

HYBRIDIZATION

Female toads engaging in adaptive hybridization prefer high-quality heterospecifics as mates

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Hybridization—interbreeding between species—is generally thought to occur randomly between members of two species. Contrary to expectation, female plains spadefoot toads (*Spea bombifrons*) can increase their evolutionary fitness by preferentially mating with high-quality males of another species, the Mexican spadefoot toad (*Spea multiplicata*). Aspects of Mexican spadefoot males' mating calls predict their hybrid offspring's fitness, and plains spadefoot females prefer Mexican spadefoot males on the basis of these attributes, but only in populations and ecological conditions where hybridization is adaptive. By selecting fitness-enhancing mates of another species, females increase hybridization's benefits and exert sexual selection across species. Nonrandom mating between species can thereby increase the potential for adaptive gene flow between species so that adaptive introgression is not simply happenstance.

Mating between species (hybridization) is widespread and has important evolutionary and ecological consequences (1–3). Although hybridization is often considered deleterious, it is increasingly recognized as potentially fitness enhancing if mates of one's own species (i.e., conspecifics) are limited (4) or if hybrids are better adapted to their environment than pure-species types (5, 6).

When hybridization is adaptive, selection can favor the evolution of traits that foster hybridization. Such traits can include mating preferences for members of a different species (i.e., heterospecifics) (4, 7, 8). However, the quality of heterospecific mates might vary such that mating with some heterospecifics versus others might result in higher fitness. Thus, mate choice among heterospecifics could allow individuals to enhance their fitness in the same way as mate choice among conspecifics (9). In such cases, sexual selection could operate between species—a possibility that has not been previously considered.

We tested the hypothesis that females exercise adaptive mate choice among members of another species by using plains spadefoot toads, *Spea bombifrons*. In the southwestern United States, this species hybridizes with Mexican spadefoot toads, *Spea multiplicata* (10). Although the resulting F₁ hybrid males are sterile and F₁ hybrid females have reduced fecundity, F₁ hybrid females can breed back to either parent species (10).

Hybridization is maladaptive for *S. multiplicata* females, but it is sometimes adaptive for *S. bombifrons* females. Spadefoot tadpoles develop in ephemeral desert ponds that often dry before the tadpoles metamorphose, resulting

in their deaths (8). Because hybrid tadpoles develop faster than pure *S. bombifrons* tadpoles, hybrids are more likely to escape drying ponds (8), thereby potentially passing on alleles from their parents to future generations. Consequently, female *S. bombifrons* have evolved mate preferences for *S. multiplicata* males but only in shallow, ephemeral ponds (8, 10); when

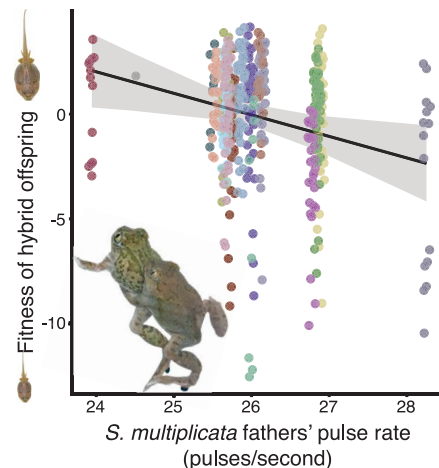


Fig. 1. Hybrid-tadpole fitness as a function of father's call. Tadpoles resulting from *S. bombifrons* females crossed with *S. multiplicata* males (inset) had the highest fitness when their fathers had calls with slow pulse rates. The fitness of hybrid offspring is the first principal component from a principal components analysis of body size, body mass, and developmental stage; all variables loaded positively on the principal component, so higher values correspond to increased fitness (table S1). The line is the predicted fitness at given pulse rates using the averaged model (Table 1) while holding all other variables at mean values; intervals are 95% confidence intervals. Different colors denote different families, and points are jittered horizontally. Tadpole photos are strictly visual representations of the variation in tadpole fitness.

a pond is deep and likely to last long enough for *S. bombifrons* tadpoles to successfully metamorphose, *S. bombifrons* females prefer males of their own species (8).

To determine if *S. bombifrons* females could exercise adaptive mate choice among *S. multiplicata* males, we first determined if the fitness consequences vary for *S. bombifrons* females mated with different *S. multiplicata* males. We then evaluated whether any aspects of *S. multiplicata* male calls (the sexual signals that females evaluate) predicted hybrid-offspring fitness. We reasoned that this may occur because attributes of *S. multiplicata* calls predict offspring fitness in conspecific matings (10).

We bred 20 *S. bombifrons* females with 20 *S. multiplicata* males that differed in mating call characteristics and reared their offspring (10). We measured tadpole body size [snout-to-vent length (SVL)], body mass, and Gosner stage (a generalized system describing anuran development) as fitness components (10). We then combined these measurements into a single principal component that explained 91.6% of the variation in our data (table S1).

This combined measure of fitness varied among families of hybrid offspring (Fig. 1 and table S2). Body size of the mothers positively predicted this fitness measure in hybrid offspring, but the pulse rate of the fathers' calls was an even better predictor of our fitness measure in the hybrid offspring (Table 1). Specifically, males with slower pulse rates sired the largest, heaviest, and most rapidly developing hybrid offspring (Table 1 and Fig. 1).

Because slower pulse rates of *S. multiplicata* calls predicted higher hybrid-offspring fitness, we next investigated whether *S. bombifrons* females preferred *S. multiplicata* male calls with slower versus faster pulse rates. We further predicted that if any such preferences are plastic [sensu (8)], they would be expressed only in shallow water, where hybridization is adaptive. Using previous methods (8, 10), we presented *S. bombifrons* females with a choice of *S. multiplicata* calls with either slow or fast pulse rates. The same females were given this choice under two conditions: shallow water (simulating ephemeral ponds where hybridization is advantageous) and deep water (simulating longer-lasting ponds where hybridization is disadvantageous).

As predicted, in shallow water, *S. bombifrons* females preferred *S. multiplicata* calls with slower pulse rates (exact binomial test, $P = 0.0026$; Fig. 2A). By contrast (and also as predicted), *S. bombifrons* females showed no such preference in deep water (exact binomial test, $P = 0.15$; Fig. 2A). The pattern of preference in deep water differed significantly from that in shallow water (McNemar binomial exact test, $P = 0.0081$; Fig. 2A). Thus, *S. bombifrons*

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females distinguish between different *S. multiplicata* male calls and prefer slower pulse rate calls that are associated with greater fitness in hybrid offspring (Fig. 1), but they exhibit this preference only when ponds are shallow and it is adaptive to hybridize.

Sympatric *S. bombifrons* females may prefer *S. bombifrons* males with slow pulse rates, and such preferences among conspecifics may be simply expressed when choosing among *S. multiplicata* males. However, we found that *S. bombifrons* females do not discriminate between conspecific calls varying in pulse rate in either shallow water (exact binomial test, $P = 1$) or deep water (exact binomial test, $P = 0.14$; Fig. 2A). Moreover, their behavior was unchanged between the two water conditions (McNemar binomial exact test, $P = 0.30$; Fig. 2A). Thus, sympatric *S. bombifrons* female preferences for males of their own species are not simply generalized to *S. multiplicata* males.

S. multiplicata females may prefer *S. multiplicata* males with slow pulse rates, and this preference might have been transferred from *S. multiplicata* into *S. bombifrons* via gene flow between the species (i.e., introgression). However, when we tested *S. multiplicata* females for their preferences for *S. multiplicata* calls that differed in pulse rate, we found no preferences in either shallow water (exact binomial test, $P = 0.49$; table S6) or deep water (exact binomial test, $P = 0.21$; table S6). Water level also did not alter female preferences (McNemar binomial exact test, $P = 0.31$). Thus, our results cannot be explained by introgression of mate preferences from *S. multiplicata* into *S. bombifrons*.

Our finding that *S. bombifrons* females prefer fitness-enhancing males of a different species suggests that these preferences evolved in sympatry (where hybridization occurs) via selection acting to optimize the fitness of their hybrid offspring. To assess whether this behavior did evolve in sympatry, we determined whether the same preferences are present in *S. bombifrons* females from allopatric populations (i.e., where they occur in the absence of *S. multiplicata*). Our rationale for doing so was that allopatric females would possess ancestral preferences that predate contact with *S. multiplicata* in the southwestern United States. The center of origin for *S. bombifrons* is the grasslands of the Great Plains, and they have apparently expanded their range into the desert Southwest, possibly because of adaptive hybridization with desert-adapted *S. multiplicata* (11, 12) (Fig. 2). Male *S. bombifrons* from the two regions produce two different call types: Males from the Great Plains produce a slow call that is more similar to *S. multiplicata* calls than to the fast call that is produced by males in the desert Southwest (Fig. 2, fig. S1, and audio S2 and S3). We presented allopatric *S. bombifrons* females with conspecific calls

of their own slow-call type that differed in pulse rate and found that they preferred slow pulse rate calls in shallow water (exact binomial test, $P = 0.029$) but not in deep water (exact binomial test, $P = 0.18$), but patterns of preference in the two environments were similar (McNemar binomial exact test, $P = 0.80$; Fig. 2B). In contrast with sympatric *S. bombifrons* females, allopatric *S. bombifrons* females did not differentiate between *S. multiplicata* calls that differed in pulse rate (exact binomial tests, shallow water: $P = 1$, deep water: $P = 0.28$;

McNemar binomial exact test, $P = 0.42$; Fig. 2B). Thus, allopatric *S. bombifrons* females potentially use pulse rate to discriminate among conspecifics, but this preference does not account for *S. bombifrons* preferences among *S. multiplicata* in sympatry. Instead, sympatric *S. bombifrons* female preferences for *S. multiplicata* calls with slow pulse rates have apparently evolved in sympatry after contact with *S. multiplicata*, possibly as a modification of their preexisting allopatric preference (9, 13).

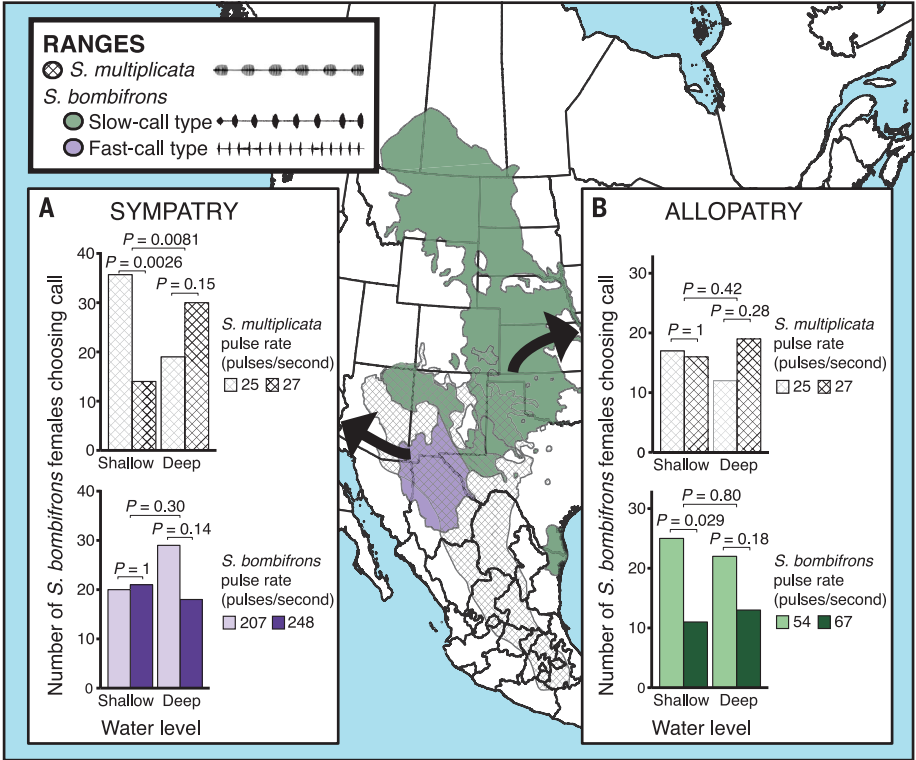


Fig. 2. Spea ranges, calls, and female preferences. *S. bombifrons* originate from the Great Plains of the United States. They have undergone contact and hybridize with *S. multiplicata* in the southwestern United States. Male *S. bombifrons* calls differ between the two regions as shown. (A) Sympatric *S. bombifrons* females prefer *S. multiplicata* male calls with slower pulse rates in shallow water (where hybridization is adaptive) but not in deep water (where hybridization is not adaptive). Sympatric *S. bombifrons* females do not distinguish between fast-call type *S. bombifrons* male calls that differ in pulse rate. (B) Allopatric *S. bombifrons* females do not distinguish between *S. multiplicata* calls that differ in pulse rate, but they do prefer slower pulse rates in the slow-call type *S. bombifrons* calls. See also table S6.

| Table 1. Standardized coefficients of model predictors for offspring fitness, after model averaging of the top nine candidate models. See table S3 for further details. SE, standard error. | | | | | |
|---|---------------------|----------|-------|---------|---------|
| Parameter | Relative importance | Estimate | SE | z value | P value |
| Male pulse rate | 0.95 | -1.44 | 0.563 | 2.56 | 0.0106 |
| Female SVL | 0.90 | -1.34 | 0.704 | 1.91 | 0.0562 |
| Male condition | 0.69 | 0.814 | 0.679 | 1.20 | 0.231 |
| Male call duration | 0.16 | 0.176 | 0.480 | 0.367 | 0.714 |
| Male genotype | 0.01 | -0.00917 | 0.110 | 0.0830 | 0.934 |

Spadefoots are unlikely to be the only group in which females can optimize the fitness consequences of hybridization by preferring heterospecific males that sire hybrid offspring with relatively higher fitness. Across taxa, individuals vary in their propensity to hybridize (14) and even prefer certain heterospecific males over others (15). Behavior to optimize hybridization may be especially likely in recently diverged groups with similar mating behaviors and for which hybridization could confer fitness benefits (4–6). However, more work is needed to determine how common this phenomenon is.

Our findings have two general implications. First, they suggest that members of one species might be able to exert sexual selection on another species. Such a pattern could affect the evolution and distribution of sexual signals, local mate competition, and even the extent to which species do or do not diverge where they co-occur (9, 16). Second, nonrandom hybridization can bias gene flow between species. To date, adaptive introgression has been considered a happenstance occurrence in which random, or possibly deleterious, hybridization is followed by the retention of adaptive alleles (17, 18). If, however, mate preferences result in nonrandom production of fitter hybrid genotypes, then nonrandom mating can enhance both the chances of adaptive introgression and

the transfer of alleles that are particularly well suited to a given habitat. In a rapidly changing world where hybridization could become increasingly common, understanding when and how adaptive introgression occurs could be key to population rescue, adaptation, or the replacement of one species by another (6, 19–21). Our results indicate that sexual selection and mate choice should be considered as integral components of these processes.

REFERENCES AND NOTES

1. R. Abbott *et al.*, *J. Evol. Biol.* **26**, 229–246 (2013).
2. K. S. Pfennig, A. L. Kelly, A. A. Pierce, *Proc. R. Soc. B* **283**, 20161329 (2016).
3. S. Lamichaney *et al.*, *Science* **359**, 224–228 (2018).
4. T. Veen *et al.*, *Nature* **411**, 45–50 (2001).
5. M. R. Jones *et al.*, *Science* **360**, 1355–1358 (2018).
6. E. M. Oziolor *et al.*, *Science* **364**, 455–457 (2019).
7. I. Lesna, M. W. Sabelis, *Nature* **401**, 581–584 (1999).
8. K. S. Pfennig, *Science* **318**, 965–967 (2007).
9. G. G. Rosenthal, *Mate Choice: The Evolution of Sexual Decision Making from Microbes to Humans* (Princeton Univ. Press, 2017).
10. Materials and methods are available as supplementary materials.
11. A. M. Rice, D. W. Pfennig, *J. Evol. Biol.* **21**, 696–704 (2008).
12. A. A. Pierce, R. Gutierrez, A. M. Rice, K. S. Pfennig, *Proc. R. Soc. B* **284**, 20170007 (2017).
13. M. J. Ryan, *Science* **281**, 1999–2003 (1998).
14. G. G. Rosenthal, *J. Evol. Biol.* **26**, 252–255 (2013).
15. M. J. Ryan, W. E. Wagner Jr., *Science* **236**, 595–597 (1987).
16. T. M. Panhuis, R. Butlin, M. Zuk, T. Tregenza, *Trends Ecol. Evol.* **16**, 364–371 (2001).
17. M. L. Arnold, K. Kunte, *Trends Ecol. Evol.* **32**, 601–611 (2017).
18. S. A. Taylor, E. L. Larson, *Nat. Ecol. Evol.* **3**, 170–177 (2019).
19. J. A. Hamilton, J. M. Miller, *Conserv. Biol.* **30**, 33–41 (2016).
20. R. P. Kovach, G. Luikart, W. H. Lowe, M. C. Boyer, C. C. Muhlfeld, *Conserv. Biol.* **30**, 428–430 (2016).
21. K. S. Pfennig, *Science* **364**, 433–434 (2019).

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SUPPLEMENTARY MATERIALS

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Female toads engaging in adaptive hybridization prefer high-quality heterospecifics as mates

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Choosing mates wisely

Hybridization between species has long been seen as an accidental contributor to evolution in some cases and as a dead end in others. New evidence is emerging, however, that hybridization may have played important, and nonrandom, roles in adaptation. Chen and Pfennig describe just such a case where female Plains spadefoot toads preferentially choose males from another toad species, the Mexican spadefoot, as mates, but only under certain environmental conditions (see the Perspective by Zuk). The offspring of this preferred hybrid mating event have higher fitness than nonhybrids in the same environment. Thus, not only do hybrids have an advantage, but females of one species are exerting a selective influence on the other species.

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