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Ecological selection and hybrid fitness: hybrids succeed on parental resources

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ABSTRACT

Hypothesis: Interspecific hybrids are competitively inferior to their parents because they possess intermediate phenotypes that are poorly adapted to either parent's niche.

Organisms: Spadefoot toads, *Spea multiplicata* and *S. bombifrons*.

Methods: Where they co-occur, tadpoles of *S. multiplicata* and *S. bombifrons* specialize on different resources (detritus and anostracan fairy shrimp, respectively). This pattern reflects ecological character displacement promoting divergence in resource use to minimize resource competition between these species. We asked whether hybrids between these species are competitively inferior for parental resources. We used a paired design to measure growth of hybrid tadpoles in the laboratory when reared in competition with pure-species tadpoles (the experimental treatment) versus when reared with siblings (the control). In separate tests we also measured: (1) foraging behaviour of hybrid and pure-species tadpoles; (2) facultative shifts in foraging behaviour by hybrids; and (3) competitive ability for the resource (shrimp) on which *S. bombifrons* tadpoles specialize.

Results: Hybrid tadpoles grew as well or better when reared in competition with pure-species tadpoles than when reared with siblings. In terms of foraging behaviour, hybrid tadpoles exhibited a wider range of phenotypes than either pure-species type. Hybrids therefore may be able to utilize resources of both parent species. Generally, the occurrence of novel hybrid phenotypes could prevent speciation if, contrary to the ecological selection hypothesis, the greater range in hybrid phenotypes enables them to occupy both parents' niches.

Keywords: competition, ecological speciation, hybridization, reinforcement, reproductive isolation.

INTRODUCTION

When two populations or species that have diverged in isolation come into secondary contact, the fitness consequences of hybridization between them will determine whether species (or sub-species) barriers arise and are maintained. Low hybrid fitness should lead to the evolution of pre-mating isolating barriers (e.g. mating behaviours) that reduce hybridization [i.e. reinforcement (reviewed in Servedio and Noor, 2003; Coyne and Orr, 2004)], whereas high

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fitness can lead to a persistent hybrid zone, a complete breakdown of isolating barriers between the interbreeding population, or the formation of new species from the hybrid gene pool (reviewed in Arnold, 1997). Assessing hybrid fitness is therefore critical for understanding how species barriers arise and are maintained.

Hybrid fitness can be affected by a potentially complex combination of factors (reviewed in Arnold, 1997; Coyne and Orr, 2004). Hybrids may have reduced fitness owing to genetic incompatibilities that disrupt development, reduce fertility, or decrease viability (reviewed in Arnold, 1997; Coyne and Orr, 2004). Alternatively, hybrid fitness may depend on the ecological circumstances in which they occur (Arnold, 1997). Indeed, hybrids may have reduced fitness because they possess intermediate phenotypes that are poorly adapted to either parent's niche and are therefore competitively inferior to pure-species types (Hatfield and Schluter, 1999; Rundle and Whitlock, 2001; Rundle and Nosil, 2005). This ecological selection hypothesis generally assumes that traits associated with resource use exhibit additive genetic variance (Rundle and Whitlock, 2001).

Traits associated with resource use do not always exhibit additive genetic variance, however. For example, hybrid phenotypes may resemble those of one parental type [as when traits are mediated by dominance (e.g. Forister, 2005)] or the maternal species (as when traits are mediated by maternal effects). Thus, contrary to the notion that hybrids may be unable to succeed in either parental species' niche, hybrid performance may be similar to that of the parent whose phenotype they most resemble, and hybrids might successfully acquire resources in that parental species' niche.

Moreover, hybrids may express phenotypes outside the range of either parent (reviewed in Rieseberg *et al.*, 1999; Seehausen, 2004). Additive genetic effects may result in the expression of novel phenotypes in advanced generation hybrids, and epistasis or pleiotropy can produce novel variants in F1 hybrids. Such variation may enable hybrids to perform as well, or better than, pure-species types in the pure species' niches and may even allow hybrids to invade and succeed in new habitats (Arnold, 1997; Rieseberg *et al.*, 1999; Seehausen, 2004).

We experimentally evaluated the fitness of hybrid tadpoles of plains spadefoot toads, *Spea bombifrons*, and Mexican spadefoot toads, *S. multiplicata*, when competing with pure-species tadpoles for the resources on which each species specializes. Although factors other than resource competition affect hybrid fitness (described below), spadefoots offer an excellent system in which to evaluate the impact of resource competition on hybrid fitness. As we describe below, competition has promoted divergent resource use by the two species where they co-occur, and we hypothesized that hybrids may have reduced fitness if they are unable to compete effectively for the resources on which each parent species specializes.

Study system and background information

Spea multiplicata and *S. bombifrons* co-occur and naturally hybridize in the southwestern USA (Sattler, 1985; Simovich, 1985, 1994; Pfennig and Simovich, 2002; Pfennig, 2003). Although hybridization between these two species has been high historically [for example, in ponds in southeastern Arizona, as many as 40% of tadpoles were F1 hybrids (Simovich, 1985)], the frequency of F1 hybrids in these same areas has decreased in the past 30 years (Pfennig, 2003). This decrease in incidence of hybridization appears to reflect reinforcement (Pfennig, 2000, 2003) – the evolution of enhanced reproductive isolation in response to selection against hybrids (Dobzhansky, 1940; reviewed in Servedio and Noor, 2003; Coyne and Orr, 2004).

Reinforcement may have occurred in the spadefoots because hybrids potentially suffer reduced fitness, at least in part, due to genetic incompatibilities: hybrid males may be sterile, and females appear to have reduced fecundity (Simovich, 1985; Simovich *et al.*, 1991; but see Forester, 1975). Moreover, survival of hybrid tadpoles of *S. multiplicata* females is lower than that of pure *S. multiplicata* tadpoles [although hybrid offspring of *S. bombifrons* females do not suffer the same effect (Pfennig and Simovich, 2002)]. As a consequence of reduced hybrid fitness, mating behaviours appear to have evolved that can preclude hybridization (Forester, 1973; Pierce, 1976; Pfennig, 2000). In particular, *S. multiplicata* females in sympatry, unlike *S. multiplicata* females in allopatry, prefer males whose calls are distinct from *S. bombifrons* (Pfennig, 2000).

In this study, we wished to determine whether competition for parental species' resources could generate reduced hybrid fitness and thereby contribute to reinforcement between these species. As we describe below, resource competition has generated divergence in resource use by tadpoles of *S. multiplicata* and *S. bombifrons* where they co-occur [i.e. they have undergone ecological character displacement (Pfennig and Murphy, 2000, 2002, 2003; Pfennig and Pfennig, 2005; Pfennig *et al.*, 2006, in press)], and hybrids may suffer reduced fitness if they cannot compete with pure-species types.

Tadpoles of *S. multiplicata* and *S. bombifrons* potentially develop into either a 'carnivore morph', which specializes on anostracan shrimp and other tadpoles, or an 'omnivore morph', which specializes on detritus (Pomeroy, 1981). Carnivores are induced when a tadpole eats shrimp (Pomeroy, 1981; Pfennig, 1990). Although the two species produce similar frequencies of these two morphs in allopatry, they differ markedly in sympatry: when they co-occur, *S. bombifrons* develop mostly into carnivores, whereas *S. multiplicata* develop mostly into omnivores (Pfennig and Murphy, 2000, 2002, 2003).

Competition between species for food has likely driven this divergence in morph production (Pfennig and Murphy, 2000, 2002, 2003; Pfennig and Pfennig, 2005; Pfennig *et al.*, 2006, in press). *Spea multiplicata* tadpoles have significantly reduced fitness when feeding on shrimp in the presence of *S. bombifrons*, whereas *S. bombifrons* tadpoles have significantly reduced fitness when feeding on detritus in the presence of *S. multiplicata* (Pfennig and Murphy, 2000; Pfennig *et al.*, 2006, in press). These differences in success may be mediated in part by species-specific differences in foraging behaviour: *S. bombifrons* omnivores spend more time swimming in the water column, whereas *S. multiplicata* omnivores spend more time grazing (Pfennig and Murphy, 2000). Presumably, increased time swimming means that *S. bombifrons* tadpoles are more likely to encounter and eat shrimp, thereby triggering development of the carnivore morphology (Pfennig and Murphy, 2000).

If hybrids between *S. multiplicata* and *S. bombifrons* are intermediate between pure-species types in resource use (or the foraging behaviours that mediate resource use), they should suffer reduced fitness when competing with *S. multiplicata* for detritus or when competing with *S. bombifrons* for shrimp. Preliminary evidence indicates that hybrids may resemble *S. bombifrons*, however. Regardless of which species is maternal, hybrids are more similar to *S. bombifrons* in their propensity to express the carnivore morphology (D.W. Pfennig, unpublished data). Thus, contrary to the expectation that hybrids will be unable to succeed on either parental species' resources, hybrids, like *S. bombifrons*, also may compete well for shrimp, but poorly for detritus.

Our goals in this study were threefold. First, we evaluated whether hybrid tadpoles experienced reduced growth in competition with pure-species tadpoles. Second, we determined if hybrids differ from the pure-species tadpoles in foraging behaviour. We did so because differences in foraging behaviour between *S. multiplicata* and *S. bombifrons* are associated with differences in resource specialization (see above; Pfennig and Murphy, 2000). If hybrids were intermediate in foraging behaviour, such a pattern could result in hybrids being inefficient at feeding on either pure species' specialty resource. Finally, we assessed whether hybrids are effective at competing for shrimp with *S. bombifrons* tadpoles. We focused on competitive ability for shrimp because it is a more nutritious resource than detritus. Indeed, feeding on shrimp as opposed to detritus appears to enhance development rate and size at metamorphosis (Pfennig and Pfennig, 2005). By evaluating competitive ability for shrimp, we could assess the ability of hybrids to take advantage of this superior resource.

MATERIALS AND METHODS

Production of pure species and hybrid offspring

Spadefoot toads were collected in sympatry near Portal, Arizona, USA and returned to the University of North Carolina, Chapel Hill. We bred these toads in the laboratory to create four types of offspring: pure *S. bombifrons* tadpoles (BB offspring); hybrid offspring for whom *S. bombifrons* was maternal (BM offspring); hybrid offspring for whom *S. multiplicata* was maternal (MB offspring); and pure *S. multiplicata* offspring (MM offspring).

To induce breeding, adults were injected with 0.07 ml of 0.01 $\mu g \cdot ml^{-1}$ GnRH agonist. Males and females were placed as pairs in separate aquaria with 10 litres of dechlorinated water and allowed to oviposit. Once oviposition was complete, the adults were removed from the aquaria and the developing eggs were aerated until the tadpoles hatched and were free-swimming. Eight days after the breeding, we placed a subset of 200 tadpoles from each sibship into separate aquaria to control for differences in density among the sibships. These were fed *ad libitum*. Because availability of *S. bombifrons* and *S. multiplicata* differed, and because not all pairs oviposited, we generated a total of three sibships of BB tadpoles, five sibships of BM tadpoles, six sibships of MB tadpoles, and 10 sibships of MM tadpoles for use in the experiments below. Although we only had three BB sibships, the sibships responded similarly in the experiments described below (i.e. no BB sibship acted as an outlier to bias the results).

Hybrid growth in competition with pure-species tadpoles

Using four pair-wise comparisons, we measured hybrid tadpole growth in competition with pure-species tadpoles for the resource on which each pure-species type specializes (i.e. detritus for *S. multiplicata* and shrimp for *S. bombifrons*). We compared growth of:

1. BM tadpoles reared with siblings versus BM tadpoles reared with MM tadpoles on a detritus diet;
2. MB tadpoles reared with siblings versus MB tadpoles reared with MM tadpoles on a detritus diet;
3. BM tadpoles reared with siblings versus BM tadpoles reared with BB tadpoles on a shrimp diet; and
4. MB tadpoles reared with siblings versus MB tadpoles reared with BB tadpoles on a shrimp diet.

Rearing the hybrids with a sibling served as a control for density effects while allowing us to measure hybrid growth in the absence of pure-species competitors. Any differences between tadpoles reared with siblings versus those reared with pure-species tadpoles could therefore be ascribed to the effects, if any, of competition with pure-species tadpoles for the resource on which the pure-species tadpoles specialized. Thus, this paired design allowed us to isolate the effects of competition with pure-species types from intrinsic genetic factors that also could suppress growth in hybrids [for full discussion of intrinsic versus extrinsic effects on hybrid fitness, see Hatfield and Schluter (1999), Rundle and Whitlock (2001), and Coyne and Orr (2004)].

To assess growth of BM and MB tadpoles in competition with MM tadpoles for a detritus diet, one of two hybrid tadpoles (the focal animals) was reared in the presence of a sibling and the other tadpole was reared in the presence of a pure-species tadpole. These focal animals were size-matched for snout-to-vent length. We began the experiment when tadpoles were 25 days old and large enough to mark. Hybrid tadpoles (the focal animals) were marked using a 26-gauge hypodermic needle to inject fluorescent coloured elastomer (Northwest Marine Technology, Inc.) into the dorsal tail membrane of each tadpole. These marks are readily observed under ultraviolet light and do not affect tadpole growth or survival (Pfennig and Murphy, 2000).

Stimulus animals (i.e. a sibling of the focal animal or a pure-species tadpole) were matched for snout-to-vent length to each other and to the focal animals. Tadpole pairs were placed in containers (34 × 21 × 11.5 cm) filled with 5 litres of water. Tadpoles were fed 20 mg of ground-up high-protein fish food every other day for 17 days. Fish food mimics naturally occurring detritus in form and nutrition (Pfennig *et al.*, 1991). At the end of the experiment, each tadpole was patted dry, its mass taken, and its snout-to-vent length measured with digital calipers.

We used the same design as above to evaluate growth of BM and MB tadpoles in competition with BB tadpoles. Here, however, tadpoles were reared exclusively on shrimp (the resource for which *S. bombifrons* is the superior competitor) rather than detritus. Tadpoles were given live brine shrimp nauplii (*Artemia* sp.; 1–2 mm in length) suspended in 20 ml of dechlorinated water daily for 17 days.

For both experiments, food levels were used that have been shown to promote competition between tadpole pairs of these species (Pfennig and Murphy, 2000, 2002; Pfennig *et al.*, in press).

We set up 50 replicate pairs for each of the four comparisons. If one of the four tadpoles in a replicate died, that replicate was excluded from subsequent analyses. Our measures of growth were snout-to-vent length (SVL) and condition. To obtain condition, we used a cubic regression of our tadpole mass measures on SVL and saved the residuals ($F_{3,384} = 1084.92$, $P < 0.0001$, $R^2 = 0.89$; the residuals were normally distributed and were entirely independent of SVL: $r = 0.0$, $n = 388$, $P = 1.0$). These residuals were operationally defined as ‘condition’ because positive values of these residuals indicated that a tadpole was more massive than expected from the relationship between mass and SVL (and therefore likely had higher fat or energy reserves), whereas negative values of this measure indicated that a tadpole was less massive than expected from the relationship between mass and SVL.

To analyse the data, we took the difference in SVL and condition between the focal hybrid with the sibling and the focal hybrid with the pure-species tadpole within each replicate. We compiled these data separately for each of the four different comparisons. We used *t*-tests (with a Bonferroni-corrected alpha level of 0.006 to minimize group-wide type I error) to compare differences in these measures to a null expectation of zero. All of our data met parametric assumptions.

Foraging behaviour of hybrids

Differences in competitive ability between *S. multiplicata* and *S. bombifrons* are mediated in part by differences in foraging behaviour: *S. multiplicata* tadpoles (the superior detritivores) spend more time grazing than do *S. bombifrons* tadpoles, whereas *S. bombifrons* tadpoles (the superior carnivores) spend more time swimming than do *S. multiplicata* tadpoles (Pfennig and Murphy, 2000). We therefore assessed how BM and MB hybrid behaviour compared with that of MM and BB tadpoles. Our goal was to determine if any differences between pure-species types and hybrids in competitive ability might reflect differences between them in foraging behaviour.

To measure the foraging behaviour of BM and MB tadpoles versus MM and BB tadpoles, we performed focal observations of single tadpoles in the presence of both detritus and shrimp. A total of 20 tadpoles from each treatment group were randomly selected without replacement from all of the available sibships (3 BB, 10 MM, 6 MB, and 5 BM). We measured each tadpole's snout-to-vent length and then placed it in an observation tank (a rectangular container 40 × 27 × 14.5 cm filled with 10 litres of dechlorinated water). The water temperature for all observations was 21°C. At the bottom of the tank, we placed a 3-cm deep layer of sand on top of which was approximately 40 mg of crushed cichlid food to mimic detritus. We also added five brine shrimp that were approximately 5 mm long and allowed to swim freely throughout the tank; these shrimp mimic naturally occurring shrimp in size and behaviour. The detritus and shrimp added were provided simply as a stimulus to induce foraging by the tadpoles. The quantities of detritus and shrimp were not meant to be equivalent to each other or to closely mimic natural conditions (though the densities we provided can be observed in nature). The amounts were sufficiently high to provide the tadpoles with a readily perceived resource that would not be eaten entirely during the observation period.

After placing each tadpole in the observation tank, we allowed it to acclimate for 5 min, and then recorded its behaviour continuously for 10 min. Specifically, we noted how much time each tadpole spent swimming in the water column, grazing (either at the water surface or on the substrate), or resting (remaining stationary on the tank bottom). Observations were performed in blocks of four tadpoles consisting of one tadpole from each treatment group randomly chosen from among the different sibships. Within a block, all tadpoles were matched for snout-to-vent length so that there were no size differences among the different treatment groups. All observations were conducted in the same room under the same white fluorescent lighting.

To analyse these data, we calculated the proportion of time that tadpoles spent resting and compared this among treatment groups to assess whether BM and MB tadpoles spent similar time foraging to pure-species tadpoles. We next assessed what proportion of time the tadpole spent swimming out of the total time it was active (i.e. either grazing or swimming). We compared this measure among treatment groups to evaluate whether BM and MB tadpoles were similar to pure-species tadpoles in foraging behaviour. Previous work suggests that greater time spent swimming is indicative of carnivorous tadpoles that are more likely to feed on shrimp (Pomeroy, 1981). Moreover, because the proportion of time a tadpole was active equalled the proportion of time it spent swimming plus the proportion of time it spent grazing, the results for the proportion of time the animals spent grazing mirrored the results for the proportion of time the animals spent swimming.

We used analysis of covariance to determine if hybrids and pure-species tadpoles differed in foraging behaviour. Tadpole body size was included as a covariate. Where a significant, or near significant (Zar, 1984), overall effect was detected, we used a Tukey HSD test to contrast pure-species tadpoles and hybrids. Proportion data were arcsine square root transformed to meet parametric assumptions.

In addition to measuring tadpole behaviours in isolation of competitive interactions, we also examined the possibility that hybrid tadpoles alter their behaviour in the presence of pure-species tadpoles during competition for food or when foraging on the different resources. We conducted four different behavioural tests using a paired design in which individual tadpole behaviours were observed with a sibling and separately with a pure-species tadpole in the presence of that pure-species tadpole's specialty resource. The four tests were as follows:

1. MB tadpoles observed with a sibling or a *S. multiplicata* tadpole in the presence of detritus;
2. BM tadpoles observed with a sibling or a *S. multiplicata* tadpole in the presence of detritus;
3. MB tadpoles observed with a sibling or a *S. bombifrons* tadpole in the presence of shrimp; and
4. BM tadpoles observed with a sibling or a *S. bombifrons* tadpole in the presence of shrimp.

Each test was replicated 20 times using tadpoles randomly chosen without replacement from the available sibships for a given treatment group.

We placed a focal hybrid in a rectangular container (13 × 18 × 8.5 cm) filled with approximately 1 litre of dechlorinated water. We then placed either a sibling or a pure-species tadpole in the container with the focal tadpole. Both stimulus tadpoles were matched for snout-to-vent length to the focal tadpole. We allowed the pair to acclimate for 3 min, and then observed the focal tadpole for 10 min, noting whether it was swimming, grazing or resting (as above). Once the observation interval was over, we then placed the focal tadpole with the alternate tadpole type (whether the tadpole was initially placed with a sibling or a pure-species tadpole was randomly determined) and repeated the procedure.

For paired observations where the contrast was behaviour with a sibling versus behaviour with a *S. multiplicata* tadpole, we placed 40 mg of ground fish food (to simulate detritus) in the container and allowed it to settle before either tadpole was introduced to the observation container. For paired observations where the contrast was behaviour with a sibling versus behaviour with a *S. bombifrons* tadpole, we placed seven live brine shrimp (5 mm in length) in the container before adding the tadpoles. We used seven shrimp in this experiment, because doing so provided a high enough density to provide a resource stimulus to the animals that would not be eaten entirely during the observation period.

As above, we measured the proportion of the observation time that the tadpole was active and the proportion of the active time that the tadpole spent swimming. To determine if the BM or MB tadpoles altered their behaviour in the presence of pure-species tadpoles versus when they were with siblings, we took the difference in these measures as those observed with the sibling minus those observed with the pure-species tadpole for each tadpole. We used *t*-tests to determine if these differences were significantly different from a null expectation of zero. We used a Bonferroni-corrected alpha level of 0.006 to minimize group-wide type I error.

Competition trials between hybrids and pure-species tadpoles

In addition to the above behavioural trials, we also determined whether hybrids could outcompete *S. bombifrons* for shrimp. To do so, we placed a single hybrid tadpole and a single *S. bombifrons* tadpole (matched for snout-to-vent length) in a rectangular container (13 × 18 × 8.5 cm) filled with approximately 1 litre of dechlorinated water. We allowed tadpoles to acclimate for 10 min. We then introduced three live brine shrimp (approximately 5 mm in length) into the container. We observed which tadpole ate the shrimp until 30 min had elapsed or until one tadpole had eaten two shrimp, whichever came first. The tadpole that ate two of the three shrimp was deemed the ‘winner’ of the competitive trial. Trials in which neither tadpole ate or in which each tadpole ate only one shrimp (i.e. ties) were excluded from the analysis. We used three shrimp in this trial to maximize the possibility that the tadpoles would eat all the shrimp during the observation period and thereby minimize the likelihood of ties.

We replicated this procedure 36 times for the BM hybrids and 43 times using the MB hybrids. No tadpole was used more than once in each experiment. For each experiment (using either the BM or MB tadpoles), we used a log-likelihood chi-square test with the null expectation that hybrids would be equally likely as the *S. bombifrons* tadpoles to win in competition for shrimp.

RESULTS

Hybrid growth in competition with pure-species tadpoles

We found no evidence that BM or MB tadpoles suffer reduced growth in competition with MM or BB tadpoles. Both hybrid types grew as well or better when in competition with a pure-species tadpole than when reared with a sibling. When reared on detritus in competition with MM tadpoles, BM hybrids had a larger snout-to-vent length than BM tadpoles reared with a sibling (Table 1). The BM tadpoles reared in competition with MM tadpoles

Table 1. Differences in hybrid growth and condition in the presence of a sibling versus a pure-species tadpole

Hybrid type	Species, diet	Mean difference in SVL, sib–pure species (S.E.M.)	$t(N)$	Mean difference in condition, sib–pure species (S.E.M.)	$t(N)$
BM	MM, detritus	−0.78 (0.19)	−4.09 (48)**	−0.02 (0.02)	−1.06 (48)
MB	MM, detritus	−0.39 (0.25)	−1.55 (46)	0.003 (0.01)	0.18 (46)
BM	BB, shrimp	−0.13 (0.23)	−0.54 (48)	−0.01 (0.02)	−0.80 (48)
MB	BB, shrimp	−0.18 (0.17)	−1.06 (49)	−0.03 (0.01)	−2.70 (49)*

Note: The null hypothesis was that mean differences equal zero. All contrasts, except those indicated, are not statistically significant ($P > 0.05$). Our alpha level was 0.006, which was Bonferroni-corrected for the number of tests performed. S.E.M. = standard error of the mean.

* $P = 0.005$; ** $P < 0.001$.

did not differ in condition from BM tadpoles reared with a sibling, however (Table 1). The MB tadpoles reared on detritus in competition with MM tadpoles did not differ from MB tadpoles reared with a sibling in either snout-to-vent length or condition (Table 1).

We found similar results for BM and MB tadpoles reared on shrimp in competition with *S. bombifrons* (BB) tadpoles. The BM tadpoles reared with BB tadpoles did not differ in growth or condition from BM tadpoles reared with a sibling (Table 1). The MB tadpoles reared with BB tadpoles also did not differ from MB tadpoles reared with a sibling in snout-to-vent length (Table 1). The MB tadpoles were in better condition, however, when reared in competition with BB tadpoles than when reared with a sibling (Table 1).

Foraging behaviour of hybrids

When we compared foraging behaviour among BM, MB, BB, and MM tadpoles, we found a marginal effect of tadpole type on time spent resting ($F_{3,72} = 2.43$, $P = 0.07$). A Tukey HSD multiple comparisons test revealed that *S. bombifrons* tadpoles spent significantly more time resting than did *S. multiplicata* tadpoles. Both hybrid types were intermediate between BB and MM tadpoles in time spent resting, but they were not significantly different from either pure-species type.

The four types of tadpoles differed significantly in the proportion of active time (i.e. when the tadpoles were either grazing or swimming) that the different tadpole types spent swimming ($F_{3,72} = 2.68$, $P = 0.05$). A Tukey HSD test revealed that *S. bombifrons* tadpoles spent significantly more time swimming than *S. multiplicata* tadpoles (Fig. 1), a result that

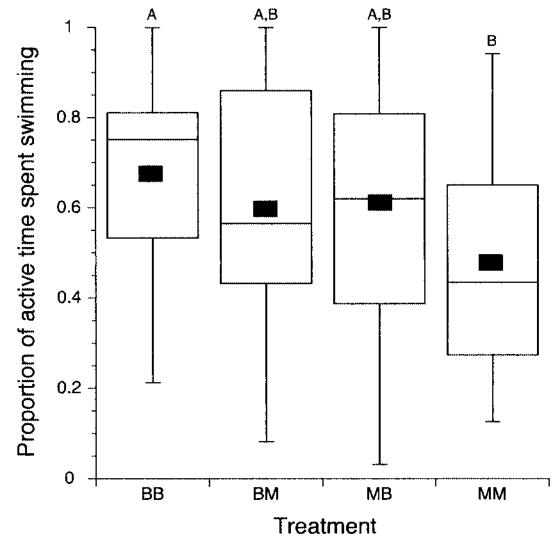

Fig. 1. Distribution of proportion of active time spent swimming (an indication of carnivorous behaviour) compared among the tadpole types. The lines in the centre of boxes show medians. Lower and upper edges of boxes show 25th and 75th quartiles, respectively. Whiskers show ranges for these data. Solid black rectangles depict means for each group. Different letters denote significantly different means (see also text). Because 100% of a tadpole's activity time equals the percentage of time spent swimming plus the percentage of time spent grazing, the proportion of time the animals spent grazing is the converse of the data shown here.

has been observed previously (Pfennig and Murphy, 2000). The BM and MB tadpoles were not significantly different from either pure-species type in time spent swimming, although time spent swimming for each hybrid type was more similar to that of *S. bombifrons* tadpoles (Fig. 1).

For most comparisons, we found no evidence that hybrids altered their foraging behaviour depending on whether they were in the presence of a sibling or a pure-species tadpole. The BM hybrids did not alter their swimming or resting behaviour in the presence of MM or BB tadpoles (Table 2). The MB hybrids also exhibited no difference in resting or swimming behaviour in the presence of MM tadpoles (Table 2). The MB tadpoles showed no differences in resting behaviour in the presence of BB tadpoles. We did find, however, that MB tadpoles spent less of their active time swimming in the presence of BB tadpoles than in the presence of siblings (Table 2).

Competition trials between hybrids and pure-species tadpoles

When BM and MB tadpoles were placed in direct competition for shrimp with BB tadpoles, we found that the hybrid tadpoles were better than or as good as BB tadpoles at capturing shrimp. In 23 of 32 trials in which there was a winner, the BM tadpole was the winner, a pattern that was significantly different from random 1 : 1 expectation (log-likelihood ratio $\chi^2_1 = 6.34$, $P < 0.01$). In contrast, in 18 of 36 trials in which there was a winner, MB tadpoles won in competition for shrimp with BB tadpoles, a pattern that was not significantly different from random 1 : 1 expectation. The BM and MB hybrids were not significantly different from each other in their likelihood of winning in competition for shrimp with BB tadpoles (log-likelihood ratio $\chi^2_1 = 2.63$, $P = 0.11$).

DISCUSSION

Spea hybrid tadpoles are apparently not at a competitive disadvantage relative to pure-species tadpoles. Indeed, BM and MB hybrids grew as large or larger in competition with pure-species tadpoles than when reared with siblings. Moreover, hybrids were as good or better at winning competitive interactions for shrimp with *S. bombifrons* (the species that is

Table 2. Differences in hybrid foraging behaviour in the presence of a sibling versus a pure-species tadpole

Hybrid type	Species type	Mean difference, sib–pure species, % time resting (S.E.M.)	t_{19}	Mean difference, sib–pure species, % active time swimming (S.E.M.)	t_{19}
BM	BB	2.2 (3.6)	0.73	−1.9 (4.2)	−0.96
BM	MM	−2.1 (4.9)	−0.36	−2.5 (7.4)	0.58
MB	BB	2.6 (3.2)	0.94	9.1 (2.6)	3.42*
MB	MM	3.7 (3.7)	1.04	3.0 (5.0)	0.45

Note: The null hypothesis was that mean differences equal zero. Actual means and errors are reported; statistical analyses performed on arcsine square root transformed data. All contrasts, except that indicated, were not statistically significant ($P > 0.05$). Our alpha level was 0.006, which was Bonferroni-corrected for the number of tests performed. S.E.M. = standard error of the mean.

* $P = 0.003$.

the superior competitor for shrimp). Thus, although hybrids may have reduced fitness relative to pure-species types because of other factors [e.g. reduced fertility (Simovich, 1985; Simovich *et al.*, 1991)], they are apparently not selectively disadvantaged when competing with pure-species tadpoles for resources. We suggest below that whether hybrids generally are at a competitive disadvantage may depend on the genetic architecture of traits associated with foraging, the range of phenotypes expressed by hybrids relative to pure species, or the interaction of different traits that contribute to successful resource use.

Although hybrids generally may be competitively inferior to pure-species types for a variety of reasons (e.g. because they show greater fluctuating asymmetry or other phenotypic anomalies that reduce competitive ability), ecologically mediated selection against hybrids often rests on the idea that hybrids will be competitively inferior to pure-species types because they possess intermediate phenotypes that are poorly adapted to either parent species' niche (Hatfield and Schluter, 1999; Schluter, 2000; Rundle and Whitlock, 2001; Rundle and Nosil, 2005). Consistent with this idea, we found that, on average, both types of hybrids were intermediate between pure-species tadpoles in the overall time they spent foraging and in the time they spent swimming (or grazing). We also found, however, that BM and MB hybrids more closely resembled *S. bombifrons* tadpoles. On average, both types of hybrids spent about 60% of their time swimming, which was more similar to the time spent swimming by *S. bombifrons* (67% of the time) than by *S. multiplicata* tadpoles (47% of the time; Fig. 1). This resemblance between hybrids and *S. bombifrons* in foraging behaviour could explain why hybrids did not suffer reduced growth when competing with *S. bombifrons* for shrimp. Generally, if dominance or maternal effects generate resemblance between hybrids and one of the pure-species types, they may succeed in exploiting the resources of that parental species.

The observed pattern of foraging behaviour does not fully explain, however, why MB hybrids were equally likely, and BM hybrids more likely, to win competitive trials for shrimp with *S. bombifrons* (see Results). One possible explanation for these results is that hybrids were intermediate between *S. bombifrons* and *S. multiplicata* in how much time they were active. The greater (although not statistically significant) tendency of hybrids to be active, coupled with their similarity to *S. bombifrons* in proportion of activity time spent swimming, could have contributed to their ability to succeed in competition with *S. bombifrons*. Generally, whether hybrids succeed relative to pure-species types may depend on how different traits combine in hybrids to affect successful resource use (Forister, 2005).

When *S. bombifrons* and *S. multiplicata* tadpoles compete for resources, they trade-off successful competitive ability on one resource with poor performance on the alternate resource: *S. bombifrons* performs poorly on detritus in the presence of *S. multiplicata*, whereas *S. multiplicata* performs poorly on shrimp in the presence of *S. bombifrons* (Pfennig and Murphy, 2000, 2003). Such trade-offs are often expected when competition drives ecological divergence of species' niches (reviewed in Schluter, 2000; Rundle and Nosil, 2005). The findings that hybrids were similar to *S. bombifrons* in swimming time and were effective competitors for shrimp suggest that hybrids, like *S. bombifrons*, should compete well for shrimp but poorly for detritus. Surprisingly, this was not the case. Hybrids did not suffer reduced growth when competing with *S. multiplicata* tadpoles for detritus.

One explanation for this lack of a trade-off in how well hybrids performed on the two resources is that variation in hybrid phenotypes allowed the hybrids to compete successfully for both shrimp and detritus. Both the BM and MB tadpoles expressed a wider range of foraging behaviour than either pure-species type (Fig. 1), in that some hybrid tadpoles spent all of their active time swimming (as occurred with some *S. bombifrons* tadpoles), whereas some hybrid tadpoles spent less time swimming (and therefore more time grazing) than *S. multiplicata*. Thus, hybrids expressed phenotypes associated with both parental species' niches. Doing so may have enabled individual hybrids to do well on different resources (e.g. hybrids that spent more time swimming did well on shrimp, whereas hybrids that spent more time grazing did well on detritus). As a group, therefore, hybrids may not have faced the trade-offs in performance on each resource type that each pure species experiences when competing with the other species. Thus, contrary to the expectation that hybrids might fall between the parental species' niches, hybrids potentially can succeed at resource acquisition in both parents' niches provided hybrids express a range of phenotypes capable of exploiting alternative resources.

Our findings suggest that resource competition among tadpoles does not strongly disfavour the production of hybrids in this system. Yet, in the southeastern Arizona populations where our study animals were collected, hybridization has declined over the last 30 years (Pfennig, 2003). Also, mating behaviours by *S. multiplicata* have apparently evolved that preclude hybridization (Pfennig, 2000; Pfennig *et al.*, 2000; Pfennig and Pfennig, 2005). Presumably, low hybrid fitness stemming from genetic incompatibilities that contribute to reduced fecundity in adult hybrids (Simovich, 1985, 1994; Simovich *et al.*, 1991; Pfennig and Simovich, 2002; but see Forester, 1975) has promoted the evolution of mating behaviours that enhanced reproductive isolation between these species [i.e. the two species have undergone reinforcement (Pfennig, 2003)].

Generally, ecological selection, whereby hybrids are unable to occupy either parental lineage's niche, is also thought to be a potentially critical selective process promoting reinforcement. Yet, whether hybrids are selectively disfavoured could depend on the interaction between the genetic architecture underlying the traits associated with resource use and the ecological conditions in which those traits are expressed. Indeed, hybrids may express novel phenotypes relative to those expressed by either pure-species type (Rieseberg *et al.*, 1999; Seehausen, 2004). If the range of phenotypes expressed by hybrids allows them to thrive in both species' niches, speciation may be precluded by favourable introgression between the two parental lines (in the absence of genetic incompatibilities). Variation in hybrid phenotypes and the fitness consequences of this variation may therefore determine when or whether ecologically mediated speciation will occur.

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