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Hybridization in leopard frogs (*Rana pipiens* complex): Variation in interspecific hybrid larval fitness components along a natural contact zone

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ABSTRACT

Although hybrid zones between North American leopard frog species pairs (*Rana pipiens* complex) are usually narrow, the contact zone between *R. blairi* and *R. sphenocephala* varies from sharp parapatry to relatively broad sympatry. I compared fitness components using larval life-history traits for *R. blairi*, *R. sphenocephala* and F_1 hybrid genotypes from Texas populations where the hybrid zone is sharply parapatric, and genotypes from Missouri populations where hybridization occurs in broadly sympatric areas. Texas F_1 hybrids had significantly reduced survival and metamorphosis relative to Missouri F_1 hybrids. Texas hybrids also demonstrated reduced performance in terms of survival, body mass and metamorphosis when reared in experimental populations with *R. sphenocephala* or both parental species, but not *R. blairi* alone. Larval fitness estimates as measured by survival, body mass at metamorphosis, proportion of survivors metamorphosing and length of larval period for Missouri F_1 hybrids were equal or higher than those of their parental genotypes in all mixtures. These patterns suggest selection may be dependent on the larval rearing environment and strongest against hybrid genotypes in the southern region of the hybrid zone (Texas), but not directional against F_1 hybrids in the northern region (Missouri). Thus, fitness data from a single location within hybrid zones may lead to inaccurate conclusions concerning the evolutionary potential of hybridization.

Keywords: amphibian larvae, endogenous selection, exogenous selection, fitness, geographic variation, hybridization, *Rana*.

INTRODUCTION

Natural hybridization can have important effects on natural patterns of variation and organismal diversity, but its evolutionary significance is uncertain. As common features of both animals and plants, hybrid zones are frequently examined for their roles in speciation events (Harrison, 1990; Bullini, 1994; Dowling and Secor, 1997). Interspecific hybridization can also have significant evolutionary effects through introgression from one diploid taxa

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into another (e.g. de Marais *et al.*, 1992). Indeed, an important component of adaptive evolution may be natural selection acting on hybrid genotypes with novel gene combinations (Grant and Grant, 1997; Arnold and Emms, 1998; Parris, 1999). Others consider natural hybridization maladaptive because of the reduced frequency and fitness of hybrids relative to parental genotypes, or the restriction of hybrids to marginal habitats (for a complete discussion, see Barton and Hewitt, 1985; Moore and Price, 1993).

In determining the evolutionary significance of hybrid genotypes, it is critical to directly measure relative fitness from multiple locations throughout areas of hybridization. Some contemporary hybrid zone studies have demonstrated geographic variation in hybrid zone widths (summarized in Barton and Hewitt, 1985), suggesting different relative genotypic fitness in distinct geographic regions. However, few studies have directly measured and compared relative fitness among localities (e.g. Butlin *et al.*, 1991; Shaw *et al.*, 1993). If hybrid genotypes have relatively high fitness in particular areas and low fitness in others, the evolutionary outcome of hybridization could vary geographically throughout the zone. Strong selection against hybrid genotypes could produce parapatric species distributions and narrow hybrid zones (Key, 1968; Remington, 1968; Barton and Hewitt, 1985), and relatively high hybrid fitness, especially under some environmental conditions, could produce more extensive or complex patterns of hybridization (Moore and Price, 1993; Arnold, 1997; Arnold and Emms, 1998).

Understanding the selective mechanisms operating in hybrid systems is crucial in determining the evolutionary potential of natural hybridization in specific environments and the nature of genetic and ecological interactions between taxa (Barton and Hewitt, 1985, 1989; Mallet and Barton, 1989). Natural selection can operate intrinsically (endogenous) against hybrid genotypes, or genotype by environment interactions (exogenous) can determine fitness. Either or both forms of selection may operate in hybrid systems. Endogenous selection typically is directional against hybrid genotypes in all environments because of inherent genetic incompatibilities between parental genomes; recombination disrupts normal organismal homeostasis (Barton and Hewitt, 1985). Exogenous selection caused by underlying ecological gradients may lead to parental genotypes being differentially adapted to a given habitat type, with hybrids showing reduced fitness everywhere (Barton and Hewitt, 1985). Alternatively, some hybrid genotypes could be successful in certain environments (Endler, 1977; Moore, 1977; Freeman *et al.*, 1999).

North American leopard frogs of the *Rana pipiens* sibling species complex are among the most widely studied vertebrates (Hillis, 1988). Hillis *et al.* (1983) and Hillis (1988) resolved the phylogenetic relationship of the 25 species or more of this group into two broad lineages (α and β). Parapatric geographic distributions and narrow zones of hybridization are long-standing features of most species pairs within lineages (Sage and Selander, 1979; Hillis, 1981). Hybrid inferiority is a common inference drawn from many leopard frog hybrid zones, but limited experimental data on relative fitness of hybrid and parental genotypes are available (Sage and Selander, 1979; Hillis, 1981; Frost and Platz, 1983; Kocher and Sage, 1986; but see Parris, 1999, 2000; Parris *et al.*, 1999). *Rana blairi* and *R. sphenocephala* are sister species within the β lineage (Hillis, 1988). The hybrid zone between these two species is unique because it varies in width in different geographic areas. Although parapatric in northern Texas, these two species are sympatric in Oklahoma and Kansas (Hillis, 1981), and broadly sympatric in central and northern Missouri (Sage, 1994).

Previous analyses of this hybrid system demonstrated F_1 and advanced-generation hybrid genotypes from Missouri populations had equivalent or higher levels of growth,

development, survival and metamorphosis relative to their parental genotypes in single- and mixed-genotype populations in experimental ponds (Parris, 1999; Parris *et al.*, 1999). Although indicative of equivalent hybrid and parental fitness, these studies examined fitness components in single-genotype or two-genotype mixtures (i.e. hybrids with only one parental genotype), which may not represent the most realistic natural community of leopard frog genotypes in Missouri (Sage, 1994). A more biologically relevant scenario would include interactions among both parental genotypes and hybrids. Furthermore, fitness components were only estimated for leopard frogs from one region of the hybrid zone.

To determine whether natural hybridization has the potential to produce unique evolutionary results in two widely separated geographic regions within a hybrid zone, I examined larval fitness components of *R. blairi*, *R. sphenocephala* and F_1 hybrids from Texas and Missouri populations in single-, two- and three-genotype mixtures. I raised larvae in experimental ponds to test for differences among genotypes and between populations in larval survival, body mass at metamorphosis, metamorph production and developmental rate. I then asked: (1) Is there phenotypic variation among genotypes in fitness-related larval traits? (2) Do F_1 hybrids perform better or worse than the parental genotypes? (3) Is F_1 hybrid performance dependent on larval rearing treatment (i.e. competitive environment)? (4) Do F_1 hybrids from Texas and Missouri populations differ in relative fitness component values, thus providing evidence for unique evolutionary potentials of hybrid genotypes in different geographic areas?

MATERIALS AND METHODS

Source of frogs and breeding design

I used adult frogs for artificial crosses. Individuals were collected in late March and early April 1998 from natural populations in northern Texas (33°8′ N, 98°1′ W) and central Missouri (38°35′ N, 92°8′ W). These two populations are in the southern and northern extremes of the hybrid zone. I assessed the genotype of each field-collected frog morphologically and confirmed it with protein electrophoresis using the diagnostic enzyme loci *LDH-B*, *MDH*, *PGM-1* and *SDH* (Hillis *et al.*, 1983; Sage, 1994).

I generated offspring of two parental genotypes (*R. blairi*, designated BB; *R. sphenocephala*, designated SS) and primary hybrids (F_1). I confirmed the pure parental status of animals after crossing using electrophoresis. Cohorts of larvae for each genotype were produced separately from Texas and Missouri populations, and each female was crossed to each male within genotypes. I used six females (three *R. blairi* and three *R. sphenocephala*) and seven males (four *R. blairi* and three *R. sphenocephala*) for Texas crosses, and six females (three *R. blairi* and three *R. sphenocephala*) and eight males (four *R. blairi* and four *R. sphenocephala*) for Missouri crosses. I performed interspecific crosses using the same males and females from parental crosses to produce the F_1 hybrid genotype. I pooled resultant larvae from reciprocal F_1 genotypes (*R. blairi* × *R. sphenocephala* and reciprocal; mother listed first) because of an earlier determination of equivalent larval viabilities (Parris *et al.*, 1999). Families within genotypes were pooled to randomly distribute the potential for maternal effects among all treatments, although it has previously been shown that maternal effects on offspring performance in this system are minimal (Parris *et al.*, 1999).

I followed standard procedures for artificial crosses (Semlitsch *et al.*, 1997; Parris, 1999). Field-captured females did not need external hormone stimulation to induce ovulation. I prepared sperm suspensions by mashing both testes of a male in a small volume of pond water in a petri dish. Fertilizations were then performed by stripping 20–50 eggs from one female at a time into a petri dish. After 4–7 min, I decanted the sperm suspension into a new petri dish and the fertilized eggs were covered with fresh pond water. Eggs of the next female were fertilized in the same manner. I performed all fertilizations in 3–5 h on 25 March for Texas frogs and on 3 April for Missouri frogs. Slight differences in timing of artificial crosses were unavoidable because of temporal separation in adult breeding phenologies between the Texas and Missouri populations. Texas larvae were transported back to the laboratory after hatching at room temperature (23°C) on 29 March, and transferred to 1-litre containers of pond water. Missouri larvae were transferred to 1-litre containers after hatching at room temperature on 10 April.

Experimental design and procedures

I raised larvae in outdoor experimental ponds (polyethylene cattle tanks, 1.83 m diameter) positioned in a rectangular array at the University of Missouri Research Park (Boone County, Missouri). The ponds are within the size range of small natural temporary ponds and are an excellent compromise between the realism of natural ponds and the experimental stringency of laboratory containers (Hurlbert, 1984). Ponds were prepared identically from late March to early April 1998 by filling them with approximately 1000 litres of tap water (50 cm depth), adding 1.0 kg of air-dried deciduous leaf litter and inoculating six times with equal-sized aliquots (500–1500 ml each) of concentrated plankton suspensions collected from several nearby natural ponds (for details, see Parris and Semlitsch, 1998; Parris, 1999).

Treatments for larvae from the Texas and Missouri populations consisted of both parental genotypes and F_1 hybrids reared separately, and in two- and three-genotype mixtures. Larvae were reared at an initial total density of 60 larvae per pond, which is representative of natural *Rana* densities (Morin, 1983; Petranka, 1989; Parris *et al.*, 1999; Parris, 1999). Two-way mixtures consisted of each parental genotype reared separately with F_1 hybrids at an initial density of 30 : 30 larvae. The three-way mixture consisted of all genotypes at an initial density of 20 : 20 : 20 larvae. Treatments were replicated 3–6 times and randomly assigned to an array of 50 ponds (25 Texas, 25 Missouri). Upon reaching free-swimming (stage 25; Gosner, 1960), the larvae were randomly assigned to ponds according to the design (day 0; Texas = 4 April, Missouri = 11 April). A 75-day hydroperiod was imposed on larvae in the Texas treatments and a 69-day hydroperiod for the Missouri treatments. The experimental hydroperiods were based on the criteria of at least one pond in which 100% of the individuals metamorphosed and every pond producing at least one metamorph (see Appendix).

Measurement of larval fitness components and statistical analyses

Phenotypic variation in growth and developmental rates are important components of fitness for larval amphibians. Larval growth rate and developmental time can affect fitness through timing of and mass at metamorphosis (Wilbur and Collins, 1973; Alford and Harris, 1988). Rapid developmental rates allow larvae to metamorphose quickly and escape desiccation in ephemeral habitats or predation in more permanent ponds (Newman, 1988;

Denver *et al.*, 1998). Short larval periods may also be advantageous in more permanent aquatic habitats because of the partial drying of edge habitat used by developing larvae (Semlitsch *et al.*, 1997). Larval performance can influence terrestrial adult fitness (Endler, 1986; Werner and Anholt, 1993). Large body mass at metamorphosis can lead to high terrestrial juvenile survival, early age and large size at first reproduction, high fecundity and high terrestrial physiological performance (e.g. Berven and Gill, 1983; Smith, 1987). Survival and metamorphosis are two critical larval performance measures because of their direct effects on juvenile recruitment (Berven, 1990). Thus, larval performance is an important fitness component for both larval and adult amphibian life-history stages (de Jong, 1994).

I measured responses separately for each genotype in mixtures and mean values per pond for each genotype were the units of analysis because measurements from individuals within ponds were not independent. During the experiment, metamorphs were dip netted out of the ponds and held in the laboratory for 2–5 days or until tail resorption was complete, and then weighed to the nearest 0.1 mg. Body mass at tail resorption is the most reliable indicator of body mass at metamorphosis (Travis, 1984). Survival was calculated as the proportion of all individuals (larvae and metamorphs) surviving to the end of the experiment from those initially added to each pond. I defined metamorphosis as emergence of one forelimb (stage 42; Gosner, 1960) and calculated it as the proportion of survivors metamorphosing by the end of the experiment. In general, survival differed across sites and was dependent on genotype and mixture (Table 1). Because differential survival may confound the measurement of other larval responses by establishing different realized densities among remaining larvae (Wilbur, 1997; Parris and Semlitsch, 1998), survival was used as a covariate in analyses of covariance for body mass at metamorphosis, the proportion of survivors metamorphosing and larval period length after testing for site, genotype, mixture and interaction effects on survival (SAS, 1990). Furthermore, to account for correlations between body mass and larval period ($r = 0.236$, $P = 0.029$), each was used as an additional covariate in the univariate analyses of the other. This correction also minimized any confounding effects caused by different experimental hydroperiods imposed on Texas and Missouri larvae, which would probably be reflected in differential growth and development. Larval responses also were analysed using multivariate analysis of covariance (survival covariate) for combined larval responses. Additional analyses of variance and covariance were used to test genotype responses separately within sites when interaction effects were significant. Conservative pairwise tests of significance among factor level means were conducted using Scheffé's multiple range tests (Scheffé, 1959; $P < 0.05$). Body mass at metamorphosis and larval period length data were log-transformed and the proportion surviving and metamorphosing data were arcsine-square root-transformed before analysis to increase additivity of effects and homogeneity of error variances (Sokal and Rohlf, 1995). At the end of the experiment (Texas = 18 June; Missouri = 19 June), I drained and thoroughly searched all ponds for remaining larvae and metamorphs. After weighing, the genotype of all individuals from mixtures was identified by electrophoresis (Sage, 1994).

RESULTS

Missouri larvae had significantly higher survival than Texas larvae (Table 1; Appendix). Although genotypes did not differ significantly in terms of survival, there were significant site × genotype and site × genotype × mixture interaction effects on survival (Table 1).

Table 1. Univariate analysis of variance (ANOVA) of proportion of individuals surviving, multivariate analysis of covariance (MANCOVA; survival covariate) and univariate analyses of covariance (ANCOVA) of body mass at metamorphosis (survival and larval period length covariates), proportion of survivors metamorphosing (survival covariate) and larval period length (survival and body mass covariates) for Texas and Missouri parental and F_1 hybrid genotypes between *R. blairi* and *R. sphenocephala* reared alone and in mixtures

ANOVA: Survival				
Source	d.f.	MS	*F*	*P*
Site	1	1.2697	47.67	0.0001
Genotype	2	0.0472	1.77	0.1777
Mixture	4	0.0680	2.55	0.0471
Site × genotype	2	0.1169	4.39	0.0162
Site × mixture	4	0.0562	2.11	0.0895
Genotype × mixture	3	0.1131	4.25	0.0084
Site × genotype × mixture	3	0.1949	7.32	0.0003
Error	66	0.0266		

MANCOVA: Body mass, metamorphosis and larval period				
Source	Wilks' λ	d.f.	*F*	*P*
Site	0.385	3,63	33.52	0.0001
Genotype	0.569	6,126	6.83	0.0001
Mixture	0.557	12,167	3.45	0.0002
Site × genotype	0.667	6,126	4.72	0.0002
Site × mixture	0.778	12,167	1.39	0.1758
Genotype × mixture	0.479	9,153	6.01	0.0001
Site × genotype × mixture	0.831	9,153	1.35	0.2177
Survival	0.742	3,63	7.32	0.0003

ANCOVA: Body mass, metamorphosis and larval period					
Response	Source	d.f.	MS	*F*	*P*
Body mass	Site	1	0.0193	3.30	0.0739
	Genotype	2	0.0221	3.78	0.0281
	Mixture	4	0.0064	1.09	0.3686
	Site × genotype	2	0.0638	10.89	0.0001
	Site × mixture	4	0.0103	1.75	0.1491
	Genotype × mixture	3	0.0293	4.98	0.0036
	Site × genotype × mixture	3	0.0143	2.44	0.0726
	Survival (covariate)	1	0.1176	20.09	0.0001
	Larval period (covariate)	1	0.0004	0.07	0.7994
	Error	64	0.0059		
Metamorphosis	Site	1	0.0470	1.25	0.2678
	Genotype	2	0.2096	5.57	0.0058
	Mixture	4	0.0062	0.17	0.9554
	Site × genotype	2	0.3201	8.51	0.0005
	Site × mixture	4	0.0671	1.78	0.1426
	Genotype × mixture	3	0.1752	4.66	0.0052

	Site × genotype × mixture	3	0.0132	0.35	0.7878
	Survival (covariate)	1	0.1899	5.05	0.0281
	Error	65	0.0376		
Larval period	Site	1	0.0174	94.17	0.0001
	Genotype	2	0.0026	13.94	0.0001
	Mixture	4	0.0015	8.21	0.0001
	Site × genotype	2	<0.0001	0.07	0.9290
	Site × mixture	4	0.0001	0.64	0.6361
	Genotype × mixture	3	0.0024	13.09	0.0001
	Site × genotype × mixture	3	0.0001	0.45	0.7212
	Survival (covariate)	1	0.0002	1.23	0.2719
	Body mass (covariate)	1	0.0001	0.75	0.3906
	Error	64	0.0002		

Scheffé's comparisons indicated that Missouri hybrids had higher survival than Texas hybrids (Fig. 1). Within sites, Texas hybrid survival was significantly lower than that of BB and SS ($F_{2,33} = 6.46$, $P = 0.0043$; see Appendix) and dependent on mixture (genotype × mixture interaction: $F_{3,33} = 7.65$, $P = 0.0005$). Texas hybrid survival was highest when reared in mixtures with BB, and lowest when reared with SS or in the three-way mixture (Fig. 1). Missouri hybrids and SS had higher survival than BB ($F_{2,33} = 3.88$; $P = 0.0306$; see Appendix). Using multivariate analysis of covariance, there were significant main effects of site, genotype and mixture and site × genotype and genotype × mixture interaction effects on combined larval responses (Table 1). Although genotypes differed significantly in body mass (Table 1), the direction of this difference varied across sites. Texas hybrids were smaller than both parental genotypes ($F_{2,31} = 14.59$, $P = 0.0001$) and smallest when reared in mixtures with SS or in the three-way mixture (genotype × mixture interaction: $F_{3,31} =$

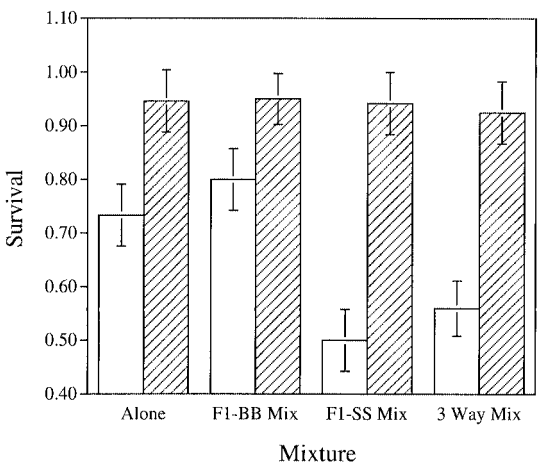

Fig. 1. Proportion of individuals surviving (larvae and metamorphs) for Texas (□) and Missouri (▨) F_1 hybrid larvae reared in experimental ponds. Values plotted are least-squares means ± 1 standard error when reared in single-genotype populations (Alone) and mixtures ($F_1 = F_1$ hybrids; BB = *R. blairi*; SS = *R. sphenocephala*).

7.66, $P = 0.0006$; Fig. 2). Missouri BB were larger than SS ($F_{2,31} = 4.40$, $P = 0.0207$), but Missouri hybrids did not differ significantly from either parental genotype. Genotypes also differed significantly in metamorphosis (Table 1), but in different directions. Texas hybrids produced fewer metamorphs than parental genotype SS ($F_{2,32} = 7.82$, $P = 0.0017$) but not BB. Missouri hybrids produced significantly more metamorphs than parental genotype BB ($F_{2,32} = 6.45$, $P = 0.0044$), but did not differ significantly from SS. Scheffé's comparisons indicated that Missouri hybrids produced a greater proportion of metamorphs than Texas hybrids (Fig. 3). There were significant main effects of site, genotype and mixture and a genotype × mixture interaction effect on length of larval period (Table 1). Texas larvae had significantly longer larval periods than Missouri larvae. Across sites, hybrids had shorter larval periods than BB, but longer than SS. Both Texas and Missouri hybrids had longer larval periods when reared with SS than in the other mixtures.

DISCUSSION

Evolutionary ecology of larval performance

The reduced performance of Texas F_1 hybrids relative to their parental genotypes in terms of survival, body mass at metamorphosis and the proportion of survivors reaching metamorphosis may be an important contributor to the narrowness of the hybrid zone in Texas. The equivalent or better performance of Missouri F_1 hybrids compared to their parentals for all life-history traits measured may be a factor responsible for the relatively broad area of hybridization in the northern region of this zone. Furthermore, for survival and metamorphosis, hybrids from Missouri outperformed Texas hybrids. Such differential performance between hybrids suggests different evolutionary potentials for hybridization in different areas of this zone. It is important to note that, although Texas F_1 hybrids had

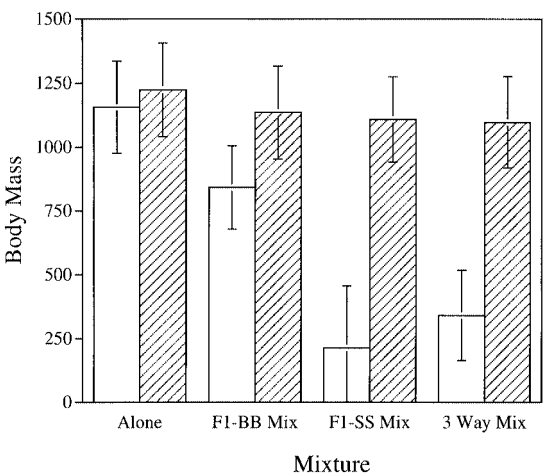

Fig. 2. Body mass at metamorphosis (mg) for Texas (□) and Missouri (▨) F_1 hybrid larvae reared in experimental ponds. Values plotted are corrected least-squares means ± 1 standard error when reared in single-genotype populations (Alone) and mixtures ($F_1 = F_1$ hybrids; BB = *R. blairi*; SS = *R. sphenocephala*).

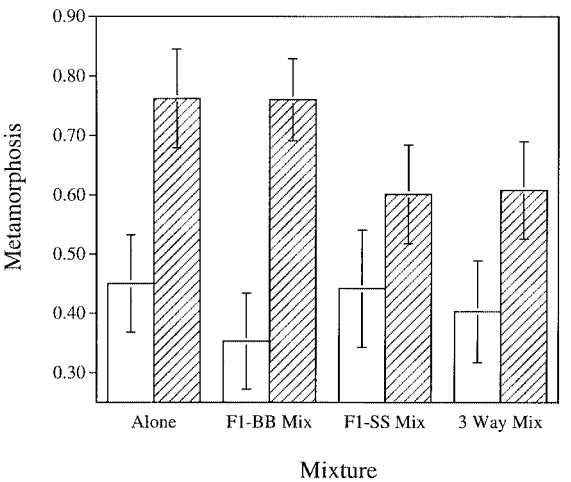

Fig. 3. Proportion of survivors metamorphosing for Texas (□) and Missouri (▨) F_1 hybrid larvae reared in experimental ponds. Values plotted are corrected least-squares means ± 1 standard error when reared in single-genotype populations (Alone) and mixtures ($F_1 = F_1$ hybrids; BB = *R. blairi*; SS = *R. sphenocephala*).

inferior performance relative to their parental genotypes for some life-history traits, they did not demonstrate a unilateral performance decrease for all traits in all mixtures, or differ significantly from Missouri F_1 hybrids for all traits (Table 1; Figs 1–3). Selection, therefore, may not be directional against F_1 hybrids in the southern region of this hybrid zone, but rather depend on the traits being measured and the genotypic make-up of the larval community and thus the competitive and resource environment (Parris, 1999).

Genetic analyses of species interactions in northern Texas indicate a differentiation of diagnostic allozyme characters over a distance of 5 km (R. Sage, unpublished data). Such a sharp genomic transition could reflect a severe reduction in hybrid fitness caused by recombination and subsequent disruption of parental gene complexes (i.e. endogenous selection). However, despite performing less well than their parental genotypes for most traits, Texas F_1 hybrids did not suffer performance decrements in all treatments, suggesting endogenous selection may not be important in determining larval performance. Natural selection could also be mediated by an environmental cline (i.e. exogenous selection) such that each parental species is most fit in its native habitat. The hybrid zone thus occurs in a transitional habitat, or ecotone, between two distinct habitat types (Remington, 1968), where hybrid fitness could be relatively high, or all genotypes may be maladapted to the transitional habitat (Moore, 1977; Barton and Hewitt, 1985). Exogenous selection can be used to explain strong associations between genotypes and putative ecological variables (e.g. Harrison and Rand, 1989; Arnold and Bennett, 1993). Even though such correlations may be useful in detecting potential selective gradients, they do not necessarily confirm action of exogenous selection (Endler, 1986). The sharp allozyme cline between *R. blairi* and *R. sphenocephala* in Texas corresponds to a sharp boundary between major physiographic and biotic provinces. The Kansan biota inhabited by *R. blairi* is a region of mixed grass and plains habitat (Udden *et al.*, 1916). The Kansan changes on its eastern edge to the alluvial agricultural and deciduous forest soils of the Texan biota (Udden *et al.*, 1916;

Blair, 1950). Thus, hybridization occurs in an ecologically intermediate region between the predominantly plains habitat of *R. blairi* and the woodland habitat of *R. sphenocephala*. Anthropogenic disturbance caused by settlement, farming and agriculture has also altered the natural environment in this region (Sage and Selander, 1979). However, this pattern of disturbance and ecological transition is not endemic to the narrow hybrid zone because the edaphic, floristic and faunisitc transitions also occur throughout a much more extensive portion of Texas, often involving complex patterns of habitat interdigitation (Udden *et al.*, 1916). In contrast, genomic transition from *R. blairi* to *R. sphenocephala* occurs over a region only 5 km wide. It is important to note that, although the experiment reported here simulated several important biotic interactions, it probably excluded some natural environmental gradients affecting overall larval fitness in this system. Future research designs incorporating additional realistic components of natural environments would best discern the environmental factors affecting differential performance for these genotypes.

The high performance of Missouri F_1 hybrid larvae is corroborated by relative fitness determinations in previous studies from the same Missouri populations. Parris *et al.* (1999) found that F_1, backcross hybrid and parental genotypes performed at equivalent levels when reared in single-genotype populations in experimental ponds. A subsequent investigation revealed that F_1, first- and second-generation backcross hybrids had increased performance in survival, growth rate and body mass at metamorphosis when reared in mixtures with *R. blairi* or *R. sphenocephala* relative to when reared in single-genotype populations (Parris, 1999). The current study expands these results by demonstrating that Missouri F_1 hybrids performed as well in mixtures with both *R. blairi* and *R. sphenocephala* as when reared with only one parental genotype or when reared alone. Collectively, these data indicate overall selection in Missouri populations is not endogenous, and some hybrid genotypes may have a selective advantage in larval habitats containing parental genotypes.

Hybridization in Missouri leopard frog populations occurs mosaic-like throughout a 160-km^2 region of sympatry in north-central Missouri (Axtell, 1976; Sage, 1994). The large range overlap between *R. blairi* and *R. sphenocephala* in Missouri may be attributable to intermixing of two broad ecological biota. Prairie and grassland habitats of central North America expanded into northeastern Missouri and Illinois 4000 years ago (Schroeder, 1982). The range expansion of *R. blairi* into Missouri probably accompanied this broad landscape transition (Brown *et al.*, 1993). The movement of prairie habitat into Missouri disrupted the indigenous forest and broad-leafed deciduous woodland habitat of *R. sphenocephala*. Anthropogenic disturbance during the past several hundred years has also altered dramatically the environmental mosaic in Missouri (Schroeder, 1982). Thus, the landscape of the hybrid zone in Missouri contains a patchy array of habitat types characteristic of discrete habitats in which *R. blairi* and *R. sphenocephala* are found in the bulk of their respective geographic ranges. Wherever contacts occur between prairie and woodland habitats in Missouri, hybridization between *R. blairi* and *R. sphenocephala* occurs (Sage, 1994).

Implications for hybrid zone evolution in leopard frogs

Geographic variation in hybrid zone width is not uncommon, but corresponding information on relative fitness of parental and hybrid genotypes generally is lacking (Barton and Hewitt, 1985; Butlin *et al.*, 1991). Relative fitness in hybrid zones is often inferred from patterns of clinal variation in diagnostic biochemical markers or disequilibria measures, or

by sampling genotype frequency transects. These approaches can describe the population genetic structure of interacting taxa, but cannot be used to infer underlying selective forces responsible for structure. The presence of significant disequilibrium or sharp clines in character states across a hybrid zone can suggest endogenous selection is occurring, but it does not confirm the hybrid zone is entirely maintained by it. This claim was supported by Arnold and Hodges (1995), who summarized studies involving several hybrid systems thought to be paradigms of endogenous selection. They found that studies that measured fitness components directly, rather than inferred fitness by indirect methods, revealed a range of fitness responses for hybrid and parental genotypes, with the general pattern of hybrids demonstrating either equivalent fitness to both parental species or higher fitness than at least one of the parentals (see Arnold and Hodges, 1995, and references therein).

Most evidence suggests the evolutionary potential of hybridization between *R. blairi* and *R. sphenocephala* is influenced by the genotypes present in the larval environment. The relative strength of selection against hybrids is stronger in southern populations (Texas). Selection against hybrid genotypes, as demonstrated here by reduced hybrid larval viability, is strongest where ecological transitions are most abrupt and where genetic differentiation between the two parental genomes is sharpest. Although the severely reduced performance of Texas F_1 hybrids seems to indicate endogenous selection, assuming this type of selection is operating is inaccurate because hybrid performance was not uniformly reduced in all treatments. The relatively high larval performance of F_1 hybrids from Missouri populations could be an important mechanism maintaining the broad sympatric area with widespread hybridization in the northern region of this hybrid zone. There are some data to support such high relative hybrid fitness (Parris, 1999; Parris *et al.*, 1999), but other data suggest some hybrid genotypes display certain aspects of infertility or inviability (Parris *et al.*, 1999; Semlitsch *et al.*, 1999; Parris, 2000). Thus, although positive selection for some hybrid genotypes may be responsible for their distribution and evolutionary trajectory in Missouri, evidence for hybrid breakdown indicates more comprehensive analyses of relative fitness in all life-history stages are needed. For example, analysis of a hybrid zone between *R. berlandieri* and *R. sphenocephala* in central Texas revealed that hybrid genotypes were relatively successful in the larval environment but the proportion of hybrid genotypes decreased in older age classes (Sage and Selander, 1979; Kocher and Sage, 1986). This analysis suggests hybrid breakdown may be accentuated post-metamorphosis in the terrestrial environment. Similar conclusions were drawn from other *Rana* hybrid zones in Arizona (Frost and Platz, 1983). Hillis (1988) and Parris *et al.* (1999) demonstrated reduced hybrid male fertility in certain *Rana* hybrid zones, but Parris (1999) found that both primary and advanced generation hybrid males from the Missouri *R. blairi* and *R. sphenocephala* hybrid zone were as fertile as parental genotypes.

It is also important to note that, in this experiment, only primary generation (F_1) hybrids were used. If recombination disrupts internally co-adapted gene complexes (i.e. endogenous selection), hybrid dysgenesis may not be manifested until the F_2 or advanced generations (Barton and Hewitt, 1985, 1989). Thus, advanced-generation hybrids may express genomic incompatibilities between parental species more severely than F_1 hybrids. Parris (1999) demonstrated F_1 and advanced-generation hybrid genotypes from Missouri performed at equivalent levels in experimental ponds, suggesting hybrid dysgenesis by this mechanism does not occur in Missouri leopard frog populations. Analogous data from Texas leopard frog populations are not available. Nevertheless, the results of the present study clearly emphasize the importance of directly measuring multiple fitness components of hybrid

and parental genotypes from more than one location within hybrid zones to test the evolutionary significance of hybridization.

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APPENDIX

Summary of uncorrected means (± 1 standard error) of proportion of individuals surviving, body mass at metamorphosis (mg), proportion of survivors metamorphosing and length of larval period length (days) for all treatment combinations for Texas and Missouri animals

Site	Mixture	*n*	Survival	Body mass	Metamorphosis	Larval period
F_1 hybrids						
Texas	Alone	4	0.73 ± 0.058	1328.8 ± 195.7	0.49 ± 0.084	69.1 ± 1.00
Missouri	Alone	4	0.95 ± 0.058	1009.5 ± 195.7	0.71 ± 0.084	61.6 ± 1.00
Texas	F_1-BB mix	4	0.80 ± 0.058	894.8 ± 195.7	0.37 ± 0.084	66.5 ± 1.00
Missouri	F_1-BB mix	6	0.95 ± 0.047	922.5 ± 159.8	0.71 ± 0.069	59.4 ± 0.82
Texas	F_1-SS mix	4	0.50 ± 0.058	832.3 ± 195.7	0.59 ± 0.084	72.7 ± 1.00
Missouri	F_1-SS mix	4	0.94 ± 0.058	883.8 ± 195.7	0.55 ± 0.084	65.9 ± 1.00
Texas	3-way mix	5	0.56 ± 0.052	863.2 ± 175.1	0.52 ± 0.075	67.8 ± 0.89
Missouri	3-way mix	4	0.93 ± 0.058	921.8 ± 195.7	0.57 ± 0.084	62.0 ± 1.00
R. blairi						
Texas	Alone	4	0.52 ± 0.058	2149.8 ± 195.7	0.76 ± 0.084	67.8 ± 1.00
Missouri	Alone	4	0.93 ± 0.058	822.3 ± 195.7	0.35 ± 0.084	62.3 ± 1.00
Texas	BB-F_1 mix	4	0.92 ± 0.058	1135.8 ± 195.7	0.58 ± 0.084	70.3 ± 1.00
Missouri	BB-F_1 mix	6	0.90 ± 0.047	1050.7 ± 159.8	0.51 ± 0.069	63.1 ± 0.82
Texas	3-way mix	5	0.94 ± 0.052	1363.6 ± 175.1	0.49 ± 0.075	71.7 ± 0.89
Missouri	3-way mix	4	0.76 ± 0.058	1003.8 ± 195.7	0.35 ± 0.084	66.9 ± 1.00
R. sphenocephala						
Texas	Alone	4	0.77 ± 0.058	1178.0 ± 195.7	0.55 ± 0.084	69.0 ± 1.00
Missouri	Alone	3	0.94 ± 0.067	783.0 ± 226.0	0.54 ± 0.097	61.5 ± 1.15
Texas	SS-F_1 mix	4	0.74 ± 0.058	1428.0 ± 195.7	0.59 ± 0.084	68.4 ± 1.00
Missouri	SS-F_1 mix	4	0.92 ± 0.058	885.5 ± 195.7	0.72 ± 0.084	61.9 ± 1.00
Texas	3-way mix	6	0.87 ± 0.052	1274.8 ± 175.1	0.80 ± 0.075	63.8 ± 0.89
Missouri	3-way mix	4	0.98 ± 0.058	854.8 ± 195.7	0.72 ± 0.084	58.5 ± 1.00

Note: *n* = number of replicate experimental ponds for each treatment.