mate status were significant (Fig. 6B).

4. Discussion

We used the recent range expansion of *D. carinulata* biocontrol agents to study the evolution of life history and dispersal traits between core and edge populations. We merged the distinct bodies of theory that focus on evolution during range expansion (e.g. Phillips *et al.* 2010b; Shine *et al.* 2011; Peischl & Excoffier 2015; Peischl *et al.* 2015) and on informed dispersal (Clobert *et al.* 2009; De Bona *et al.* 2019) to refine predictions for how dispersal may differ across a range expansion in different ecological contexts (Fig. 1C). We examined dispersal traits of mated and unmated individuals reared at high and low densities. We also tested divergent theoretical predictions regarding fitness at range edges (Fig. 1A, B). We found that core and edge populations diverged in fecundity, age at first reproduction, female body size, and dispersal traits.

Low population densities on the edge of expanding ranges lead to two alternative evolutionary outcomes for life history traits such as body size, fecundity, and age at first reproduction, depending on whether selection at low densities or expansion load is dominant at the edge. We found that edge populations laid on average one more egg per day than core populations on the first day of reproduction. This could sum to a substantial difference in fitness if the difference persists throughout the lifespan of the insect, as *D. carinulata* continue to reproduce throughout their multi-week adult lifespan. We used weight as an additional gauge of fecundity, since insect body size is related to how many eggs females can fit in their bodies and egg production in general (Berger *et al.* 2012). We found this association to hold in *D. carinulata*. While female weight increased at the edge, male weight did not shift across the range. Based on our results from female weight, fecundity, and age at first reproduction, theory suggests that evolution may be driven by lower density at the edge compared to the core, leading to a shift in selection as the dominant process acting across this range

expansion (Fig. 1A), rather than expansion load (Fig. 1B).

Several characteristics of *D. carinulata* and its range expansion are consistent with finding no evidence of expansion load at the edge. Expansion load occurs when genetic diversity is low at the edge, but neutral genetic diversity has been retained along *D. carinulata* expansion fronts from core to recently colonized populations (Stahlke *et al.* 2021, in revision). Ecologically important genetic variation also appears to be high at the edge, as during this range expansion, the timing of diapause induction has rapidly evolved (Bean *et al.* 2012). Even though population densities may be lower at the edge than in the core, this species tends to aggregate (Cossé *et al.* 2005; Bean *et al.* 2007b), which may allow it to maintain high enough population densities on the range edge to reduce the consequences of serial founder events and gene surfing inherent in range expansion. Finally, biocontrol agents in general might maintain more genetic variation than invasive species, since agents are often collected from multiple source populations and population sizes are deliberately large before field releases to avoid reducing variation that could increase establishment in the field (Szűcs *et al.* 2017).

The evolution of dispersal traits during range expansion has been a topic of great interest in invasion biology. Other studies have shown an increase in dispersal traits in edge populations, including an increase in long-winged morphs of the Australian field cricket in edge populations (Simmons & Thomas 2004) and longer legged cane toads at the edge of the invasion front (Phillips *et al.* 2006). Dispersal evolution during range expansion has been studied within the context of density-dependence (Altwegg *et al.* 2013; Bitume *et al.* 2013; Fronhofer *et al.* 2017), but rarely in the context of other external or internal conditions. Without explicitly taking these factors into account, measurements of dispersal can be difficult to interpret. Across all metrics of dispersal, we found that density and mating status interacted as predicted by informed dispersal theory: unmated individuals at low density and mated individuals at high density dispersed more often and further. For dispersal distance, edge beetles flew further than core beetles across all density and mating treatments, which shows the additive effect of the edge predicted by spatial sorting theory.

In contrast to dispersal distance, when we measured emigration potential, core and edge beetles responded differently to density and mating contexts. For presence of flight, beetles from edge populations were especially sensitive to mating status and were more likely to initiate flight when unmated. For number of flights, beetles from edge populations were more sensitive to density and took more flights when at low density. These results suggest that core and edge beetles have evolved to initiate dispersal based on different density and mating status cues. The conditions at the edge of the range that have given rise to these patterns in emigration are not yet clear. Population density has not been measured across the range, but if population densities are lower at the edge than the core, as assumed by most range expansion theory, dispersal from low densities would be beneficial to unmated edge beetles looking for a mate. But, at the higher density core, emigrating from low density may not increase the chances of finding a mate. Beetles from core and edge may have also adapted to different density environments, which would impact their responses to these factors (De Bona *et al.* 2019). Our dispersal results show the effects of both spatial sorting of dispersal ability and informed dispersal at the edge of the *D. carinulata* range expansion. The context of dispersal decisions should be accounted for in the design of future studies since core and edge populations can evolve different responses to these conditions.

During range expansion, heterogeneous environments exert selection pressures, which can interact with the spatial processes of range expansion. For example, maladaptation during range expansion can slow down the expansion, which in turn would impede spatial sorting by dispersal ability (Gilbert *et al.* 2017). During its range expansion, *D. carinulata* was maladapted to photoperiod cues at southern latitudes (Bean *et al.* 2007a, 2012) and possibly to higher temperatures in southern latitudes (Herrera *et al.* 2005). Despite this, we found that *D. carinulata* showed the signatures of spatial sorting and selection for increased fecundity. Our results suggest that spatial processes during range expansion can be important to natural range expansions over heterogeneous environments.

We have demonstrated evolution of both dispersal and life history traits in *D. carinulata* and we do not see evidence of a trade-off between these dispersal and life-history traits, though we were unable to measure

all traits within the same individuals. Dispersal traits increased while female weight and fecundity also increased and age at first reproduction decreased at the edge of the range expansion. Other study systems have shown a trade-off between dispersal and reproduction traits at the edge (Hughes *et al.* 2003; Kelehear & Shine 2020). As Bonte & Doherty (2017) argue, dispersal can be seen as a life-history trait that evolves independently, and that may be the case here. Alternatively, there may exist trade-offs between the traits we studied and other traits not measured here, such as lifespan or immune system development or function (reviewed in Chuang & Peterson 2016).

Long-term success of the *Tamarix-Diorhabda* biocontrol program requires *D. carinulata* to continue its spread to cover the range of the target weed and to adapt to new environments. Our results contribute further evidence that there is sufficient genetic variation for adaptive evolution to occur. We show an increase in both reproductive and dispersal ability at the edge, which will enable *D. carinulata* to continue and possibly accelerate its range expansion in the future (Phillips *et al.* 2010a). As the first test of evolutionary theory of range expansions in a biocontrol agent, we show that these theoretical predictions can be applied to range expansions across heterogeneous environments, especially when the ecological context the individuals experience is included.

As range expansions of invasive species, biocontrol agents, and threatened species tracking changing climates all become more common in the modern age, predicting the dynamics of range expansions is becoming increasingly important. By utilizing the *Tamarix-Diorhabda* biocontrol system, we demonstrate an evolved increase in weight and fecundity and a decrease in age at first reproduction at the edge of the range expansion. Additionally, we demonstrate a density- and mating status-dependent increase in emigration potential and an overall increase in flight distance at the edge of the range expansion. Our results suggest that evolutionary processes impacting range expansion can act simultaneously with adaptation to environmental gradients.

5. Acknowledgements

We thank undergraduate research assistants Jenna Galvin, Tori Applehans, Itai Boneh, Sam Chaney, Katie Calsyn, Jordan Fleming, Chelsey Gilley-Dunn, and Kora Kastengren for their help in collecting the dispersal and life history data; the Colorado State University Physics Shop for their assistance in designing and building the flight mills; and Brandon Dyck for the Python program to process the flight mill data. This research was supported by USDA Agriculture and Food Research Initiative grant COLO-2016-09135 to RAH, EVB, DWB, and PAH, and RAH additionally acknowledges support from USDA NIFA Hatch project 1012868.

6. References

- Altwegg, R., Collingham, Y.C., Erni, B. & Huntley, B. (2013). Density-dependent dispersal and the speed of range expansions. *Divers. Distrib.* , 19, 60–68.
- Ashenden, T., Rooke, A.C. & Fox, M.G. (2017). Boldness and dispersal tendency of native and invasive pumpkinseed (*Lepomis gibbosus*): is spatial sorting creating superior invaders? *Aquat. Invasions* , 12, 311–320.
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using {lme4}. *J. Stat. Softw.* , 67, 1–48.
- Bean, D. & Dudley, T. (2018). A synoptic review of *Tamarix* biocontrol in North America: tracking success in the midst of controversy. *BioControl* , 63, 361–376.
- Bean, D.W., Dalin, P. & Dudley, T.L. (2012). Evolution of critical day length for diapause induction enables range expansion of *Diorhabda carinulata*, a biological control agent against tamarisk (*Tamarix* spp.). *Evol. Appl.* , 5, 511–523.
- Bean, D.W., Dudley, T.L. & Keller, J.C. (2007a). Seasonal timing of diapause induction limits the effective range of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) as a biological control agent for tamarisk (*Tamarix* spp.). *Environ. Entomol.* , 36, 15–25.

- Bean, D.W., Wang, T., Bartelt, R.J. & Zilkowski, B.W. (2007b). Diapause in the leaf beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent for tamarisk (*Tamarix* spp.). *Environ. Entomol.* , 36, 531–540.
- Bénichou, O., Calvez, V., Meunier, N. & Voituriez, R. (2012). Front acceleration by dynamic selection in Fisher population waves. *Phys. Rev. E* , 86, 041908.
- Berger, D., Olofsson, M., Friberg, M., Karlsson, B., Wiklund, C. & Gotthard, K. (2012). Intraspecific variation in body size and the rate of reproduction in female insects - adaptive allometry or biophysical constraint? *J. Anim. Ecol.* , 81, 1244–1258.
- Berthouly-Salazar, C., van Rensburg, B.J., Le Roux, J.J., van Vuuren, B.J. & Hui, C. (2012). Spatial sorting drives morphological variation in the invasive bird, *Acridotheris tristis*. *PLoS One* , 7, 1–9.
- Bitume, E. V., Bonte, D., Ronce, O., Bach, F., Flaven, E., Olivieri, I., *et al.* (2013). Density and genetic relatedness increase dispersal distance in a subsocial organism. *Ecol. Lett.* , 16, 430–437.
- De Bona, S., Bruneaux, M., Lee, A.E.G., Reznick, D.N., Bentzen, P. & López-Sepulcre, A. (2019). Spatio-temporal dynamics of density-dependent dispersal during a population colonisation. *Ecol. Lett.* , 22, 634–644.
- Bonte, D. & Doherty, M. (2017). Dispersal: a central and independent trait in life history. *Oikos* , 126, 472–479.
- Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., *et al.* (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *R J.* , 9, 378–400.
- Brooks, M.E., Kristensen, K., Darrigo, M.R., Rubim, P., Uriarte, M., Bruna, E., *et al.* (2019). Statistical modeling of patterns in annual reproductive rates. *Ecology* , 100, 1–7.
- Brown, G.P., Kelehear, C. & Shine, R. (2013). The early toad gets the worm: Cane toads at an invasion front benefit from higher prey availability. *J. Anim. Ecol.* , 82, 854–862.
- Burton, O.J., Phillips, B.L. & Travis, J.M.J. (2010). Trade-offs and the evolution of life-histories during range expansion. *Ecol. Lett.* , 13, 1210–1220.
- Chuang, A. & Peterson, C.R. (2016). Expanding population edges: Theories, traits, and trade-offs. *Glob. Chang. Biol.* , 22, 494–512.
- Clobert, J., Le Galliard, J.F., Cote, J., Meylan, S. & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecol. Lett.* , 12, 197–209.
- Cossé, A.A., Bartelt, R.J., Zilkowski, B.W., Bean, D.W. & Petroski, R.J. (2005). The aggregation pheromone of *Diorhabda elongata*, a biological control agent of saltcedar (*Tamarix* spp.): Identification of two behaviorally active components. *J. Chem. Ecol.* , 31, 657–670.
- Cwynar, L.C. & MacDonald, G.M. (1987). Geographical Variation of Lodgepole Pine in Relation to Population History. *Am. Nat.* , 129, 463–469.
- Dällenbach, L.J., Glauser, A., Lim, K.S., Chapman, J.W. & Menz, M.H.M. (2018). Higher flight activity in the offspring of migrants compared to residents in a migratory insect. *Proc. R. Soc. B* , 285.
- DeLoach, C.J., Lewis, P.A., Herr, J.C., Carruthers, R.I., Tracy, J.L. & Johnson, J. (2003). Host specificity of the leaf beetle, *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) from Asia, a biological control agent for saltcedars (*Tamarix*: Tamaricaceae) in the Western United States. *Biol. Control* , 27, 117–147.
- Endriss, S.B., Vahsen, M.L., Bitume, E. V., Grey Monroe, J., Turner, K.G., Norton, A.P., *et al.* (2019). The importance of growing up: juvenile environment influences dispersal of individuals and their neighbours. *Ecol. Lett.* , 22, 45–55.

- Fisher, R.A. (1937). The wave of advance of advantageous genes. *Ann. Eugen.* , 7, 355–369.
- Fronhofer, E.A. & Altermatt, F. (2015). Eco-evolutionary feedbacks during experimental range expansions. *Nat. Commun.* , 6.
- Fronhofer, E.A., Gut, S. & Altermatt, F. (2017). Evolution of density-dependent movement during experimental range expansions. *J. Evol. Biol.* , 30, 2165–2176.
- Gilbert, K.J., Sharp, N.P., Angert, A.L., Conte, G.L., Draghi, J.A., Guillaume, F., *et al.* (2017). Local Adaptation Interacts with Expansion Load during Range Expansion: Maladaptation Reduces Expansion Load. *Am. Nat.* , 189, 368–380.
- González-Martínez, S.C., Ridout, K. & Pannell, J.R. (2017). Range Expansion Compromises Adaptive Evolution in an Outcrossing Plant. *Curr. Biol.* , 27, 2544–2551.e4.
- Hartig, F. (2020). DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.3.3.0.
- Hastings, A., Cuddington, K., Davies, K.F., Dugaw, C.J., Elmendorf, S., Freestone, A., *et al.* (2005). The spatial spread of invasions: new developments in theory and evidence. *Ecol. Lett.* , 8, 91–101.
- Heimpel, G.E. & Mills, N.J. (2017). *Biological Control: Ecology and Applications* . Cambridge University Press.
- Herrera, A.M., Dahlsten, D.D., Tomic-Carruthers, N. & Carruthers, R.I. (2005). Estimating Temperature-Dependent Developmental Rates of *Diorhabda elongata* (Coleoptera: Chrysomelidae), a Biological Control Agent of Saltcedar (*Tamarix* spp.). *Environ. Entomol.* , 34, 775–784.
- Hill, J.K., Griffiths, H.M. & Thomas, C.D. (2011). Climate change and evolutionary adaptations at species' range margins. *Annu. Rev. Entomol.* , 56, 143–159.
- Hughes, C.L., Hill, J.K. & Dytham, C. (2003). Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. *Proc. R. Soc. B Biol. Sci.* , 270, 147–150.
- Joe, H. & Zhu, R. (2005). Generalized poisson distribution: The property of mixture of poisson and comparison with negative binomial distribution. *Biometrical J.* , 47, 219–229.
- Jones, H.B.C., Lim, K.S., Bell, J.R., Hill, J.K. & Chapman, J.W. (2016). Quantifying interspecific variation in dispersal ability of noctuid moths using an advanced tethered flight technique. *Ecol. Evol.* , 6, 181–190.
- Kelehear, C. & Shine, R. (2020). Tradeoffs between dispersal and reproduction at an invasion front of cane toads in tropical Australia. *Sci. Rep.* , 10, 1–7.
- Van Klinken, R.D. & Edwards, O.R. (2002). Is host-specificity of weed biological control agents likely to evolve rapidly following establishment? *Ecol. Lett.* , 5, 590–596.
- Klopfstein, S., Currat, M. & Excoffier, L. (2006). The Fate of Mutations Surfing on the Wave of a Range Expansion. *Mol. Biol. Evol.* , 23, 482–490.
- Kot, M. (1996). Dispersal data and the spread of invading organisms. *Ecology* , 77, 2027–2042.
- Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). {lmerTest} Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* , 82, 1–26.
- Lenth, R. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means.
- Lewis, P.A., DeLoach, C.J., Knutson, A.E., Tracy, J.L. & Robbins, T.O. (2003). Biology of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae), an Asian leaf beetle for biological control of saltcedars (*Tamarix* spp.) in the United States. *Biol. Control* , 27, 101–116.

- Lombaert, E., Estoup, A., Facon, B., Joubard, B., Grégoire, J.C., Jannin, A., *et al.* (2014). Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. *J. Evol. Biol.* , 27, 508–517.
- Maes, S., Massart, X., Grégoire, J.C. & De Clercq, P. (2014). Dispersal potential of native and exotic predatory ladybirds as measured by a computer-monitored flight mill. *BioControl* , 59, 415–425.
- Merwin, A.C. (2019). Flight capacity increases then declines from the core to the margins of an invasive species' range. *Biol. Lett.* , 15, 20190496.
- Minter, M., Pearson, A., Lim, K.S., Wilson, K., Chapman, J.W. & Jones, C.M. (2018). The tethered flight technique as a tool for studying life-history strategies associated with migration in insects. *Ecol. Entomol.* , 43, 397–411.
- Monty, A. & Mahy, G. (2010). Evolution of dispersal traits along an invasion route in the wind-dispersed *Senecio inaequidens* (Asteraceae). *Oikos* , 119, 1563–1570.
- Mustin, K., Benton, T.G., Dytham, C. & Travis, J.M.J. (2009). The dynamics of climate-induced range shifting; perspectives from simulation modelling. *Oikos* , 118, 131–137.
- Ochocki, B.M. & Miller, T.E.X. (2017). Rapid evolution of dispersal ability makes biological invasions faster and more variable. *Nat. Commun.* , 8, 14315.
- Ozsoy, A.Z., Stahlke, A.R., Jamison, L. & Johnson, M.J. (2019). Genetic Identification and 892 Hybrid Analysis of Tamarisk Leaf Beetle (*Diorhabda* spp.) and Tamarisk Weevil (*Coniatus* 893 spp.) along the Rio Grande River NM watershed. *Army Corps Eng. Contract No. W912PP-14-P-0041* .
- Peischl, S., Dupanloup, I., Kirkpatrick, M. & Excoffier, L. (2013). On the accumulation of deleterious mutations during range expansions. *Mol. Ecol.* , 22, 5972–5982.
- Peischl, S. & Excoffier, L. (2015). Expansion load: Recessive mutations and the role of standing genetic variation. *Mol. Ecol.* , 24, 2084–2094.
- Peischl, S., Kirkpatrick, M. & Excoffier, L. (2015). Expansion Load and the Evolutionary Dynamics of a Species Range. *Am. Nat.* , 185, E81–E93.
- Van Petegem, K.H.P., Boeye, J., Stoks, R. & Bonte, D. (2016). Spatial Selection and Local Adaptation Jointly Shape Life-History Evolution during Range Expansion. *Am. Nat.* , 188, 485–498.
- Pettit, L.J., Greenlees, M.J. & Shine, R. (2016). Is the enhanced dispersal rate seen at invasion fronts a behaviourally plastic response to encountering novel ecological conditions? *Biol. Lett.* , 12, 20160639.
- Phillips, B.L. (2015). Evolutionary processes make invasion speed difficult to predict. *Biol. Invasions* , 17, 1949–1960.
- Phillips, B.L., Brown, G.P. & Shine, R. (2010a). Evolutionarily accelerated invasions: The rate of dispersal evolves upwards during the range advance of cane toads. *J. Evol. Biol.* , 23, 2595–2601.
- Phillips, B.L., Brown, G.P. & Shine, R. (2010b). Life-history evolution in range-shifting populations. *Ecology* , 91, 1617–1627.
- Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature* , 439, 803.
- R Core Team. (2018). R: A language and environment for statistical Computing.
- Shine, R., Brown, G.P. & Phillips, B.L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proc. Natl. Acad. Sci. U. S. A.* , 108, 5708–5711.
- Simmons, A.D. & Thomas, C.D. (2004). Changes in Dispersal during Species' Range Expansions. *Am. Nat.* , 164, 378–395.

- Stahlke, A.R., Bitume, E. V, Ozsoy, A.Z., Bean, D.W., Veillet, A., Clark, M.I., *et al.* (2021). Hybridization and range expansion in tamarisk beetles (*Diorhabda* spp.) introduced to North America for classical biological control. *bioRxiv* , 2021.05.18.444725.
- Szűcs, M., Melbourne, B.A., Tuff, T., Weiss-Lehman, C. & Hufbauer, R.A. (2017). Genetic and demographic founder effects have long-term fitness consequences for colonising populations. *Ecol. Lett.* , 20, 436–444.
- Szűcs, M., Vahsen, M.L., Melbourne, B.A., Hoover, C., Weiss-Lehman, C., Hufbauer, R.A., *et al.* (2017). Rapid adaptive evolution in novel environments acts as an architect of population range expansion. *Proc. Natl. Acad. Sci. U. S. A.* , 114, 13501–13506.
- Szűcs, M., Vercken, E., Bitume, E. V. & Hufbauer, R.A. (2019). The implications of rapid eco-evolutionary processes for biological control - a review. *Entomol. Exp. Appl.* , 167, 598–615.
- Tanaka, K. & Murata, K. (2017). Genetic basis underlying rapid evolution of an introduced insect *Ophraella communa* (Coleoptera: Chrysomelidae): Heritability of photoperiodic response. *Environ. Entomol.* , 46, 167–173.
- Thomas, C.D., Bodsworth, E.J., Wilson, R.J., Simmons, A.D., Davies, Z.G., Musche, M., *et al.* (2001). Ecological and evolutionary processes at expanding range margins. *Nature* , 411, 577–581.
- Travis, J.M.J. & Dytham, C. (2002). Dispersal evolution during invasions. *Evol. Ecol. Res.* , 4, 1119–1129.
- Travis, J.M.J., Munkemuller, T., Burton, O.J., Best, A., Dytham, C. & Johst, K. (2007). Deleterious Mutations Can Surf to High Densities on the Wave Front of an Expanding Population. *Mol. Biol. Evol.* , 24, 2334–2343.
- Travis, J.M.J., Mustin, K., Benton, T.G. & Dytham, C. (2009). Accelerating invasion rates result from the evolution of density-dependent dispersal. *J. Theor. Biol.* , 259, 151–158.
- Weiss-Lehman, C., Hufbauer, R.A. & Melbourne, B.A. (2017). Rapid trait evolution drives increased speed and variance in experimental range expansions. *Nat. Commun.* , 8, 14303.
- Wolz, M., Klockmann, M., Schmitz, T., Pekár, S., Bonte, D. & Uhl, G. (2020). Dispersal and life-history traits in a spider with rapid range expansion. *Mov. Ecol.* , 8, 2.
- Wright, M.G. & Bennett, G.M. (2018). Evolution of biological control agents following introduction to new environments. *BioControl* , 63, 105–116.

Hosted file

Dispersal fecundity figures v11.pdf available at <https://authorea.com/users/428255/articles/532154-evolution-of-life-history-and-dispersal-traits-during-the-range-expansion-of-a-biological-control-agent>