Journal of Animal Ecology

Journal of Animal Ecology 2017, **86,** 21–34 doi: 10.1111/1365-2656.12567

Genetic mixture of multiple source populations accelerates invasive range expansion

Natalie K. Wagner[1]†, Brad M. Ochocki[1], Kerri M. Crawford[2], Aldo Compagnoni[1] and Tom E.X. Miller[1]*

[1]*Department of BioSciences, Program in Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA; and* [2]*Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA*

Summary

1. A wealth of population genetic studies have documented that many successful biological invasions stem from multiple introductions from genetically distinct source populations. Yet, mechanistic understanding of whether and how genetic mixture promotes invasiveness has lagged behind documentation that such mixture commonly occurs. We conducted a laboratory experiment to test the influence of genetic mixture on the velocity of invasive range expansion.

2. The mechanistic basis for effects of genetic mixture could include evolutionary responses (mixed invasions may harbour greater genetic diversity and thus elevated evolutionary potential) and/or fitness advantages of between-population mating (heterosis). If driven by evolution, positive effects of source population mixture should increase through time, as selection sculpts genetic variation. If driven by heterosis, effects of mixture should peak following first reproductive contact and then dissipate.

3. Using a laboratory model system (beetles spreading through artificial landscapes), we quantified the velocity of range expansion for invasions initiated with one, two, four or six genetic sources over six generations. Our experiment was designed to test predictions corresponding to the evolutionary and heterosis mechanisms, asking whether any effects of genetic mixture occurred in early or later generations of range expansion. We also quantified demography and dispersal for each experimental treatment, since any effects of mixture should be manifest in one or both of these traits.

4. Over six generations, invasions with any amount of genetic mixture (two, four and six sources) spread farther than single-source invasions. Our data suggest that heterosis provided a 'catapult effect', leaving a lasting signature on range expansion even though the benefits of outcrossing were transient. Individual-level trait data indicated that genetic mixture had positive effects on local demography (reduced extinction risk and enhanced population growth) during the initial stages of invasion but no consistent effects on dispersal ability.

5. Our work is the first to demonstrate that genetic mixture can alter the course of spatial expansion, the stage of invasion typically associated with the greatest ecological and economic impacts. We suggest that similar effects of genetic mixture may be a common feature of biological invasions in nature, but that these effects can easily go undetected.

Key-words: biological invasions, dispersal, genetic diversity, heterosis, spatial spread

Introduction

Identifying sources of variation in the outcomes of species introductions is a fundamental goal of invasion biology. There is long-standing interest in the role of multiple introductions as a possible catalyst of biological invasion (Baker & Stebbins 1965; Ellstrand & Schierenbeck 2000; Dlugosch & Parker 2008; Simberloff 2009; Whitney & Gering 2015). A large body of population genetic studies has revealed that many successful invaders harbour genetic signals that are consistent with contributions from multiple, disparate source populations from the native range or other invasive ranges (e.g. Kolbe *et al*. 2004;

*Correspondence author. E-mail: tom.miller@rice.edu
†Present address: Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands

Kelly *et al.* 2006; Roman 2006; Simon-Bouhet, Garcia-Meunier & Viard 2006; Lavergne & Molofsky 2007; Konečný *et al.* 2013). Multiple introductions can clearly leave a fingerprint on the genetic composition of invasive populations, but whether they are a driver or passenger of invasiveness remains an open question (Rius & Darling 2014). Invasions with high source population diversity are likely those that are also subject to high propagule pressure (the very process that delivers source diversity), which can have positive demographic effects independent of any genetic contributions (Hufbauer *et al.* 2013; Szűcs *et al.* 2014). Understanding whether and how genetic mixture exerts a causal influence on invasiveness requires experimental manipulations, though these remain rare (Forsman 2014). In this article, we describe an experiment to test the influence of genetic mixture on invasiveness, holding propagule pressure constant. We quantify 'invasiveness' as the velocity of range expansion following introduction, as it integrates two processes that are often interpreted as proxies for invasive potential: the intrinsic rate of increase and dispersal ability (Kot, Lewis & van den Driessche 1996; Neubert & Caswell 2000). We consider two non-exclusive mechanisms by which genetic mixture associated with multiple introductions might promote invasiveness.

First, multiple introductions can increase genetic diversity and thus increase potential for adaptive evolution (Lee 2002; Roman & Darling 2007; Dlugosch & Parker 2008). Invasive populations stemming from multiple introductions tend to harbour greater allelic diversity (but not necessarily greater additive genetic variance) than those arising from a single introduction (Dlugosch & Parker 2008). Increases in adaptive potential associated with source population diversity may promote rapid invasion (Lavergne & Molofsky 2007). In some cases, increased evolutionary potential may allow invasive populations to adapt more quickly to novel environments that they encounter outside their native range (e.g. Kelly *et al.* 2006; Krehenwinkel & Tautz 2013). However, even in the absence of strong environmental selective pressures, evolution of ecologically important traits can promote rapid invasion.

In an expanding population, dispersal ability can evolve by 'spatial selection', an evolutionary process that requires a genetic basis to dispersal ability and includes several components (Phillips *et al.* 2008; Phillips, Brown & Shine 2010b; Perkins *et al.* 2013). First, alleles conferring dispersal ability may become spatially sorted, with high-dispersal alleles over-represented at the leading edge of the invasion wave, where they are carried by long-distance dispersers. Secondly, spatial structure may promote assortative mating by dispersal ability, causing good dispersers to leave good-dispersing offspring. Finally, the low-density environment and high per capita resource availability of the leading edge may allow good dispersers to leave more descendants, causing an increase in the frequency of high-dispersal alleles. Spatial selection can cause accelerating rates of range expansion because the leading edge becomes increasingly dominated by high-dispersal genotypes, generation after generation. For example, the evolution of dispersal ability by spatial selection is thought to have caused the accelerating invasion of cane toads across northern Australia (Phillips *et al.* 2006, 2008; Lindström *et al.* 2013). While there is much current interest in the evolution of dispersal and its influence on the ecological dynamics of range expansion (Kubisch *et al.* 2014; Chuang & Peterson 2016), no previous studies have evaluated whether multiple introductions could amplify the evolutionary potential of dispersal ability or reproductive traits that may also be selected at the leading edge (Perkins *et al.* 2013).

The second mechanism through which multiple introductions may promote invasiveness is intraspecific hybridization and its potential fitness benefits (Ellstrand & Schierenbeck 2000; Facon *et al.* 2005; Hovick & Whitney 2014). Reproductive contact between genetically disparate populations may cause heterosis (fitness advantage associated with heterozygosity) in their offspring, particularly if individual source populations experience strong bottlenecks and high genetic load. Heterosis following reproductive contact may have lasting effects on long-term invasion dynamics even if the fitness benefits of out-breeding are short-lived. For example, in a retrospective analysis of bird introductions, Drake (2006) found evidence for a 'catapult effect' of heterosis: a transient fitness benefit during the early stages of introduction that increased the odds of long-term establishment. However, sexual contact between multiple sources is not always associated with heterosis (e.g. Benvenuto *et al.* 2012) and could even generate maladaptive phenotypes if source populations are locally adapted to contrasting environmental conditions (Travis, Hammershøj & Stephenson 2005; Verhoeven *et al.* 2011; Rius & Darling 2014). While hybridization is often considered an evolutionary process, we treat hybridization as a distinct mechanism because fitness benefits of outcrossing do not necessarily involve change in population allele frequency.

Little is known about the relative contributions of evolution and heterosis to the overall effects of genetic mixture on invasiveness. These two mechanisms are expected to leave different signatures on the temporal dynamics of range expansion (Fig. 1); our experiment was designed to distinguish between these expectations. If driven by increased evolutionary potential, positive effects of multiple introductions on invasion velocity should increase through time, as selection sculpts the genetic variation that multiple introductions delivered. In particular, the evolution of dispersal ability by spatial selection is expected to cause acceleration of spread, such that invasion velocities in later generations exceed those in early generations (Phillips *et al.* 2008). Alternatively, if driven by heterosis, the influence of multiple introductions should peak immediately following first reproductive

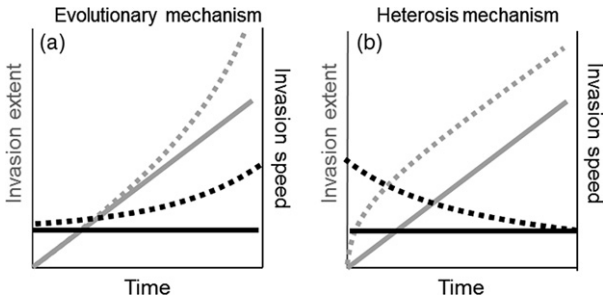

Fig. 1. Predictions for invasion trajectories of single-source (solid lines) and multi-source (dashed lines) invasions in terms of spatial extent (left axis, grey lines) and instantaneous velocity (right axis, black lines). (a) If source population mixture promotes evolution by selection, effects of mixture on invasion velocity should increase through time, causing accelerating change in spatial extent. (b) If source population mixture causes heterosis (heterozygote fitness advantage), positive effects of mixture should peak early in the invasion following first reproductive contact and then dissipate, leading to increasingly similar velocities of single- and multi-source invasions but different long-term invasion extents reflecting the early difference in speed.

contact, when heterozygosity would be maximized, and then decline if mating is random (Facon *et al.* 2005; Drake 2006). Thus, our goal was to contrast the expansion dynamics of single- and multi-source invasions and determine whether differences emerge after several generations of spread (consistent with an evolutionary mechanism) or immediately following introduction (consistent with short-term effects of heterosis). We also compared dispersal and local population growth, the traits that underlie invasive spread, between single- and multi-source invasions at the beginning and end of range expansion. We predicted that a heterosis effect of genetic mixture would enhance trait values at the beginning of the invasion process while evolution would lead to trait differences detectable after several generations of spread (Fig. 1). It is also possible for these mechanisms to combine – hybridization could cause heterosis and create novel genotypes that are favoured by selection (Ellstrand & Schierenbeck 2000; Hovick & Whitney 2014) – leading to short- and long-term effects of multiple introductions.

Under both the hybridization and evolutionary mechanisms, effects of multiple introductions may depend on the number of source populations that contribute to the invasion. For example, greater source richness could promote allelic variation (Dlugosch & Parker 2008) and increase the odds of including high-fitness genotypes that are favoured by selection, a type of ecological sampling effect called a 'selection effect' (Hughes *et al.* 2008). Selection effects may also reduce variance of mixed populations because favoured genotypes increase in frequency through time. Effects of source population richness on the heterosis mechanism are less clear, but it is possible that source richness could increase potential for between-population hybridization if mating is random. Thus, in addition to the contrast between single- and multi-source invasions, our experiment included quantitative variation in source population richness.

Our work focused on the beetle *Callosobruchus maculatus*, a powerful laboratory system for the study of spatial population dynamics and range expansion (e.g. Strevens & Bonsall 2011; Miller & Inouye 2013). We manipulated the genetic composition of beetle invasions by sampling one, two, four or six sources from a pool of genetically differentiated but reproductively compatible populations from throughout the species' global distribution. We quantified the spread of experimental invasions through artificial landscapes over six generations. We also tracked dispersal and demography traits from the beginning to the end of the experiment to quantify life-history responses to source population diversity and identify evolutionary change. Ecological theory for spreading populations tells us that dispersal (individual displacement) and demography (local population growth) are the two determinants of invasion velocity (Okubo 1980; Kot, Lewis & van den Driessche 1996); if there is an effect of source population mixture on beetle invasions, it should occur via one or both of these two traits. Finally, we used model selection methods to evaluate support for competing expectations regarding the effects of source diversity. Thus, the motivating questions of this study were as follows: (1) Does genetic mixture modify the dynamics of invasive range expansion? (2) If so, which mechanism(s) – evolution, heterosis or both – best explain(s) the overall influence of genetic mixture? (3) Does the effect of genetic mixture depend on the number of source populations that contribute to the invasion? (4) Which of the two main traits underlying invasion speed – demography and/or dispersal – responded to genetic mixture?

Materials and methods

STUDY SYSTEM AND SOURCE POPULATIONS

Callosobruchus maculatus (Coleoptera: Chrysomelidae) is a widely distributed pest of stored grains. Gravid females deposit eggs on seeds (beans) of cultivated legumes (Fabaceae). Larvae burrow into beans, pupate and emerge as adults; the egg-to-adult developmental period is no longer than ~ 32 d under our incubator conditions (27·5 °C and a 16:8-h photoperiod, Percival I-36VL). The adult life span is approximately one week, and adults do not eat during that time. Our populations were reared on black-eyed peas (*Vigna unguiculata*).

Our experiment relied on ten source populations of *C. maculatus* that were obtained from localities distributed world-wide, primarily Africa and Asia but including populations from North and South America (Table 1). These populations exhibit significant genetic structure at neutral mitochondrial and nuclear loci (Tuda *et al.* 2014; Downey *et al.* 2015) and differ in phenotypic traits (Dowling, Abiega & Arnqvist 2007; Arnqvist & Tuda 2010) and dispersal behaviour (Downey *et al.* 2015). All populations readily inter-breed (M.H. Downey and T.E.X. Miller, unpublished data). At the time of our experiments, beetle populations had been maintained in laboratory culture for many (>100) generations.

Table 1. Experimental design to test effects of source population diversity on range expansion

Replicate	No. source populations	Composition A	B	C	D	E	F	G	H	I	J
1	1	■									
2	1		■								
3	1			■							
4	1				■						
5	1					■					
6	1						■				
7	1							■			
8	1								■		
9	1									■	
10	1										■
11	2		■			■					
12	2				■						■
13	2			■				■			
14	2	■					■				
15	2			■					■		
16	2									■	■
17	4		■	■			■			■	
18	4			■			■		■	■	
19	4		■		■				■		■
20	4	■		■			■				■
21	4					■	■	■			■
22	4	■				■	■	■			
23	6	■		■	■	■		■		■	
24	6		■	■		■	■		■	■	
25	6	■	■				■		■	■	■
26	6		■	■	■				■	■	■
27	6	■	■	■		■			■		■
28	6	■		■	■			■	■		■

Composition indicates the source populations (letter codes) included in an invasion replicate (filled box). Population codes: A – Benin; B – Brazil; C – California; D – IITA; E – Mali; F – Nigeria; G – South India; H – Uganda; I – Burkina Faso; J – Yemen. See Dowling, Abiega & Arnqvist (2007) and Arnqvist & Tuda (2010) for additional information about source populations.

INVASION EXPERIMENTS

We conducted multi-generation invasion experiments in which populations of varying genetic composition spread through experimental 'landscapes', following the basic protocol of Miller & Inouye (2013). Landscapes consisted of 48 consecutive 'patches' (Petri dishes) connected by tubing, allowing for movement in one spatial dimension. All patches contained 5 g of black-eyed peas. The beetle life cycle was discretized into a 24-h dispersal period during which adults moved between patches, followed by a 31-day demography period during which adults mated, laid eggs and died, and their offspring emerged prior to the next dispersal period. (Most adults emerge after 28 or 29 days of pupal development. The 31-day development window balances inclusion of late-emerging beetles against exclusion of early beetles that may die before dispersing.) For a given invasion replicate, beetles occupied the spatial landscape only for the 24-h dispersal phase. Following dispersal, beetles were transferred to a separate set of unconnected Petri dishes for the demography phase of the life cycle, preserving their spatial locations. Beetles of the next generation were returned to their parents' respective locations for dispersal. This approach allowed us to include more invasion replicates than we had spatial landscapes.

The experiment ran for six generations. By the third generation, most invasion replicates had begun to approach the end of the 48-patch landscapes. To keep the invasions from hitting the ends, we dropped patches at the 'interior' end of the invasion waves and shifted the remaining patches backward. We assumed that dropped patches contributed negligibly to advance of the invasion front. The number of interior patches that were dropped differed for each replicate in each generation, according to the rule that leading-edge beetles had at least 27 empty patches ahead of them. The numbers of dropped patches were recorded so that we could translate locations in the landscapes to total patches travelled, much like a treadmill reports total distance travelled even though the person goes nowhere.

GENETIC DIVERSITY MANIPULATION

Invasion replicates were assigned to a genetic diversity treatment: one-, two-, four- or six-source populations were combined in the founding generation (Table 1). The 'monoculture' treatment (one source) was replicated once for each of the 10 source populations. Each of the mixture treatments (two, four or six sources) was replicated six times. For each mixture replicate, we assigned source populations from the pool of ten. These draws were random, but we intentionally re-randomized source assignments to achieve maximum dissimilarity between all pairs of replicates within a treatment (dissimilarity was assessed with the Jaccard index). This approach maximized the independence of invasion replicates, given the constraint that some sharing of sources was unavoidable (Table 1). Three replicates were dropped from the experiment in generation 3 following accidents that compromised any data that we might subsequently collect from them.

The experiment began in the local demography phase of the life cycle with 24, 12, 6 or 4 beetles from the assigned number of unique source populations (one, two, four or six, respectively); thus, all replicates began with 24 founder beetles split evenly between females and males. Founder beetles were introduced to the third patch from the end of the landscape, to minimize edge effects during the dispersal of their offspring. Thus, the first dispersal phase of the experiment involved not the founder beetles but their offspring.

DATA COLLECTION

Invasion extent

We recorded the densities and locations of all beetles following dispersal in each generation so that we could track change in spatial extent of the invasions. We were primarily interested in the locations of the wave fronts across generations. For this reason, beginning in generation 4, we focused data collection only on the wave fronts and did not record beetles in the high-density interior patches of the invasion waves (where counting beetles was very time-consuming). To standardize how much of the wave we recorded, we applied the rule that beetle densities and locations were recorded starting from the farthest patch with any beetles present and working towards the interior of the invasion wave until three consecutive patches with ≥10 total beetles were encountered.

Dispersal

The invasion extent data from generation 1 allowed us to construct dispersal distance kernels – distributions of dispersal

distances – for each replicate. Unlike later generations, all beetles observed in generation 1 had dispersed from the same location (the founding patch), so the net distance travelled by each individual was known. Because invasions were initiated three patches from the end of the array, many beetles had accumulated near the boundary. For the dispersal analysis, only beetles that had moved away from the array boundary were included.

At the end of the experiment, we remeasured dispersal using beetles that had been reared through one generation in a common environment to test for dispersal evolution. Following dispersal of generation 6 (the last generation), we collected the five farthest females and five farthest males from each replicate and transferred them into a new container with 5 g of beans. One month later, we counted their offspring and released them into the starting patch of the invasion arrays, three patches in from the boundary. After 24 h, we recorded the dispersal distances of all beetles that moved away from the array boundary. Thus, these data were collected in the same way as the generation 1 data, facilitating direct comparison. In total, we recorded the dispersal distances of 3711 beetles in generation 1 and 1762 beetles in generation 7.

Demography

Prior to dispersal in generation 2, we collected 'pre-dispersal' data on beetle densities in each patch, which represent the offspring produced by the generation 1 dispersers. We identified patches that went locally extinct and, for patches that did not, we calculated the population growth rate as $\log_e(N_{t+1}/N_t)$. At the end of the experiment, we allowed the post-dispersal beetles in generation 6 to reproduce and then counted their offspring. As above, this provided estimates of patch-level recruitment for direct comparison with generation 1, though these beetles were not reared through a common environment. All dispersal, demography and invasion extent data are available from the Dryad Digital Repository (Wagner *et al.* 2016).

DATA ANALYSIS

Invasion extent and speed

To analyse effects of genetic mixture on the spread of beetle invasions, we needed to designate a location of the invasion front in each generation so that we could map the advance of the front from one generation to the next. We designated the invasion front as the farthest patch to meet or exceed a density threshold (see Miller & Inouye 2013). We found that our results were not sensitive to the choice of the threshold; we present results based on a threshold of 10 beetles per patch. Dispersing beetles occasionally reached the last patch in the landscape (i.e. they exceeded the 27-patch dispersal 'buffer'). We applied the rule of excluding invasion extent observations if the invasion front occurred in the final patch, since it likely would have gone farther had we provided more space. However, with a threshold density of 10 beetles defining the invasion front, this never occurred. It was nonetheless possible for one or a few beetles to reach the end of the landscape even if the invasion front, as we define it, did not. Due to the experimental constrains on very long-distance dispersal events, our estimates of invasion speed are likely conservative.

We used model selection to evaluate the effects of genetic mixture on range expansion in two ways that focus on different response variables: final invasion extent and invasion velocity. First, we analysed the final range invaded after six generations of spread using four candidate linear models that included a null model and three treatment models that alternatively included effects of genetic mixture: (1) as a slope with respect to number of source populations, (2) as unique factor levels for each of the four diversity levels or (3) as a contrast between 'monocultures' (single-source invasions) and 'mixtures' (combining treatments with 2, 4 or 6 sources) (Table 1A). The slope would provide the best fit if there was a dosage effect, such that each additional source population was associated with a mean increase in range extent, as might occur under a selection effect. Factor levels would provide the best fit if the treatments differed in invasion extent in a way that was nonlinear with respect to number of sources; by assigning treatments to factor levels, we avoid having to arbitrarily choose a particular form of nonlinearity. Finally, the monoculture/mixture contrast would provide the best fit if the effect of source diversity was qualitative, such that any amount of mixing equally affected the invasion trajectory. Invasion extent (patch number) after the last generation of spread was modelled as a Gaussian response variable and models were compared by AIC. While final invasion extent (No. patches invaded) is a discrete variable, analysis of residuals indicated that the data conformed well to model assumptions. We calculated the coefficient of variation (CV) in invasion extent across replicate invasions for each treatment in each generation. We also used the invasion extent data to ask whether the source composition of mixtures predicted their final range size. We tested for a positive correlation between the final ranges of mixtures and the maximum range of their corresponding parental source populations. If selection effects promote the evolution of rapid invasion traits, then a mixture should exhibit similar invasion dynamics as the fastest monoculture that contributed to the mixture.

Secondly, to analyse the temporal component of genetic mixture effects, we analysed the velocity of expansion through time and with respect to treatment. Velocity was calculated as the change in location of the invasion front between successive generations. Candidate models included a null model with constant invasion velocity through time and across treatments, and alternative models that included a generation effect only (speed changes through time), a genetic mixture effect only (speed depends on treatment), and both generation and genetic mixture as additive and interactive effects, allowing invasion speed to vary through time in different ways for different treatments (Fig. 1). For models with a genetic mixture effect, we considered the three types of mixture effects described above, for a total of 11 models (Table 1B). We excluded from this analysis the first generation of spread (the initial dispersal from the invasion origin) because its lower variance violated model assumptions and because we explore these data in the dispersal analysis (below). Where necessary, we used model-averaging to account for uncertainty in model rankings (Burnham & Anderson 2002), though we could not average models that included different forms of source diversity effects (e.g. slope and factor levels).

Dispersal

We used a hierarchical Bayesian approach to fit dispersal distance kernels to the data. Following Miller & Inouye (2013), we fit negative binomial kernels, allowing the mean and dispersion to vary across source diversity treatments and between the first and last generations of the experiment. We fit the negative binomial kernel as a Poisson–gamma mixture (Hilbe 2011). The observations

Y_{ijkl} represent the dispersal distance (No. patches) of individual i in replicate j, genetic mixture treatment k and generation l. For a full model that included effects of treatment (as discrete levels) and generation, the observations were modelled as:

$$Y_{ijkl} \sim \text{Poisson}(\mu_{jkl} \cdot \rho_{ijkl})$$

$$\rho_{ijkl} \sim \text{Gamma}(\alpha_{jkl}, \alpha_{jkl}).$$

The Poisson mean is the product $\mu_{jkl}\rho_{ijkl}$ where ρ_{ijkl}, an individual-level random effect, is gamma-distributed with a mean of unity (equal shape and scale parameters α_{jkl}). We modelled replicate as a random effect to account for the non-independence of data from each replicate in the first and last generations. We allowed replicate-to-replicate variance to affect both the mean (variance σ^2_μ) and dispersion (variance σ^2_α), so that:

$$\log(\mu_{jkl}) = \bar{\mu}_{kl} + \varepsilon_j$$

$$\varepsilon_j \sim \text{Normal}(0, \sigma^2_\mu),$$

and

$$\log(\alpha_{jkl}) = \bar{\alpha}_{kl} + \gamma_j$$

$$\gamma_j \sim \text{Normal}(0, \sigma^2_\alpha).$$

With this mixture parameterization, the observations follow a negative binomial distribution with mean dispersal distance $e^{\bar{\mu}_{kl}}$ and dispersion $e^{\bar{\alpha}_{kl}}$ (smaller dispersion corresponds to greater variance). We fit all combinations of competing models in which kernel parameters $\bar{\mu}$ and $\bar{\alpha}$ could vary across mixture treatments and between the first and last generations. As above, candidate models included an influence of genetic mixture as a slope with respect to source richness, as four unique factor levels, and as a contrast between monocultures and mixtures (Table 2C). For slope effect of genetic mixture, the model structure changed slightly such that parameters $\bar{\mu}$ and $\bar{\alpha}$ were expressed as linear functions of source richness. For tractability, we considered models including treatment effects on neither or both parameters, and not all possible combinations. There were 11 models in total and they were fit in the Bayesian software Stan (Stan Development Team 2016). We used 'flat' (uninformative) prior probabilities for all parameters. We selected the best-fit model(s) using the information criterion WAIC, which is appropriate for hierarchical Bayesian models (Hooten & Hobbs 2015) and is interpreted similarly as AIC (lower is better).

Demography

We decomposed the analysis of local demography into two steps: the probability of local (patch-level) extinction and, for patches that did not go extinct, the population growth rate.

First, we asked whether source diversity affected the probability of local extinction during the early phase of invasion. We analysed the probability of local (patch-level) extinction in relation to distance from the invasion front, expecting extinction risk to increase towards the invasion's leading edge, where densities were lowest (often a single beetle per patch) and mate limitation potentially strongest (Miller & Inouye 2013). Based on the criteria for identifying the location of the invasion front described above, we were able to standardize the spatial position of each patch relative to the invasion front for each replicate in each generation; because the invasion front was not literally the farthest beetle, these values could be positive or negative. We used generalized linear mixed-effects models (R package LME4: Bates *et al.* 2015) with extinction as a Bernoulli response variable, a logit link function and the random effect of invasion replicate. We fit seven candidate models (Table 2D), all of which included spatial position (distance from invasion front) as a predictor variable. The remaining models additionally included main or interaction effects (with spatial position) of source diversity. As above, source diversity was modelled alternatively as a slope with respect to source richness, as four unique factor levels or as a contrast between monocultures and all mixtures combined. We compared candidate models by AIC. This analysis was limited to generation 1 because extinction was much rarer in generation 6 (33% of patches in generation 1 vs. 6% in generation 6). The difference was likely a reflection of beetles hitting the end of the spatial array in the later generation, cutting short the 'tail' of singly occupied patches and making it difficult to compare between generations.

Second, for populations that did not go extinct, we examined how population growth varied across the invasion waves, from the core to the leading edge. We expected population growth rates to increase towards the front, as density decreased. We asked whether and how the source diversity treatment affected the shape of the relationship between population growth and wave position, whether this relationship differed between the first and last generations of the experiment, and whether the change through time varied across treatments. As above, we conducted a model selection analysis using linear mixed-effects models with the local population growth rates ($\log_e(N_{t+1}/N_t)$) from generations t = 1 and t = 6 as the response variable, a Gaussian error distribution and a random effect of replicate. All models included distance from the invasion front as a predictor variable. Candidate models also included source diversity treatment and/or generation (1 or 6) as independent or interactive factors (Table 2). For example, an interaction between spatial position and source diversity treatment would mean that source diversity modified how population growth responds to density dependence along wave fronts, whereas independent effects would mean that source diversity modifies the reproductive rate but not the way it responds to density. As above, the candidate models included three possibilities for effects of source diversity: as a slope, as factor levels for each treatment or as a contrast between monocultures and mixtures. In total, there were 24 candidate models (Table 2).

We focus our demographic analyses on wave location as a proxy for population density, which declines from core to edge (Miller & Inouye 2013). We supplemented the analysis of wave position-dependent demography with additional models that directly quantified density dependence in extinction risk and population growth of persistent patches (Appendix S1, Supporting information).

Table 2. Candidate models and model selection results for the total range invaded (No. patches) after six generations (A), the invasion velocity (patches per generation) (B), dispersal kernel parameters (C), local extinction probability across the invasion waves (D) and local population growth across the invasion waves, conditional on persistence (E)

Source mixture effect	Model	Expression for fixed effects	No. parameters	ΔIC	IC weight	ΣIC weight
(A)						
None	A1	Extent ~ 1 (null)	1	8·7	0·01	
Slope	A2	Extent ~ Mixture	2	5·1	0·06	
Factor levels	A3	Extent ~ Mixture	4	2·7	0·19	
Monoculture vs. mixture	**A4**	**Extent ~ Mixture**	**2**	**0·0**	**0·74**	
(B)						
None	B1	Speed ~ 1 (null)	1	5·2	0·02	0·06
	B2	Speed ~ Generation	2	4·0	0·04	
Slope	B3	Speed ~ Mixture	2	5·8	0·02	0·07
	B4	Speed ~ Generation + Mixture	3	4·5	0·03	
	B5	Speed ~ Generation × Mixture	4	5·8	0·02	
Factor levels	B6	Speed ~ Mixture	4	4·1	0·04	0·17
	B7	Speed ~ Generation + Mixture	5	2·1	0·11	
	B8	Speed ~ Generation × Mixture	8	5·7	0·02	
Monoculture vs. mixture	B9*	Speed ~ Mixture	2	1·8	0·13	0·69
	B10*	**Speed ~ Generation + Mixture**	**3**	**0·0**	**0·31**	
	B11*	Speed ~ Generation × Mixture	4	0·4	0·25	
(C)						
None	C1	Kernel ~ 1 (null)	2	284·1	<0·001	<0·001
	C2	Kernel ~ Generation	4	132·5	<0·001	
Slope	C3	Kernel ~ Mixture	4	284·4	<0·001	<0·001
	C4	Kernel ~ Generation + Mixture	6	133·5	<0·001	
	C5	Kernel ~ Generation × Mixture	6	118·7	<0·001	
Factor levels	C6	Kernel ~ Mixture	8	284·6	<0·001	0·99
	C7	Kernel ~ Generation + Mixture	12	133·1	<0·001	
	C8	**Kernel ~ Generation × Mixture**	**16**	**0·0**	**0·99**	
Monoculture vs. mixture	C9	Kernel ~ Mixture	4	283·9	<0·001	<0·001
	C10	Kernel ~ Generation + Mixture	6	133·4	<0·001	
	C11	Kernel ~ Generation × Mixture	8	66·5	<0·001	
(D)						
None	D1*	logit(Extinction) ~ Location	2	0·6	0·25	0·25
Slope	D2	logit(Extinction) ~ Location + Mixture	3	2·3	0·1	0·15
	D3	logit(Extinction) ~ Location × Mixture	4	3·9	0·05	
Factor levels	D4	logit(Extinction) ~ Location + Mixture	5	2·6	0·09	0·14
	D5	logit(Extinction) ~ Location × Mixture	6	3·6	0·05	
Monoculture vs. mixture	D6*	logit(Extinction) ~ Location + Mixture	3	1·8	0·13	0·46
	D7*	**logit(Extinction) ~ Location × Mixture**	**4**	**0·0**	**0·33**	
(E)						
None	E1	$\log(N_{t+1}/N_t) \sim$ Location	2	49·6	<0·001	0·01
	E2	$\log(N_{t+1}/N_t) \sim$ Location + Generation	3	9·4	0·003	
	E3	$\log(N_{t+1}/N_t) \sim$ Location × Generation	4	7·8	0·007	
Slope	E4	$\log(N_{t+1}/N_t) \sim$ Location + Mixture	3	47·3	<0·001	0·14
	E5	$\log(N_{t+1}/N_t) \sim$ Location × Mixture	4	48·4	<0·001	
	E6	$\log(N_{t+1}/N_t) \sim$ Location + Generation + Mixture	4	6·4	0·014	
	E7	$\log(N_{t+1}/N_t) \sim$ Location + Generation × Mixture	5	6·2	0·016	
	E8	$\log(N_{t+1}/N_t) \sim$ Location × Generation + Mixture	5	4·1	0·045	
	E9	$\log(N_{t+1}/N_t) \sim$ Location × Generation + Mixture	5	4·4	0·038	
	E10	$\log(N_{t+1}/N_t) \sim$ Location × Generation × Mixture	8	5·4	0·023	
Factor levels	E11	$\log(N_{t+1}/N_t) \sim$ Location + Mixture	5	49·7	<0·001	0·28
	E12	$\log(N_{t+1}/N_t) \sim$ Location × Mixture	8	47·3	<0·001	
	E13	$\log(N_{t+1}/N_t) \sim$ Location + Generation + Mixture	6	7·2	0·0094	
	E14	$\log(N_{t+1}/N_t) \sim$ Location + Generation × Mixture	9	2·2	0·11	
	E15	$\log(N_{t+1}/N_t) \sim$ Location × Generation + Mixture	9	4·2	0·04	
	E16	$\log(N_{t+1}/N_t) \sim$ Location × Generation + Mixture	7	5·0	0·02	
	E17	$\log(N_{t+1}/N_t) \sim$ Location × Generation × Mixture	16	2·5	0·1	
Monoculture vs. mixture	E18	$\log(N_{t+1}/N_t) \sim$ Location + Mixture	3	48·2	<0·001	0·56
	E19	$\log(N_{t+1}/N_t) \sim$ Location × Mixture	4	49·8	<0·001	
	E20	$\log(N_{t+1}/N_t) \sim$ Location + Generation + Mixture	4	6·3	0·015	

(continued)

Table 2. (continued)

Source mixture effect	Model	Expression for fixed effects	No. parameters	ΔIC	IC weight	ΣIC weight
	E21	$\log(N_{t+1}/N_t) \sim$ Location + Generation × Mixture	5	6·8	0·012	
	E22*	$\mathbf{\log(N_{t+1}/N_t) \sim}$ **Location × Generation + Mixture**	**5**	**0·0**	**0·35**	
	E23	$\log(N_{t+1}/N_t) \sim$ Location × Generation + Mixture	5	4·2	0·043	
	E24*	$\log(N_{t+1}/N_t) \sim$ Location × Generation × Mixture	8	1·8	0·14	

Expressions and number of parameters show fixed effects. Models in B–D also included random effects of replicate. The generation effect in (B) is a continuous slope for change in invasion speed per generation. The generation effect in (C) and (E) is a contrast between the first and last generations of spread. Information criteria (IC) are AIC for (A, B, D, E) and WAIC for (C). IC weights are given for each model within a candidate set and, for (B–E), are summed within model groups for type of source mixture effects (none, slope with respect to number of sources, factor levels for each of four treatments or a monoculture/mixture contrast that combines mixtures with 2, 4 and 6 sources). Bolded model shows the best fit within each analysis. Asterisks indicate models that were averaged to generate predictions shown in Figs 2, 4 and 5, respectively. Dispersal kernel analysis in (C) includes fixed effects on both the mean and overdispersion parameters.

Results

INVASION EXTENT AND SPEED

Over six generations, beetle invasions derived from mixed source populations spread farther and faster than single-source invasions (Fig. 2a). We used model selection to evaluate the type of source diversity effect on final range size (none, slope, factor levels, mixture effect). The data supported an overall mixture effect, such that replicates with two-, four- or six-source populations were qualitatively consistent in their final range size but different from monoculture invasions (Table 2A, Fig. 2a). Genetic mixture also reduced the variance in spatial extent across replicate invasions, estimated as the coefficient of variation (CV; Fig. 2a,b). The reduction in CV associated with genetic mixture occurred during the first few generations of spread and then stabilized (Fig. 2b). There was no significant correlation between the final extent of the multi-source invasions and the final extent of the farthest single-source invasion that contributed to each mixture ($r = 0{\cdot}16$, $P < 0{\cdot}58$).

The velocities of multi- and single-source invasions differed in ways that were consistent with the difference in final invasion extent (Fig. 2c). Cumulatively, 69% of AIC support indicated a monoculture/mixture contrast for invasion speed (Table 2B). Among the monoculture/mixture models, there was mixed support for additive vs. interactive effects of treatment and generation on spreading speeds (Table 2B, models B9–B11). The model-averaged predictions, shown in Fig. 2c, indicated that differences in the velocities of monoculture and mixture invasions were greatest early in the experiment. In fact, the raw mean ± SD wave velocity for mixtures in generation 2 (the first generation to include F1 offspring) was 21·2 ± 2·6 patches/generation, nearly twice that of monocultures (12·8 ± 1·9 patches/generation). In the last generation, monocultures and mixtures had very similar velocities (20·7 ± 3·9 and 22·4 ± 2·1, respectively). Thus, there was some evidence for accelerating spread, but it was the monoculture invasions whose velocity increased over the first few generations, eventually catching up to the mixture treatments. The mixtures, by contrast, reached their long-run velocity in the very early stage of range expansion (Fig. 2c).

DISPERSAL

Model selection supported an interaction between mixture treatment (as factor levels) and generation (Table 2C), meaning that the four levels of source richness exhibited different changes in dispersal ability from the beginning to the end of range expansion. Figure 3 shows raw dispersal distributions (Fig. 3a–d) and parameter estimates for the fitted kernels (mean and overdispersion) (Fig. 3e). Within generations, 95% credible intervals for kernel parameters largely overlapped across source mixture treatments, although invasions with two source populations had a greater mean dispersal distance than invasions with one source population at the start of the invasions (Fig. 3e, grey circles). Across generations, there was some evidence consistent with the evolution of dispersal ability: after six generations, the descendants of founding populations exhibited greater mean dispersal distance than that of their ancestors. However, change in dispersal distance occurred only in the monocultures and six-source mixtures (Fig. 3e). Two- and four-source invasions showed no differences across generations, nor between each other.

DEMOGRAPHY

During the first generation of spread, extinction risk increased from the core to the leading edge (Fig. 4). In fact, extinction was virtually guaranteed for the most-distantly colonized patches. Population densities during the early phase of invasion were low and leading-edge patches were often colonized by a single, mateless individual. There was an influence of source population mixture on extinction risk, with the contrast between monocultures and mixtures receiving the most statistical support,

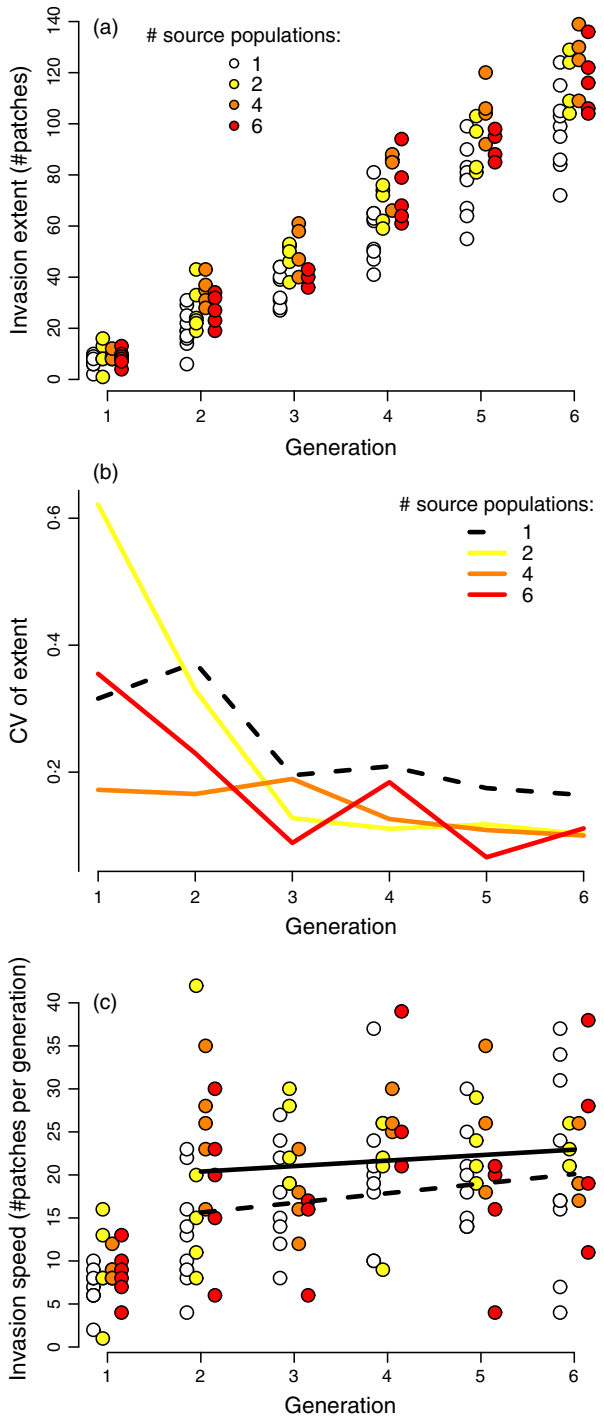

Fig. 2. (a) Spatial extent of beetle invasions through time. Point colours represent the number of source populations included in the invasion replicate (see legend). (b) The coefficient of variation (CV) in invasion extent across treatments and generations. (c) The velocity of expansion (patches/generation) across treatments and generations; points as in (a). Lines show model-averaged predictions (models B9, B10, B11: Table 2B), differentiating 'monocultures' (1 source, dashed line) and 'mixtures' (2, 4 and 6 sources combined, solid line).

though the null model was a close second (Table 2D). We averaged the top three models (D1, D6 and D7), collectively representing 71% of AIC support, to generate the predictions in Fig. 4. For both monocultures and mixtures, persistence was virtually guaranteed in the invasion core and extinction was guaranteed at the invasion vanguard. However, in transitional patches behind the leading edge but in front of the core, mixtures had a lower extinction risk than monocultures.

For local patches that did not go extinct, their population growth rates increased from the core of the waves to the leading edges, as densities decreased (Fig. 5). Genetic mixture enhanced population growth along the invasion wave. Models that included source diversity as a contrast between monocultures and mixtures provided the best fit to the data, cumulatively receiving the majority of AIC support (Table 2). We averaged the top two models (E22 and E24), which included interacting effects of wave location, genetic diversity and generation. The model-averaged predictions indicated that mixed invasions experienced greater population growth than monocultures equally across the invasion wave in generation one (Fig. 5a). Thus, mixed invasions had a population growth advantage in both density-dependent and density-independent environments. This effect was greatly diminished by the end of the experiment (Fig. 5b), consistent with an early, transient advantage of genetic mixture.

The role of population density in extinction risk and population growth mirrored the influence of wave position, as expected (Appendix S1). Extinction risk in the first generation of spread strongly increased with decreasing population density and was lower in mixture invasions than monocultures (Fig. S1, Table S1). For local populations that persisted, their growth rate was negatively density-dependent and was greater in mixture invasions than in monocultures, but the mixture effect diminished from the first to the last generation of spread (Fig. S2, Table S1).

Discussion

Genetic mixture following multiple introductions is a common feature of biological invasions. Yet, mechanistic understanding of whether and how genetic mixture affects invasion dynamics has lagged behind documentation that such mixture occurs. This study provides experimental evidence that genetic mixture can influence the spatial trajectories of incipient invasions. Most experimental studies have focused on effects of genetic mixture on colonization success (reviewed in Forsman 2014). Additionally, Szűcs *et al.* (2014) showed that genetic mixture can increase dispersal in the first generation following successful colonization. Our work is the first to demonstrate that genetic mixture can alter the course of range expansion, the stage of invasion typically associated with the greatest ecological and economic impacts. While our laboratory-based experimental system does not attempt to capture the full complexity of invasive range expansion in nature, it provides a powerful distillation of its key ingredients: within-patch population dynamics and between-patch movement.

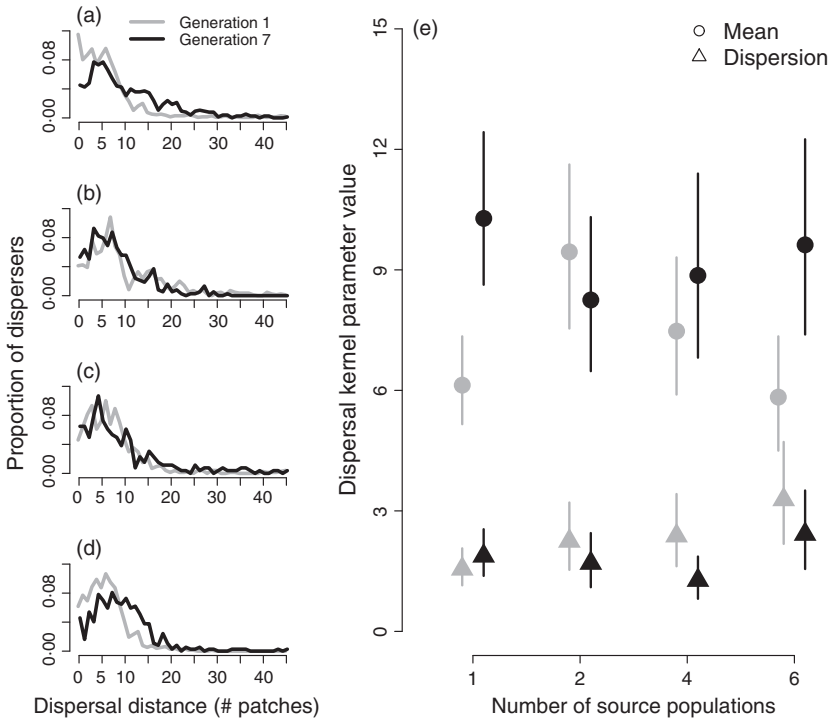

Fig. 3. (a–d) Observed distributions of dispersal distances at the beginning (generation 1; grey lines) and end (generation 7; black lines) of the invasion experiment. Panels show invasions with 1 (a), 2 (b), 4 (c) and 6 (d) source populations. (e) Parameter values of fitted negative binomial dispersal kernels. Points show Bayesian estimates of the mean dispersal distance (circles) and dispersion parameter (triangles) for generations 1 (grey) and 7 (black). Lower values of the dispersion parameter correspond to greater variance. Bars show 95% Bayesian credible intervals.

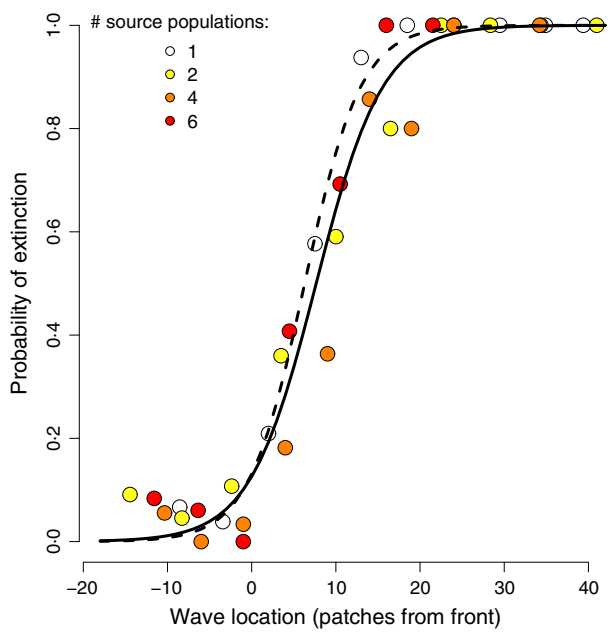

Fig. 4. Probability of local patch extinction in relation to wave location (patches from invasion front) during the first generation of spread. Lines show model-averaged predictions (models B1, B11 and B12: Table 2B), differentiating 'monocultures' (1 source, dashed line) and 'mixtures' (2, 4 and 6 sources combined, solid line).

In this way, our results provide proof of concept that genetic mixture can exert a causal influence on invasiveness, independent of any demographic effects of propagule pressure.

Our data are most consistent with the hypothesis that mixture effects were driven by heterosis following reproductive contact of distinct genetic lineages. Differences in the velocities of single-source and mixed invasions were greatest during the early stages of spread and then diminished (Fig. 2a,c). Under a dominant role for heterosis, we expected that population mean heterozygosity and its potential fitness benefits would have peaked in the F1 offspring following the initial reproductive contact of independent source populations (Fig. 1b). By contrast, if effects of mixture were due to increased evolutionary potential, trajectories of monoculture and mixture invasions should have increasingly diverged through time (Fig. 1a), the opposite of what we observed. Furthermore, our results indicate that the overall effects of genetic mixture were not strongly sensitive to 'dosage' or sampling effects associated with the number of donor populations: invasions with two, four or six sources – any amount of mixture – were qualitatively consistent in their spread dynamics (Table 2A). If mating was random, we may have expected inter-population crosses to increase with the number of source populations included. The lack of source richness effects suggests the possibility that beetles preferentially select mates from different populations leading to similar amounts of outcrossing at different levels of source richness, a hypothesis that merits further study.

The spatial dynamics of invasive spread are driven by the interplay of dispersal and local demography. In addition to identifying heterosis as the most probable driver of genetic mixture effects, we can go a step further and identify local population growth, not dispersal, as the trait most likely responsible for accelerating mixed invasions in our experiment. First, invasions with source population diversity were more resistant to local extinction at the

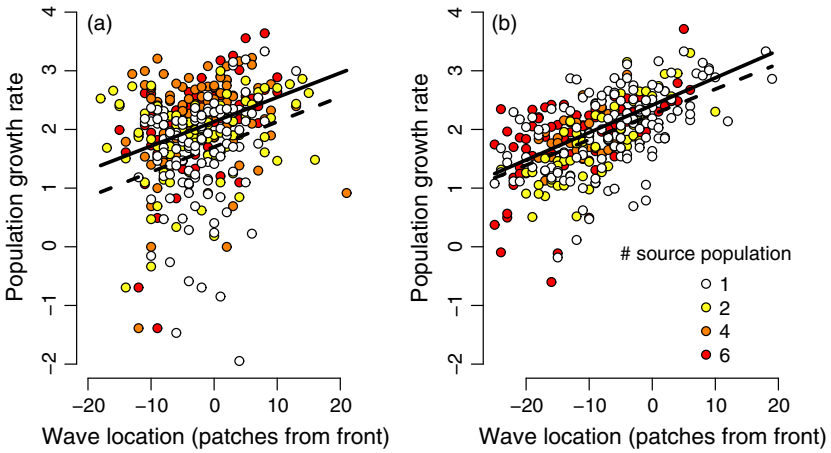

Fig. 5. Population growth rate (log (N_{t+1}/N_t)), conditional on persistence ($N_{t+1} > 0$), in relation to wave location (patches from invasion front) in generation 1 (a) and 6 (b). Point shading represents the number of source populations included in the invasion replicate. Lines show model-averaged predictions (models C22 and C24: Table 2C), differentiating 'monocultures' (1 source, dashed line) and 'mixtures' (2, 4 and 6 sources combined, solid line).

low-density vanguard of the invasion waves, particularly in the first generation of spread (Fig. 4, Appendix S1). These density-dependent extinction events were consistent with Allee effects, which are known to decelerate invasion (Kot, Lewis & van den Driessche 1996). We speculate that sex-biased dispersal, which is well documented in *C. maculatus*, contributed to low-density extinction risk at the invasion fronts, since it causes leading-edge patches to be dominated by a single sex (Miller & Inouye 2013). The mechanism by which genetic mixture reduced extinction risk at the low-density invasion fronts is unclear but may involve improved mate tracking during dispersal that alleviated mate-finding Allee effects. Secondly, for patches that did not go extinct, genetic mixture elevated rates of local population growth during the first generation of spread and this effect largely dissipated by the end of the experiment (Fig. 5, Appendix S1). By contrast, we did not find consistent dispersal differences between monoculture and mixture invasions nor consistent trends of change in dispersal through time that could explain the observed effect of genetic mixture on spread dynamics (Fig. 3). Collectively, the data suggest that hybrid offspring produced following first reproductive contact of multiple genetic sources had reduced low-density extinction risk and elevated reproductive rates that provided a demographic 'catapult'. The catapult effect allowed mixed invasions to establish an early lead over monocultures, leaving a lasting signature on range expansion even though mixtures and monocultures eventually converged on similar population growth rates and invasion velocities. Benefits of heterosis need not be transient, in general. Changes in ploidy level and other factors that reduce opportunities for recombination with parental lineages could lead to a sustained hybrid advantage, as is the case for invasive hybrid snails, for example (Facon *et al.* 2005). However, for intraspecific hybridization, as expected for multiple introductions of a single species, decay of heterozygosity due to backcrossing seems most likely.

Our results should be interpreted in the light of the history of our study populations. We worked with populations that had been maintained in laboratory culture for many generations. They likely experienced strong genetic bottlenecks and historical inbreeding that could make them poorly representative of natural populations. In prior work, we found that most of these populations exhibited very little inbreeding depression following full-sib mating (Downey *et al.* 2015), further suggesting a history of inbreeding that may have been sufficient to purge deleterious alleles. The low genetic diversity expected for inbred laboratory lines may have increased the chances of finding support for a heterosis effect, as we did. However, our results are qualitatively consistent with a recent meta-analysis of the effects of interspecific hybridization on invasiveness that showed fecundity benefits in wild hybrid taxa (Hovick & Whitney 2014). We nonetheless emphasize that the importance of our work lies in the proof of concept that genetic mixture can have important ecological effects on spread dynamics, likely through the mechanism of heterosis. Whether these effects would play out in more realistic, natural contexts remains an open question and an important direction for future work.

A second caveat of our results is that, independent of the history of our experimental lines, the experimental design imposed a strong genetic bottleneck at the start of the experiment (24 founder beetles per replicate) and restricted resources that kept local population sizes small (5 g of beans per patch). It is possible that the experimental limitation of initial population size also affected our results. However, natural invasions typically begin with strong genetic bottlenecks, especially for accidental introductions, so it is possible that our bottleneck was a reasonable way to initiate experimental invasions. Studies that cross genetic diversity with founding population size (e.g. Szűcs *et al.* 2014) would be useful direction for resolving understanding of this issue.

The genetic mixture effect not only increased the mean invasion extent after six generations, but also reduced the variability in invasion extent (Fig. 2a,b). The reduction in variance could be interpreted as a signature of evolution, since selection effects are expected to 'cherry-pick' the alleles and traits of the most fit monocultures that contribute to a mixture; this process would yield a

distribution of outcomes for mixture invasions with lower variance and a mean centred at the upper range of the distribution of monocultures, precisely the pattern in Fig. 2a. However, several lines of evidence argue against a role for evolution in generating this pattern. First, there was no clear evidence for trait evolution that differed between monoculture and mixture invasions in ways that could explain the difference in spread dynamics between these two groups. Secondly, there was little evidence for a quantitative effect of source richness in any of our analyses. An influence of source richness is expected under a selection effect because more diverse mixtures would be more likely to include trait values with a strong influence on spreading speed. Thirdly, we find no evidence that 'fast' mixtures were non-randomly composed of 'fast' monocultures, another prediction of the selection effect hypothesis. These lines of evidence all point us back towards demographic benefits of mixture early in the invasion process (Figs 4,5), likely due to heterosis, as the most likely explanation for our results.

Lacking strong evidence for an evolutionary mechanism, we suggest an alternative explanation for the reduced variation of mixture invasions. The timing of the variance reduction for mixtures coincided with positive effects of mixture on extinction risk and population growth rates, in the early stages of invasion (Figs 2b, 4 and 5). In fact, patterns of variability across treatments were relatively constant after the second generation of spread. We therefore hypothesize that demographic benefits of genetic mixture reduced variability in range extent during the early generations. Demographic stochasticity arising from the discrete nature of individuals causes dispersal kernels to be 'sampled' imperfectly, especially the low-probability long-distance tails, and this effect is greatest with low numbers of dispersing individuals (Snyder 2003; Miller & Inouye 2013). We speculate that elevated reproductive rates reduced the noisiness in kernel sampling, generating more consistent spread dynamics of mixtures early in the experiment that carried through to later generations via the spatiotemporal autocorrelation inherent to range expansion.

The dominant role for short-term benefits of heterosis in our experiment does not eliminate potential longer term effects of hybridization. Indeed, most studies of intra- or interspecific hybridization in invasive organisms focus on the long-term evolutionary potential of hybrid lineages (Ellstrand & Schierenbeck 2000; Schierenbeck & Ellstrand 2009). While heterosis is our best explanation for the six generations of spread observed in our experiments, increased evolutionary potential of mixed invasions might have become apparent with a longer time series of expansion. Nonetheless, we suggest that short-term, non-evolutionary effects of intraspecific hybridization warrant greater attention, as others have recently argued (Rius & Darling 2014). These effects are best detected with finely resolved time series of invasions with known genetic sources (Drake 2006) and/or experimental recreation of hybrid lineages from multiple sources (Facon *et al.* 2005; Hardiman & Culley 2010; Turgeon *et al.* 2011); both approaches are very rare in the current literature. Consequently, we suggest that the long-term consequences of heterosis following genetic mixture early in the invasion process are potentially widespread but easily undetected.

The environment of our experiment was simple and homogenous. Different effects of source diversity might have manifest in different environmental contexts. For example, a more heterogeneous resource environment could have set the stage for diversity effects such as niche complementarity, whereby mixed populations partition resources according to genotype-specific niches (Crawford & Whitney 2010). With only one available resource (cowpeas) and virtually no habitat complexity, our experiment provided little opportunity for niche complementarity to manifest. In addition to heterogeneity, environmental novelty may be an important factor that modifies the role of genetic mixture. In our experiment, the resource environment of the 'invasive' range was identical to the 'native' ranges of all source populations. An invasive environment that more strongly contrasts with the native environment could amplify the importance of evolutionary mechanisms. Indeed, positive effects of multiple introductions on adaptive potential have been implicated in cases of successful invasion into novel abiotic environments, such as the invasion of amphipods from brackish environments into freshwater (Kelly *et al.* 2006). Additionally, the abiotic context could modify the consequences of outcrossing among multiple sources. Inbreeding depression is generally stronger under more stressful environmental conditions (Fox & Reed 2011), suggesting that outcrossing may be more beneficial for introduced organisms that encounter challenging environments. In the light of the many ways that the environment can modify effects of genetic mixture, experimental designs that cross source population diversity with environmental context are an important next step for future research.

Even in simple and benign environments, there is potential for dispersal to evolve in expanding populations via spatial selection, as long as there is a genetic basis to dispersal behaviour. Our ongoing studies of *C. maculatus* indicate an additive genetic basis to dispersal distance (28% narrow-sense heritability; B.M. Ochocki, unpublished data). This heritability estimate is quantitatively very similar to the heritability of dispersal behaviour in cane toads in Australia (Phillips, Brown & Shine 2010a), perhaps the best-studied example of evolutionarily accelerated invasive range expansion (Phillips *et al.* 2008; Lindström *et al.* 2013). Yet, we did not find strong evidence that dispersal evolution accelerated range expansion or that multiple introductions amplified the evolutionary potential of dispersal. Our direct estimation of dispersal distance kernels at the beginning and end of the invasion experiments did show an increase in mean dispersal distance by about 4 patches, but only for 1- and 6-source

treatments (Fig. 3). It is not clear why the 2- and 4-source treatments did not show similar responses, but the results suggest that there was sufficient genetic variation, even in single-source invasions, for the evolution of dispersal ability. This result is consistent with the hypothesis that genetic bottlenecks associated with single introductions do not significantly reduce genetic variation underlying quantitative traits (Dlugosch & Parker 2008). To our knowledge, our study is the first to test whether multiple introductions can amplify the evolutionary potential of dispersal and its ecological consequences. More studies are needed to build a general picture of dispersal evolution in invasions stemming from multiple introductions.

We do see evidence for accelerating expansion in single-source invasions (Fig. 2c), but this is unlikely to reflect dispersal evolution. Instead, the acceleration appears to be due to an increase in population growth rate over the first few generations of spread, suggesting that monocultures were more vulnerable than mixtures to Allee effects (positive density dependence) early in the invasion process. This interpretation is supported by direct evidence for density-dependent extinction risk that was lower in mixtures than in monocultures (Table 2B, Fig. 4, Appendix S1). The stronger influence of Allee effects on monoculture than mixture invasions likely explains why observed patterns of range expansion do not perfectly match our idealized predictions (Fig. 1): single-source invasions required several generations of population growth before reaching their long-term speed, while mixtures quickly reached their long-term speed. Allee effects are commonly invoked to explain lags in invasive spread (Veit & Lewis 1996; Taylor & Hastings 2005). Interestingly, multi-source invasions did not, on average, exceed the long-run velocity of single-source invasions; they simply reached it more quickly, we think because they were buffered against Allee effects. Our results suggest that the genetic consequences of multiple introductions may be an important but under-recognized source of variation in the occurrence and duration of lags in spatial expansion.

CONCLUSIONS

The genetics of colonizing species has long attracted the interest of evolutionary ecologists. Widespread evidence for multiple introductions of exotic organisms, accumulated over the past several decades, has sparked ongoing discussion of whether and how genetic mixture modifies the trajectories of biological invasions (Lee 2002; Roman & Darling 2007; Dlugosch & Parker 2008; Verhoeven *et al.* 2011; Forsman 2014; Rius & Darling 2014). We provide novel experimental evidence that multiple introductions of disparate genetic sources have a positive effect on the spatial expansion phase of invasion. Furthermore, our data support a transient 'catapult effect' of heterosis associated with intraspecific hybridization; the catapult effect reduced extinction risk of leading-edge low-density patches and enhanced population growth but did not strongly influence dispersal. While the fitness benefits of outcrossing were apparently short-lived, long-term patterns of spatial expansion retained the memory of early gains due to genetic mixture. We suggest that similar effects of genetic mixture may be a common feature of biological invasions in nature, but that these effects can easily go undetected.

Acknowledgements

We thank G. Arqvist for generously sharing beetle populations. Shannon Carter, Michelle Sneck, Emily Schultz, Ben Toscano and four anonymous reviewers provided valuable comments that improved the manuscript. This research was supported by USDA-CSREES-2007-02270, NSF-DEB-1501814 and the Godwin Assistant Professorship at Rice University.

Data accessibility

Data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.3813d (Wagner *et al.* 2016).

References

Arnqvist, G. & Tuda, M. (2010) Sexual conflict and the gender load: correlated evolution between population fitness and sexual dimorphism in seed beetles. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1345–1352.

Baker, H.G. & Stebbins, G.L. (1965) *The Genetics of Colonizing Species*. Academic Press, New York, NY, USA.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Benvenuto, C., Cheyppe-Buchmann, S., Bermond, G., Ris, N. & Fauvergue, X. (2012) Intraspecific hybridization, life history strategies and potential invasion success in a parasitoid wasp. *Evolutionary Ecology*, **26**, 1311–1329.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York, NY, USA.

Chuang, A. & Peterson, C.R. (2016) Expanding population edges: theories, traits, and trade-offs. *Global Change Biology*, **22**, 494–512.

Crawford, K. & Whitney, K. (2010) Population genetic diversity influences colonization success. *Molecular Ecology*, **19**, 1253–1263.

Dlugosch, K. & Parker, I. (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Dowling, D.K., Abiega, K.C. & Arnqvist, G. (2007) Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. *Evolution*, **61**, 194–201.

Downey, M.H., Searle, R., Bellur, S., Geiger, A., Maitner, B.S., Ohm, J.R. *et al.* (2015) A comparative approach to testing hypotheses for the evolution of sex-biased dispersal in bean beetles. *Ecology and Evolution*, **5**, 4819–4828.

Drake, J.M. (2006) Heterosis, the catapult effect and establishment success of a colonizing bird. *Biology Letters*, **2**, 304–307.

Ellstrand, N.C. & Schierenbeck, K.A. (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7043–7050.

Facon, B., Jarne, P., Pointier, J. & David, P. (2005) Hybridization and invasiveness in the freshwater snail *Melanoides tuberculata*: hybrid vigour is more important than increase in genetic variance. *Journal of Evolutionary Biology*, **18**, 524–535.

Forsman, A. (2014) Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 302–307.

Fox, C.W. & Reed, D.H. (2011) Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution*, **65**, 246–258.

Hardiman, N.A. & Culley, T.M. (2010) Reproductive success of cultivated *Pyrus calleryana* (Rosaceae) and establishment ability of invasive, hybrid progeny. *American Journal of Botany*, **97**, 1698–1706.
Hilbe, J.M. (2011) *Negative Binomial Regression*. Cambridge University Press, Cambridge, UK.
Hooten, M. & Hobbs, N. (2015) A guide to Bayesian model selection for ecologists. *Ecological Monographs*, **85**, 3–28.
Hovick, S.M. & Whitney, K.D. (2014) Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. *Ecology Letters*, **17**, 1464–1477.
Hufbauer, R., Rutschmann, A., Serrate, B., Vermeil de Conchard, H. & Facon, B. (2013) Role of propagule pressure in colonization success: disentangling the relative importance of demographic, genetic and habitat effects. *Journal of Evolutionary Biology*, **26**, 1691–1699.
Hughes, A.R., Inouye, B.D., Johnson, M.T., Underwood, N. & Vellend, M. (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.
Kelly, D.W., Muirhead, J.R., Heath, D.D. & Macisaac, H.J. (2006) Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. *Molecular Ecology*, **15**, 3641–3653.
Kolbe, J.J., Glor, R.E., Schettino, L.R., Lara, A.C., Larson, A. & Losos, J.B. (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.
Konečný, A., Estoup, A., Duplantier, J.M., Bryja, J., Bâ, K., Galan, M. *et al*. (2013) Invasion genetics of the introduced black rat (*Rattus rattus*) in Senegal, West Africa. *Molecular Ecology*, **22**, 286–300.
Kot, M., Lewis, M.A. & van den Driessche, P. (1996) Dispersal data and the spread of invading organisms. *Ecology*, **77**, 2027–2042.
Krehenwinkel, H. & Tautz, D. (2013) Northern range expansion of European populations of the wasp spider Argiope bruennichi is associated with global warming–correlated genetic admixture and population-specific temperature adaptations. *Molecular Ecology*, **22**, 2232–2248.
Kubisch, A., Holt, R.D., Poethke, H.J. & Fronhofer, E.A. (2014) Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, **123**, 5–22.
Lavergne, S. & Molofsky, J. (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3883–3888.
Lee, C.E. (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.
Lindström, T., Brown, G.P., Sisson, S.A., Phillips, B.L. & Shine, R. (2013) Rapid shifts in dispersal behavior on an expanding range edge. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 13452–13456.
Miller, T.E.X. & Inouye, B.D. (2013) Sex and stochasticity affect range expansion of experimental invasions. *Ecology Letters*, **16**, 354–361.
Neubert, M.G. & Caswell, H. (2000) Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations. *Ecology*, **81**, 1613–1628.
Okubo, A. (1980) *Diffusion and Ecological Problems: Mathematical Models*. Springer-Verlag, Berlin, Germany.
Perkins, A.T., Phillips, B.L., Baskett, M.L. & Hastings, A. (2013) Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. *Ecology Letters*, **16**, 1079–1087.
Phillips, B., Brown, G. & Shine, R. (2010a) Evolutionarily accelerated invasions: the rate of dispersal evolves upwards during the range advance of cane toads. *Journal of Evolutionary Biology*, **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.
Phillips, B.L., Brown, G.P., Travis, J.M. & Shine, R. (2008) Reid's paradox revisited: the evolution of dispersal kernels during range expansion. *The American Naturalist*, **172**, S34–S48.
Rius, M. & Darling, J.A. (2014) How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology & Evolution*, **29**, 233–242.
Roman, J. (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2453–2459.
Roman, J. & Darling, J.A. (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution*, **22**, 454–464.
Schierenbeck, K.A. & Ellstrand, N.C. (2009) Hybridization and the evolution of invasiveness in plants and other organisms. *Biological Invasions*, **11**, 1093–1105.
Simberloff, D. (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 81–102.
Simon-Bouhet, B., Garcia-Meunier, P. & Viard, F. (2006) Multiple introductions promote range expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from mitochondrial sequence data. *Molecular Ecology*, **15**, 1699–1711.
Snyder, R. (2003) How demographic stochasticity can slow biological invasions. *Ecology*, **84**, 1333–1339.
Stan Development Team (2016) Stan Modeling Language Users Guide and Reference Manual.
Strevens, C.M. & Bonsall, M.B. (2011) Density-dependent population dynamics and dispersal in heterogeneous metapopulations. *Journal of Animal Ecology*, **80**, 282–293.
Szűcs, M., Melbourne, B.A., Tuff, T. & Hufbauer, R.A. (2014) The roles of demography and genetics in the early stages of colonization. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141073.
Taylor, C. & Hastings, A. (2005) Allee effects in biological invasions. *Ecology Letters*, **8**, 895–908.
Travis, J., Hammershøj, M. & Stephenson, C. (2005) Adaptation and propagule pressure determine invasion dynamics: insights from a spatially explicit model for sexually reproducing species. *Evolutionary Ecology Research*, **7**, 37–51.
Tuda, M., Kagoshima, K., Toquenaga, Y. & Arnqvist, G. (2014) Global genetic differentiation in a cosmopolitan pest of stored beans: effects of geography, host-plant usage and anthropogenic factors. *PLoS One*, **9**, e106268.
Turgeon, J., Tayeh, A., Facon, B., Lombaert, E., De Clercq, P., Berkvens, N. *et al*. (2011) Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. *Journal of Evolutionary Biology*, **24**, 1044–1052.
Veit, R.R. & Lewis, M.A. (1996) Dispersal, population growth, and the Allee effect: dynamics of the house finch invasion of Eastern North America. *The American Naturalist*, **148**, 225–274.
Verhoeven, K.J., Macel, M., Wolfe, L.M. & Biere, A. (2011) Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 2–8.
Wagner, N.K., Ochocki, B.M., Crawford, K.M., Compagnoni, A. & Miller, T.E.X. (2016) Data from: Genetic mixture of multiple source populations accelerates invasive range expansion. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.3813d.
Whitney, K.D. & Gering, E. (2015) Five decades of invasion genetics. *New Phytologist*, **205**, 472–475.

Received 27 June 2015; accepted 2 June 2016
Handling Editor: Kim Cuddington

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Density dependence in local extinction risk and population growth.

Table S1. Model selection for effects of density and genetic mixture treatment on extinction risk (A) and per-capita growth rate (B).

Fig. S1. Density-dependent extinction risk.

Fig. S2. Population growth ($\log_e(N_{t+1}/N_t)$) in relation to local patch density (N_t) following dispersal in generations 1 (a) and 6 (b) of the invasion experiment.