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Genetic variation during range expansion: effects of habitat novelty and hybridization

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How species' ranges evolve remains an enduring problem in ecology and evolutionary biology. Species' range limits are potentially set by the inability of peripheral populations to adapt to range-edge habitat. Indeed, peripheral populations are often assumed to have reduced genetic diversity and population sizes, which limit evolvability. However, support for this assumption is mixed, possibly because the genetic effects of range expansion depend on two factors: the extent that habitat into which expansion occurs is novel and sources of gene flow. Here, we used spadefoot toads, *Spea bombifrons*, to contrast the population genetic effects of expansion into novel versus non-novel habitat. We further evaluated gene flow from conspecifics and from heterospecifics via hybridization with a resident species. We found that range expansion into novel habitat, relative to non-novel habitat, resulted in higher genetic differentiation, lower conspecific gene flow and bottlenecks. Moreover, we found that hybridizing with a resident species introduced genetic diversity in the novel habitat. Our results suggest the evolution of species' ranges can depend on the extent of differences in habitat between ancestral and newly occupied ranges. Furthermore, our results highlight the potential for hybridization with a resident species to enhance genetic diversity during expansions into novel habitat.

1. Introduction

Explaining the evolution of species' ranges is fundamental to understanding how biodiversity is distributed and maintained [1–3]. Species' ranges are influenced by biotic (e.g. predation, parasitism and competition), and abiotic factors (e.g. climate) [4,5]. Yet, we still do not fully know how species' geographical ranges evolve and what factors fuel range expansions [6,7].

Generally, species' ranges are limited by the inability of populations at the range edge to adapt to environmental pressures before going extinct [6,8,9]. Range expansions often result in smaller population sizes at the range periphery and decreased genetic diversity [10–12]. Thus, peripheral populations' adaptive potential is low and their risk of extinction high. Adaptive evolution that prevents extinction could occur in such populations via new mutations or gene flow [8,13]. However, the waiting time for adaptive mutations is potentially too long to rescue edge populations, and gene flow from conspecifics will most likely consist of alleles from the range centre [14,15], which may be poorly adapted to the range periphery [8,16].

Whether such gene flow has positive or negative effects will differ depending on the novelty of the habitat into which expansion occurs. When expansion occurs into relatively non-novel habitat, gene flow from other conspecific populations inhabiting similar environments can provide an increase in genetic diversity or adaptive alleles to foster local adaptation in peripheral populations [8,17]. By contrast, when expansion occurs into novel habitat, theoretical and empirical work has shown that gene flow from the centre of the range can have an opposite effect, generating an influx of maladaptive alleles that prevent local adaptation in peripheral populations [8,16].

An alternative to conspecific gene flow as a source of genetic variation is hybridization with a resident species. Although hybridization is often deleterious [18,19], it is sometimes beneficial [20,21]. In such cases, introgression of heterospecific alleles may provide populations at the range edge with a source of genetic variation [22,23], including the transfer of specific adaptive alleles from one species to another [20,24–28]. This can result in rapid adaptation by peripheral populations, allowing for further expansion into the novel habitat [29,30].

Whether hybridization plays an important role in range expansion remains an open question, especially in animals, as most tests of the hypothesis have been in plants [21,29,31]. Yet, evaluating hybridization's role in the evolution of species' ranges is important for ascertaining hybridization's role in the origins and distribution of biodiversity. Indeed, understanding the relationship between hybridization and range expansion is increasingly important for practical reasons as evidence shows that global change is altering the distribution of animal and plants species around the world [32–35] and hybridization events could become more common as a result [36].

We addressed the above issues with two goals for this study. Using a population genetic approach, we: (i) ascertained whether encountering a novel environment might limit range expansion as theory predicts; and (ii) evaluated the potential role of hybridization in expansion into a novel habitat.

To achieve these goals, we used Plains spadefoot toads, *Spea bombifrons*, as a model system. *Spea bombifrons* occupy a wide range throughout the southwestern and central United States (figure 1) and are thought to be ancestral to the central plains region [37]. After the most recent glacial retreat, *S. bombifrons* appears to have expanded its range northward [37] through grassland habitat similar to the ancestral region, with further northern expansion taking place in current populations [38,39]. By contrast, *S. bombifrons* also may have expanded their range southwestward into an entirely different biome: the desert [37]. Museum collections record *S. bombifrons* in the Southwest USA in the late 1800s, so this expansion is not contemporary. However, in some populations in Arizona, the relative abundance of *S. bombifrons* increased within the last 30 years [40].

Spadefoot toads breed, and their tadpoles develop, in ephemeral ponds that potentially dry before the tadpoles successfully metamorphose. This putative southwestward expansion of *S. bombifrons* is therefore striking because a limiting environmental factor for these amphibians is ponds that last long enough for tadpole metamorphosis. Indeed, a congener, *S. multiplicata* (Mexican spadefoot toad), that is ancestral to the desert region has shorter developmental times that enable tadpoles to more likely metamorphose before their desert ponds rapidly dry [41].

Where *S. multiplicata* and *S. bombifrons* co-occur, they potentially hybridize and produce viable offspring. Female hybrids can backcross to both parent species (hybrid males are sterile; [42,43]), thereby generating introgression between the two species [42,44]. Critically, hybrid tadpoles develop faster than pure *S. bombifrons* tadpoles, resulting in a fitness benefit for the expanding species to hybridize in a dry, desert environment [45]. In fact, *S. bombifrons* females that occur in sympatry with *S. multiplicata* have evolved facultative mate preference where they prefer conspecifics when breeding in deep, long-lasting ponds, but switch their preference to *S. multiplicata* in shallow, ephemeral ponds [45]. Consequently, *S. bombifrons* females primarily contribute to the production of F1 hybrids and the incidence of hybridization increases with decreasing pond size [46].

Because *S. bombifrons* appears to have undergone two distinct range expansions, and because they hybridize with a resident species in the context of one of those expansions, *S. bombifrons* is ideally suited to address the issues raised above. We specifically compared the genetic effects of range expansion into a novel, desert environment with expansion into a non-novel grassland environment, and ascertained whether hybridization has potentially facilitated the expansion of *S. bombifrons* into the southwestern USA. To do so, we used microsatellites and population genetic analyses to: (i) investigate patterns of genetic diversity and population structure within *S. bombifrons*; and (ii) evaluate patterns of introgression between *S. bombifrons* and *S. multiplicata*. Because the desert provides a novel habitat, we expected populations there to suffer genetic effects from bottlenecks and reduced gene flow compared to populations in the non-novel grassland habitat. Our findings support these predictions. Our results also suggest that hybridization has enhanced genetic variation in populations of southwestern *S. bombifrons*; this hybridization might have enabled their expansion into novel habitat.

2. Methods

(a) Sample collections

We obtained 217 samples of *S. bombifrons* from 21 locations across the USA through collection efforts and museum samples (figure 1). Locations ranged from a grassland environment in Nebraska to a desert environment in Arizona and included one location in Arizona that did not overlap locally with *S. multiplicata* (i.e. allotopy) (table 1). Additionally, we obtained 93 *S. multiplicata* samples from three sympatric locations in Texas and Arizona (electronic supplementary material, table S1). Genotype data for the Arizona *S. multiplicata* individuals were previously reported in [37]. Adult specimens of *S. bombifrons* from Arizona sympatry and both *S. bombifrons* and *S. multiplicata* from Texas sympatry were used to ensure accurate species identification (see figure 1 inset photos). Museum sample IDs are provided in electronic supplementary material, table S2.

(b) Microsatellite analysis

We genotyped each sample using 10 polymorphic microsatellite markers that were previously shown to not be in linkage disequilibrium (electronic supplementary material, table S3; method details in electronic supplemental materials) [47–49]. We used the software Arlequin v 3.5.1.2 [50] to calculate observed and expected heterozygosity for each location (electronic supplementary material, table S4). Deviation from Hardy–Weinberg equilibrium was calculated with an exact test contrasting observed and expected heterozygosity in Arlequin using a Markov chain with a chain length of 1 000 000 and 100 000 dememorization steps. We then corrected for multiple testing using a sequential Bonferroni correction at $\alpha = 0.05$ for each locus in each population. All of our loci were in Hardy–Weinberg equilibrium in at least 70% of our sampling locations (electronic supplementary material, table S4).

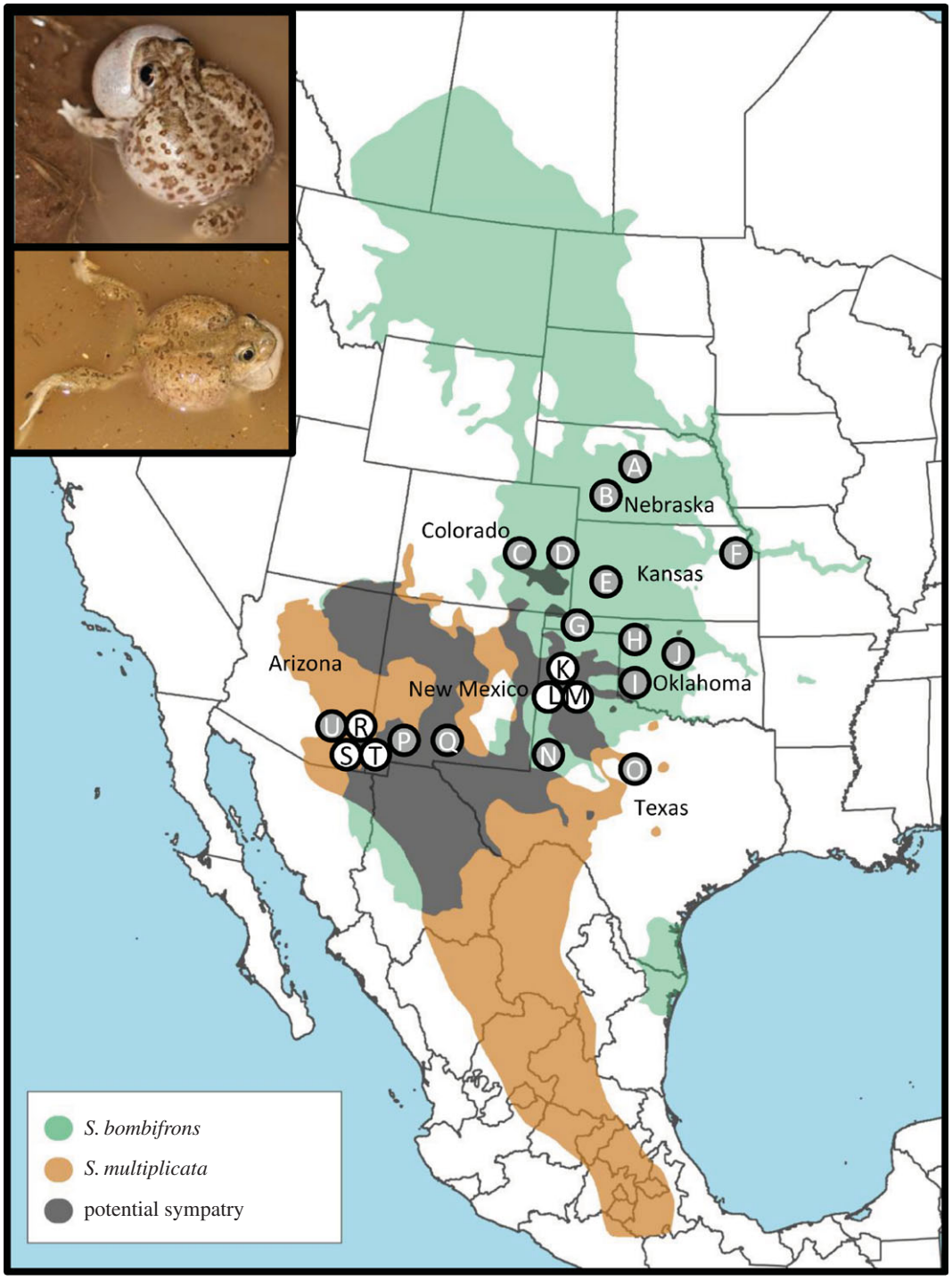

Figure 1. Map showing species' ranges and sampling locations of *S. bombifrons* and *S. multiplicata*. Central Oklahoma is the likely origin of the *S. bombifrons* range and grey circles represent *S. bombifrons* only sampling sites. White circles represent sites with both species sampled. Inset shows breeding *S. bombifrons* (top) and *S. multiplicata* (bottom) males. Sampling site key located in table 1. (Photos by David W. Pfennig; map by Travis Taggart.)

(c) Population genetics of range expansion across different habitats

To understand the impact of habitat novelty on range expansion, we first examined population structure of *S. bombifrons* across non-novel grassland and novel desert environments using the software STRUCTURE v. 2.3.3 [51]. For the STRUCTURE analysis we also included samples of *S. multiplicata* individuals from seven locations. Because we included *S. multiplicata* in this analysis, we used only the eight microsatellite loci that could be amplified in both species (electronic supplementary material, table S3). We implemented 100 000 burn-ins followed by 200 000 Markov chain Monte Carlo runs. We also used an admixture model with uncorrelated allele frequencies to avoid the risk of overestimating the number of populations and the LOCPRIOR model to provide the software with collection information for each toad to ensure the detection of subtle population structure. We started simulations with K values of 1–28, to reflect the 28 sampling locations (table 1; electronic supplementary material, table S1). For each K, we ran 10 simulations to check for consistency between runs, and used the log likelihood [51] and delta K method [52] to determine the most likely number of genetic populations (electronic supplementary material, figure S1). To confirm our results, we used all 10 loci and calculated F_{ST} and R_{ST} statistics [53,54] to measure genetic differentiation between *S. bombifrons* populations. Permutation tests (using 10 000 permutations) implemented in ARLEQUIN v. 3.5.1.2 [50] were used to determine whether pairwise F_{ST} and R_{ST} values were significantly different from 0. We also performed an analysis of molecular variance (AMOVA) and

Table 1. *Spea bombifrons* sampling location with numbers and habitat information. 'Type' indicates whether the locality is sympatric (both species present), allopatric (outside of the range of one species), or allotopic (within the region of sympatry, but only one species present).

map key	sampling location	state	*N*	type	habitat
A	Purdhum	Nebraska	18	allopatry	grassland
B	Twin Stars	Nebraska	8	allopatry	grassland
C	Limon	Colorado	15	allopatry	grassland
D	Burlington	Colorado	6	allopatry	grassland
E	Finney	Kansas	7	allopatry	grassland
F	Johnson	Kansas	6	allopatry	grassland
G	Cimarron	Oklahoma	8	allopatry	grassland
H	Ellis	Oklahoma	10	allopatry	grassland
I	Roger Mills	Oklahoma	11	allopatry	grassland
J	Payne	Oklahoma	7	allopatry	grassland
K	Amarillo	Texas	13	sympatry	grassland
L	Hereford	Texas	5	sympatry	grassland
M	Springlake	Texas	15	sympatry	grassland
N	Kermit	Texas	12	allopatry	grassland
O	Arnett	Texas	6	allopatry	grassland
P	Lordsburg	New Mexico	11	sympatry	desert
Q	NMHwy9	New Mexico	11	sympatry	desert
R	Sulphur Draw	Arizona	11	sympatry	desert
S	Shrimp	Arizona	14	sympatry	desert
T	Zent	Arizona	12	sympatry	desert
U	Wilcox	Arizona	11	allotopy	desert

calculated *p*-values based on permutation tests (using 1000 permutations) in ARLEQUIN for the northern, central, and desert regions to examine differences in the level of population structure across the range. Finally, using R_{ST} statistics we performed a Principal Coordinate Analysis using the cmdscale function in R (v. 3.0.1).

To understand if habitat novelty results in different colonization mechanisms and demographic effects, we used Poptools [55] to standardize sample sizes to seven individuals per collection site before comparing levels of genetic diversity (using the value 1-Qinter, the inter-individual diversity within populations), which were measured using Genepop v. 4.1.0 [56]. Locations with fewer than seven samples were excluded resulting in 17 sites used in the analysis. A sample size of seven was chosen to optimize statistical power with number of sites.

We also calculated allelic richness using ADZE-1.0 [57], which uses a rarefaction approach to account for unequal sample sizes. For this analysis, we set the minimum sample size per site to seven individuals with no missing data across all loci. By setting the minimum samples size as seven individuals with no missing data, we optimized the number of sites used (12 total for this analysis) while minimizing bias that could result from including sites with too few individuals. We compared values between grassland and desert regions using a Welch two sample *t*-test implemented in R v. 3.1.2.

To examine possible deleterious effects of a novel environment, we tested for recent population bottlenecks in *S. bombifrons* populations with at least 10 individuals samples using a Wilcoxon test [58] for heterozygosity excess across loci and a two-phase mutation model in the software Bottleneck [59]. We accounted for possible null alleles and determined corrected allele frequencies using the Brookfield 1 estimator [60] implemented in Micro-checker, v. 2.2.3 [61]. We also performed this analysis with uncorrected frequencies; however results were not qualitatively different so we only report results based on corrected frequencies. If novel habitats limit expansion, we expected signatures of bottlenecks in the desert, but not grassland, *S. bombifrons* populations.

(d) Examining genetic effects of hybridization

To calculate the amount of hybridization across the range, we calculated gene flow between the species in Texas and Arizona using likelihood ratio tests implemented in the coalescent-based software package MIGRATE-N 3.2 [62]. For this analysis we used the Brownian motion approximation to the ladder ('stepwise' or 'one-step') mutation model and Bayesian inference with multiple heating chains to jointly estimate parameters with three replicates [63,64]. This also allowed us to determine the directionality of gene flow.

We identified specific loci showing patterns of introgression by comparing hybrids with both parental species using F_{ST}. Hybrids were identified as admixed individuals (more than 10% assignment to heterospecific populations) based on inferred ancestry by STRUCTURE. Using the F_{ST} calculations, we could detect when admixed individuals were more genetically similar to the heterospecific, indicating introgression at that locus. We further identified signatures of introgression at locus SpeaC7 with alleles primarily found in the desert toad, *S. multiplicata* appearing in *S. bombifrons* individuals. We resampled without replacement to obtain population sizes of 10 and examined changes in frequencies of the putative heterospecific allele across the *S. bombifrons* range. Allele frequency values at sampling sites were used to generate an allele frequency surface

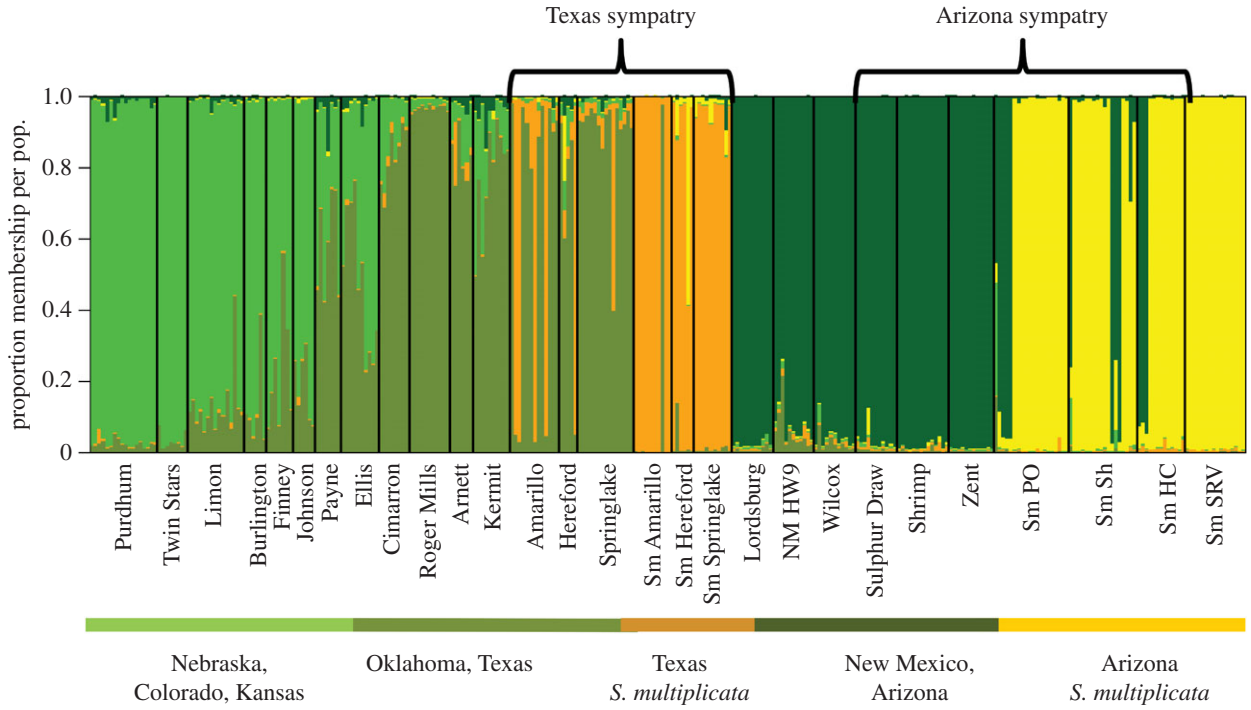

Figure 2. Structure plot showing that *K* (number of distinct populations) = 5 for *S. bombifrons* and *S. multiplicata*. The northern range of *S. bombifrons* shows increasing differentiation from the central range whereas the most southwestern portion of the range is highly differentiated. Individual toads are indicated by vertical bars and colour denotes population membership.

map by inverse distance weighted (IDW) interpolator in ArcGIS v. 10.4.1 (ESRI, Redlands, California, USA). IDW estimates values by averaging nearby data points, with closer points carrying more influence.

3. Results

(a) Range expansion across similar habitat

Across grassland populations (table 1 and figure 1), STRUCTURE analysis revealed a clinal pattern of increasing membership to the 'green' group with increasing distance northward into Nebraska from Oklahoma, the putative centre of the range (figure 2). This is consistent with a northward range expansion as suggested by previous observational and genetic data [37–39]. Despite evidence of a range expansion, *S. bombifrons* populations showed stable levels of heterozygosity ($F_{14,135} = 0.54$, $p = 0.91$; figure 3*a*), genetic diversity ($F_{10,99} = 1.26$, $p = 0.27$; figure 3*b*) and allelic richness ($F_{7,72} = 0.50$, $p = 0.83$; figure 3*c*) across the grassland portion of their range from Texas to Nebraska (figure 3). These latter findings contrast with previous empirical and theoretical work in other systems showing that an expanding species will exhibit decreasing genetic diversity [11,12,65–68]. We further found that F_{ST} and R_{ST} values were low across much of the northern and central portions of the range (electronic supplementary material, table S5 and figure S2) of *S. bombifrons*. An AMOVA analysis using R_{ST} confirmed that differences among sites did not account for a significant amount of observed variation in the northern (Kansas, Nebraska, Colorado: 3.4% variation, $p = 0.17$) or central (Oklahoma, Texas: 5.18% variation, $p = 0.06$) populations. Thus, where *S. bombifrons* has expanded its range across similar habitat, it maintains stable levels of genetic diversity and allelic richness, likely via gene flow among other grassland populations.

(b) Range expansion across novel habitat

Contrary to the non-novel grassland populations, allelic richness decreased among desert populations in New Mexico and Arizona ($t_{4.77} = 6.90$, $p < 0.01$; figure 3*c*). Additionally, we found the desert-inhabiting populations to be highly genetically differentiated compared to grassland populations in our F_{ST}, R_{ST} and STRUCTURE analyses (figure 2; electronic supplementary material, table S5 and figure S2). Not only are the southwestern populations significantly differentiated from the northern and central populations, they are significantly differentiated from one another. An AMOVA analysis using R_{ST} confirmed that differences among sites accounted for increased population structure in the desert populations (New Mexico, Arizona; 9.33% variation; $p < 0.001$). Additionally, we found evidence for bottlenecks in two Arizona populations (Zent, $p < 0.01$ and Wilcox, $p < 0.01$). Outside of Arizona, we did not detect evidence for bottlenecks. The decline in allelic richness, high population structure, and evidence for bottlenecks is consistent with the desert habitat limiting gene flow and restricting movement among conspecific populations. Nevertheless, genetic diversity and heterozygosity appear to be maintained among desert populations of *S. bombifrons* as neither measure was significantly different from values found in grassland populations (genetic diversity: $t_{4.66} = 2.54$, $p = 0.06$; heterozygosity: $t_{6.73} = 1.28$, $p = 0.24$; however the marginal *p*-value for diversity could reflect insufficient power to detect a difference).

(c) Hybridization's effects on range expansion

To investigate if hybridization might have enhanced genetic diversity in *S. bombifrons* in the novel desert habitat, we surveyed *S. multiplicata* from two areas of sympatry—Texas and Arizona. We found that outlier *S. bombifrons* individuals located in Texas were genetically similar to *S. multiplicata*,

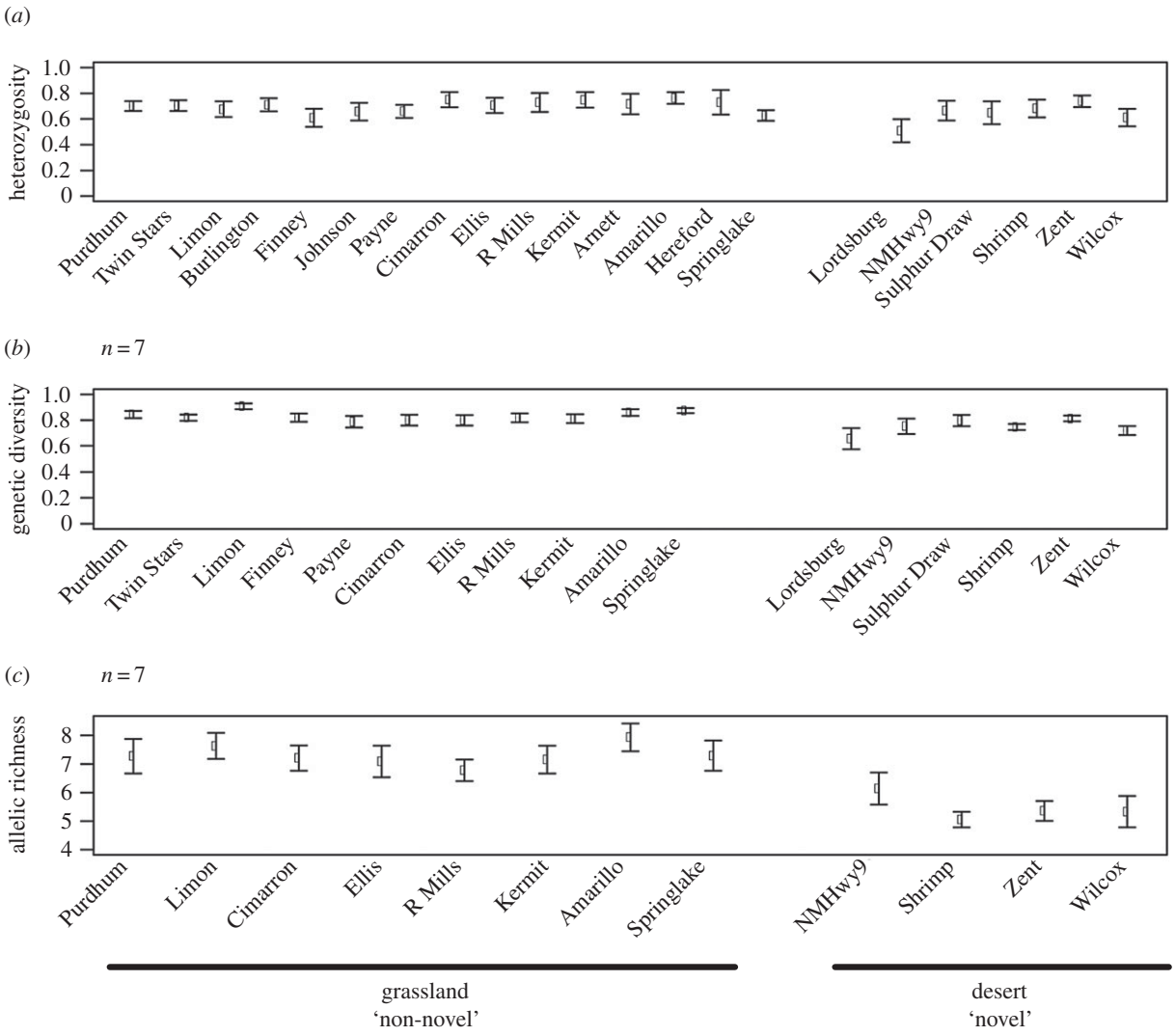

Figure 3. Genetic trends across *S. bombifrons* range. Sampling sites are ordered from north to southwest. (*a*) High levels of heterozygosity (H_o) are maintained throughout the range. (*b*) Genetic diversity (using the value 1-Qinter, the inter-individual diversity within populations) was also maintained. (*c*) Allelic richness declines in desert populations.

indicative of hybridization (figure 2). Additionally, we found outlier *S. multiplicata* individuals in Arizona appearing genetically similar to Arizona *S. bombifrons*, again pointing towards hybridization. Rather than equal gene flow between the species, we found asymmetrical gene flow with the recipient species differing based on sympatric location. Migrate-n confirmed these findings, indicating a higher level of gene flow from resident *S. multiplicata* to *S. bombifrons* in Texas (*S. multiplicata* → *S. bombifrons* 5.43 immigrants/generations; *S. bombifrons* → *S. multiplicata* 1.40 immigrants/generation) but a higher level of gene flow from invading *S. bombifrons* to native *S. multiplicata* in Arizona (*S. bombifrons* → *S. multiplicata* 4.09 immigrants/generation; *S. multiplicata* → *S. bombifrons* 1.89 immigrants/generation).

Additionally, individual examination of the markers revealed introgression across multiple loci (electronic supplementary material, table S6). For example, in sympatry, admixed individuals were genetically indistinguishable from heterospecifics at multiple loci, while showing significant differentiation from their conspecific population. For locus SpeaC7 in particular, a *S. multiplicata* allele was not found in high frequencies in central or northern *S. bombifrons* populations outside of sympatry, but was maintained at a relatively high frequency in *S. bombifrons* throughout the desert habitat (figure 4). Given that we detected introgression across multiple loci with only a handful of microsatellite markers, it is possible that hybridization has introduced a larger amount of genetic variation than observed here.

4. Discussion

We used Plains spadefoot toads, *S. bombifrons*, to examine the population genetic effects of habitat novelty during range expansion and to evaluate how hybridization with a resident species can impact genetic variation during expansion into novel habitat. In contrast to populations in the ancestral grassland environment, we found that the novel desert environment was associated with reduced gene flow and recent population bottlenecks in *S. bombifrons*. Although these factors led to a reduction in allelic richness, hybridization with the resident *S. multiplicata* appears to have led to the transfer of genetic variation in the novel desert habitat. Such transfer of genetic variation via hybridization might have facilitated the range expansion of *S. bombifrons* into a novel habitat.

Generally, species are expected to evolve expanded ranges when edge populations adapt to local conditions

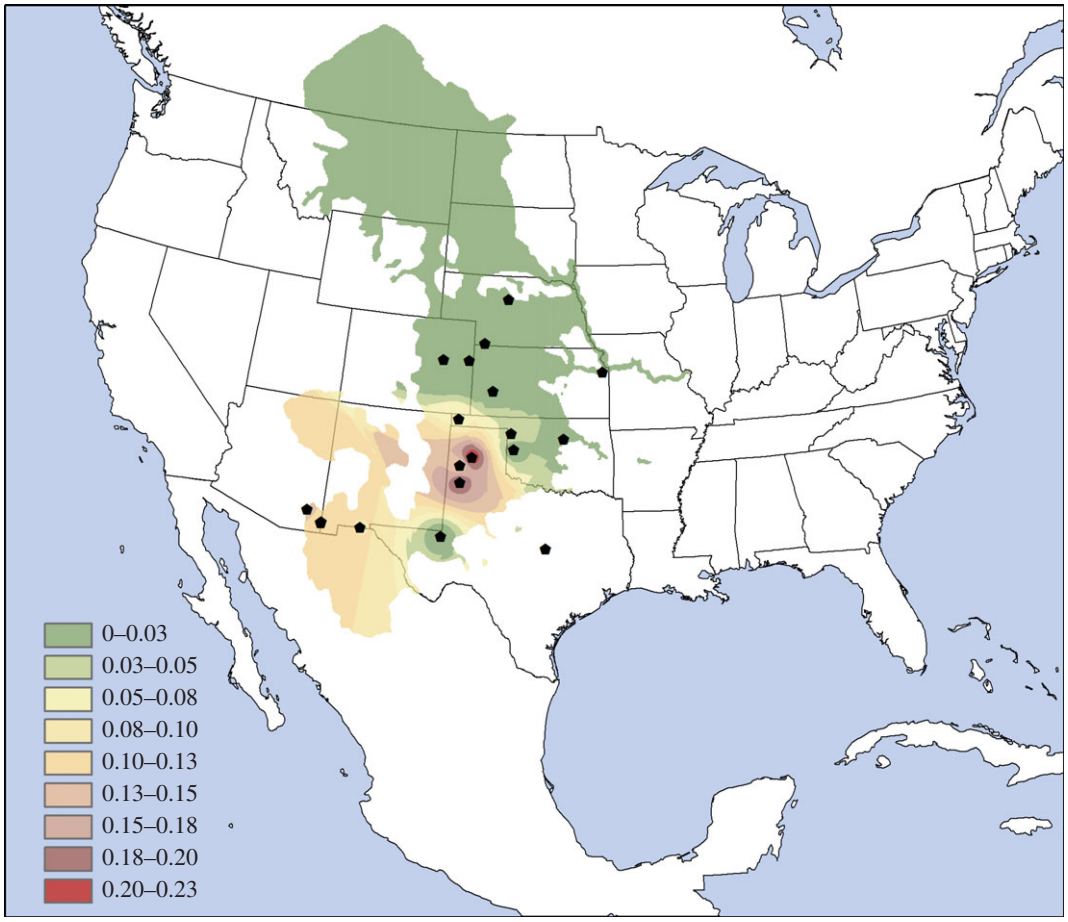

Figure 4. Introgression and maintenance of heterospecific allele in *S. bombifrons*. ArcGIS frequency surface map of heterospecific (*S. multiplicata*) allele at locus *SpeaC7* over the range of *S. bombifrons* based on observed population frequency at collection sites (black pentagons). See Methods for details. This heterospecific allele first appears in Texas sympatry and is maintained throughout the desert region. It is present even in the most westward *S. bombifrons* desert population, which is allotopic.

and become sources of dispersers [6,8,9,69]. Adaptability of peripheral populations therefore sets the limits of a species' range. A key factor that limits adaptability is genetic diversity: in the absence of genetic diversity, populations are unable to evolve in response to local selective pressures [70,71]. Ironically, a common signature of range expansion is reduced genetic diversity because edge populations are often the result of serial founder events or suffer population crashes (and, concomitantly, genetic bottlenecks) [10–12]. Although dispersal and the resulting gene flow among conspecific populations can reintroduce genetic variation into peripheral populations [72,73], such gene flow can inhibit adaptation if alleles from the range centre are maladaptive at the range edge [8,16]. Moreover, dispersal might be limited across novel habitat [74–76]. Therefore, the novelty of the habitat into which expansion occurs might critically impact the adaptability of peripheral populations.

Our results illustrate this dynamic between range expansion and habitat novelty. In the northward range expansion into a relatively non-novel grassland habitat, allelic richness and genetic diversity levels remain high among *S. bombifrons* populations (figure 3). Additionally, F_{ST} and R_{ST} values throughout the grassland regions are relatively low, suggesting ample movement of toads throughout the grassland range, which maintains a high level of genetic diversity and a low level of population differentiation (electronic supplementary material, table S5). Conversely, the southwestward range expansion by *S. bombifrons* into novel desert habitat showed a different pattern. Desert populations were highly genetically differentiated not only in comparison with the rest of the range (figure 2), but also between closely located populations in the desert itself (electronic supplementary material, table S5). Such strong population differentiation was likely influenced by bottleneck events.

The differences we observe between the northern and southwestern range expansions by *S. bombifrons* highlight how expansion into novel versus non-novel habitats can generate variation in gene flow and population genetic patterns. Indeed when a species expands into a similar habitat, gene flow among populations is more likely to result in maintenance of allelic richness and adaptive potential [8,17]. By contrast, our results indicate that a novel habitat might restrict movement, so that populations are less likely to receive migrants (and genetic rescue) and are more likely subject to population crashes and extinction. The novel habitat thus generates a negative genetic impact, which—without some counterforce—could limit peripheral population adaptability.

One such counterforce is hybridization with a resident species that is locally adapted [21,30]. In spadefoots, hybridization with desert-adapted *S. multiplicata* may be one way in which *S. bombifrons* maintains genetic variation in the novel habitat. Patterns of introgression are consistent with this, as is our finding that desert populations of *S. bombifrons* contain high levels of genetic diversity despite evidence of recent bottlenecks.

Specifically, we examined two areas of sympatry to evaluate the effects, if any, of hybridization on *S. bombifrons* desert expansion. Interestingly, we found that directionality of introgression differed between these regions (figure 2). In Texas, introgression was from the resident *S. multiplicata* into the expanding *S. bombifrons*, whereas the opposite was true in Arizona. Although theory predicts that the rarer, expanding species should receive massive introgression from the resident species, the female driven hybridization and hybrid male sterility seen in *S. bombifrons* may result in the inverse pattern in Arizona (*sensu* [77]). Additionally, a study examining hybrid mate choice in this system found that hybrid females had no mate preference between parental species [78]. Thus, the relative abundance of parental species might drive patterns of backcrossing and introgression in a given region [78]. Indeed in Texas, *S. bombifrons* are more common, resulting in a higher likelihood for hybrid females to mate with *S. bombifrons*, thereby moving *S. multiplicata* alleles into the *S. bombifrons* population. In Arizona, by contrast, there are relatively fewer *S. bombifrons*, so hybrid female behaviour might contribute to introgression of *S. bombifrons* alleles into the *S. multiplicata* population. Whether hybrid behaviour contributes to the patterns of introgression we observed requires further study. Nevertheless, our data suggest that *S. bombifrons* has received *S. multiplicata* alleles in Texas, where they initially encountered *S. multiplicata*.

Furthermore, we found that *S. bombifrons* has not only received heterospecific alleles, but has maintained an introgressed allele following their southwestward expansion deeper into the desert region (figure 4). This allele is maintained despite evidence of bottlenecks and low gene flow among Arizona populations of *S. bombifrons* (but see below), and despite introgression primarily from *S. bombifrons* into *S. multiplicata* in Arizona. We also observed a relatively high frequency of this heterospecific allele in an allotopic *S. bombifrons* population at the western edge of the species' range where *S. multiplicata* is absent. This latter result emphasizes that ongoing hybridization is not necessary to maintain this genetic variation. Why this allele persists is not clear, but one explanation is that it is linked to a functional locus under selection. Such a pattern would be expected if *S. bombifrons* acquired adaptive alleles from *S. multiplicata* that enabled them to expand into the desert habitat. Although this explanation is speculative at this point, we can conclude that the allele is not being purged from the populations as would be expected if it were associated with reduced fitness hybrids.

The exception to this pattern of heterospecific allele maintenance was the sympatric Zent population in Arizona in which the heterospecific allele is absent. However, Zent also shows one of the strongest signatures of a recent bottleneck and is a newly discovered (and possibly newly established) site with very low population size (N = approx. 12–20 adults of both species in recent samplings). The allele may therefore have recently been lost through genetic drift.

Although it is possible that this putatively heterospecific allele was a result of convergent evolution or shared ancestry, rather than introgression, both possibilities seem unlikely. Texas *S. multiplicata* samples have a high frequency of this allele (approx. 50% in some populations), making it likely to be shared during hybridization. Additionally, we do not see significant frequencies of this allele in any *S. bombifrons* populations north of the Texas sympatric zone. Given the spatial pattern of the allele frequency (figure 4), and that hybridization between these two species occurs [46], the most parsimonious explanation for its presence in *S. bombifrons* is introgression.

As *S. bombifrons* expanded into the novel desert environment of the southwestern US, the receipt of *S. multiplicata* alleles in Texas could have provided *S. bombifrons* with adaptive genetic variation that enabled them to colonize the novel habitat and further expand southwestward. Future work examining differential levels of introgression across the genome and its adaptive significance, if any, are underway to evaluate this possibility. Regardless, this study indicates that hybridization with *S. multiplicata* has altered the population genetics of *S. bombifrons*. Our findings suggest that hybridization with a resident species may be a way in which expanding species can maintain levels of genetic diversity in a novel habitat, which could enable further expansion. Given shifting species' ranges [32–35,79] and the likelihood that hybridization will become increasingly common [36], the need to evaluate hybridization's role in range expansion is more pressing now than ever [30].

Data accessibility. Data are provided as electronic supplementary material.

Authors' contributions. K.S.P. and A.A.P. conceived of the project and its design. K.S.P. collected samples in addition to those provided by the museums acknowledged below. A.A.P. and R.G. genotyped samples and analysed data. A.M.R. provided microsatellite data for *S. multiplicata*. A.A.P. wrote the paper in collaboration with K.S.P. R.G. and A.M.R. approved and edited the final versions.

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