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Michael J. Ryan, William E. Wagner

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Asymmetries in Mating Preferences Between Species: Female Swordtails Prefer Heterospecific Males

MICHAEL J. RYAN AND WILLIAM E. WAGNER, JR.

In male swordtails (*Xiphophorus nigrensis*) there are three size classes that derive from allelic variation at the pituitary locus on the Y chromosome. Progeny analysis and preference tests suggest that females prefer to mate with larger males. In the closely related *X. pygmaeus*, there is no allelic variation at this locus; this species consists of males similar in size only to smaller *X. nigrensis* males. In addition to being smaller than most *X. nigrensis* males, these *X. pygmaeus* males also lack both the swordtail and a major component of the courtship display common in most *X. nigrensis* males. Usually, female *X. pygmaeus* prefer to mate with heterospecific males rather than conspecifics, regardless of body size and the presence of a swordtail. However, the smallest *X. nigrensis* males lack the same courtship component as do the *X. pygmaeus* males, and in this comparison female *X. pygmaeus* show no preference. Although sexual selection, through its action on divergence of courtship displays, has been implicated as a factor leading to speciation, in this case sexual selection could lead to the congealing of gene pools between heterospecifics.

IN MANY SPECIES, MATING ATTEMPTS BY a male can be successful only with the cooperation of the female. Female choice can have important effects at two levels: it can result in mating with conspecifics instead of heterospecifics (interspecies discrimination), and it can enhance the mating success of some conspecific males relative to others (intraspecies discrimination). Mate choice usually is hierarchical; intraspecies discrimination, or sexual selection, acts within the constraints of interspecies discrimination, or species recognition. Furthermore, researchers have suggested that divergence of courtship signals under sexual selection can lead to speciation as individuals from different populations fail to recognize one another as conspecific (*1*). We report that females of two species of swordtail (*Xiphophorus*, Poeciliidae family) exhibit preference for mates and that in one species females prefer heterospecific males to their own conspecifics. This heterospecific preference results from lack of a courtship display component in conspecific males combined with similar preference by females of both species for full courtship display. These data suggest that sexual selection can not only have a diversifying effect, as suggested above (*1*), but can also override species recognition and potentially act as a congealing force between closely related species.

Many species of *Xiphophorus* are characterized by considerable variation in body size (*2*). Much of this variation is heritable [for example, greater than 90% for the Rio Choy, Mexico, population of *X. nigrensis* (*3*)], and it results from allelic variation at the pituitary (P) locus on the Y chromosome (*2*). In species with a greater number of alleles at the P locus, body size is distributed continuously, but in *X. nigrensis* from the Rio Choy, there are only three alternative alleles at the P locus, which results in three discrete body size classes (*2*). Swordtails have internal fertilization, females choose their mates, and less preferred males attempt to force copulation with females (*4*, *5*). Paternity analysis of progeny from females collected in the field demonstrates a mating advantage for larger males in *X. nigrensis* (*6*). Laboratory tests reveal female mating preferences for these larger males that are consistent with the greater mating success of larger males in nature (*6*).

We wanted to determine if this preference for large males in *X. nigrensis* could be generalized to closely related species. If so, females of closely related species that did not have large males should prefer to mate with heterospecifics. This preference would constitute a unique example of mate choice overriding considerations of species recognition, and would demonstrate sexual selection that potentially gives rise to heterospecific preference. *Xiphophorus pygmaeus* and *X. nigrensis* are allopatric and closely related (*7*). In the former species, the P locus also influences male body size, but only the allele that results in smaller males is present (*3*). Therefore, we tested the hypothesis of heterospecific preference by giving female *X. pygmaeus* a choice between their own conspecific and a larger *X. nigrensis* male.

Xiphophorus pygmaeus contains gold and blue males; thus in these initial tests females were tested with either a gold [26 mm standard length (SL)] or a blue conspecific male (26 mm SL) against a larger heterospecific (37 mm SL). Eleven females were tested in four trials: twice with a blue conspecific-heterospecific pair, and twice with a gold conspecific-heterospecific pair. The testing apparatus was an aquarium (45 by 90 by 41 cm) that was divided into five equal sections. The sections at each end were separated from the three central sections by plexiglass. A male was placed in each of these end sections. The plexiglass partition ensured that females were exposed only to

Department of Zoology, University of Texas, Austin, TX 78712.

Table 1. The amount of time spent by female *X. nigrensis* and *X. pygmaeus* with courting males of different sizes and species. Abbreviations: Mean s, mean number of seconds; C, conspecific (relative to the female tested); Cb, blue conspecific; Cg, gold conspecific; H, heterospecific (relative to the female tested); Hns, heterospecific with swordtail removed; *t*, paired *t* test statistic; df, degrees of freedom; and *P*, probability.

Test males, mean s (SE)		*t*	df	*P*
Conspecific	Heterospecific			
	Female Xiphophorus pygmaeus			
Cg, 26 mm; 302 s (39.2)	H, 37 mm; 744 s (63.9)	10.9	10	<0.001
Cb, 26 mm; 285 s (24.7)	H, 37 mm; 764 s (24.4)	4.3	10	<0.01
Cb, 26 mm; 351 s (39.2)	H, 26 mm; 740 s (46.9)	4.6	9	<0.01
Cb, 26 mm; 331 s (81.3)	Hns, 26 mm; 801 s (85.5)	2.8	9	<0.05
Cb, 26 mm; 571 s (65.7)	H, 24 mm; 503 s (72.6)	0.5	9	>0.25
Cb, 23 mm; 533 s (51.7)	H, 23 mm; 555 s (64.2)	0.2	9	>0.25
	Female Xiphophorus nigrensis			
C, 26 mm; 711 s (44.4)	H, 26 mm; 412 s (47.6)	3.3	9	<0.01

visual cues. An opaque cylinder (11 cm in diameter) was placed in the middle section; a female was placed in the cylinder and allowed to acclimate for 10 minutes. After the female had acclimated, the cylinder was removed and the female had the opportunity to be in one of the three sections—either the center section or one of the sections adjacent to a male. We recorded the amount of time the female spent in each section during the following 10 minutes. After the first trial, the female was returned to the cylinder, the males were switched between the end sections of the tank, and the trial was repeated. This switching controlled for side biases. In all trials described below, males and females attempted to court one another with species-typical behaviors through the glass partition; thus this test appears to measure courting preference. We used a two-tailed paired *t* test to evaluate the null hypothesis that the amount of time a female spent with each male was equal (*8*). In both comparisons females preferred heterospecific males (Table 1). In subsequent tests, only blue *X. pygmaeus* males were used.

The above results suggest that *X. pygmaeus* females, like *X. nigrensis* females, prefer larger males, even though these larger males are heterospecifics and greatly exceed the size of any male *X. pygmaeus*. However, other differences are apparent between males of these two species: differences in general body form and the lack of the swordtail in *X. pygmaeus*. Also, Franck (*4*) reported that *X. pygmaeus* males do not exhibit the elaborate sexual display associated with courtship—the most conspicuous component of the full courtship display. In this respect, *X. pygmaeus* males resemble small *X. nigrensis* males and differ from larger *X. nigrensis* males. To test the hypothesis that body size was the cause of the heterospecific preference, we presented ten female *X. pygmaeus* with a choice between a heterospecific and conspecific of the same size (26 mm SL). Females showed a preference for the heterospecific in this comparison in which body size differences were eliminated (Table 1). In the same comparison, female *X. nigrensis* similarly preferred the male *X. nigrensis*, its conspecific (Table 1). Although the males were the same size, the male *X. nigrensis* possessed a swordtail. To test the importance of this variable, we surgically removed the swordtail and again tested female *X. pygmaeus*; again, the *X. pygmaeus* females preferred the *X. nigrensis*, the heterospecific. Thus neither body size nor swordtail alone accounts for the heterospecific preference.

As noted above, males also differ in their courtship behavior. The hypothesis that the display accounts for the female preference would indicate that when female *X. pygmaeus* are given a choice between a conspecific and a small male *X. nigrensis*, which also fails to exhibit the sexual display, the heterospecific preference would be eliminated. This was the case when *X. pygmaeus* and small *X. nigrensis* males were the same size and when *X. nigrensis* males were smaller (Table 1).

These results demonstrate that, in most comparisons, *X. pygmaeus* females prefer heterospecifics. This preference seems to be based on the presence or absence of full courtship behavior, since it is eliminated when small, noncourting *X. nigrensis* males are tested.

One possible explanation for the heterospecific preference is that the female preference for full courtship was shared by an ancestor common to *X. nigrensis* and *X. pygmaeus*, and it has been retained in females of both species even though this courtship trait is lacking in male *X. pygmaeus*. Mating asymmetries also have been reported in *Drosophila*. Unlike those in *X. pygmaeus*, however, the mating asymmetries in *Drosophila* are characterized by random mating between conspecifics and heterospecifics rather than preference for heterospecifics as we have demonstrated here (*9*). In *Drosophila*, female preference combined with the lack of a courtship component also is suggested to be responsible for the mating asymmetry, but the efficacy of female choice and differences in courtship behaviors have not been demonstrated. Similar results are found among stickleback populations. McPhail (*10*) showed that female sticklebacks from populations with either red or black nuptial colors both preferred red males. The taxonomic status of the red and black male populations of sticklebacks is not clear, but they are generally considered the same species (*10*).

Our study of interspecific mating preferences in *Xiphophorus* demonstrates that females prefer to mate with heterospecific males rather than their own conspecifics. Recent theoretical models, discussions, and data suggest that sexual selection on courtship traits generated by female choice can result in speciation as conspecifics in adjacent populations fail to respond to each other as members of the same species (*1*). In contradistinction, sexual selection could act as a congealing force for the species pair of *X. nigrensis* and *X. pygmaeus*. These species readily hybridize in the laboratory with no obvious deleterious effects on hybrid offspring. If these allopatric species were to become sympatric, preference of *X. pygmaeus* females for *X. nigrensis* males could result in extensive introgression, and perhaps the convergence of these two species into one cohesive gene pool.

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Cloning of Complementary DNA for GAP-43, a Neuronal Growth-Related Protein

LARRY R. KARNS, SHI-CHUNG NG, JOHN A. FREEMAN, MARK C. FISHMAN*

GAP-43 is one of a small subset of cellular proteins selectively transported by a neuron to its terminals. Its enrichment in growth cones and its increased levels in developing or regenerating neurons suggest that it has an important role in neurite growth. A complementary DNA (cDNA) that encodes rat GAP-43 has been isolated to study its structural characteristics and regulation. The predicted molecular size is 24 kilodaltons, although its migration in SDS-polyacrylamide gels is anomalously retarded. Expression of GAP-43 is limited to the nervous system, where its levels are highest during periods of neurite outgrowth. Nerve growth factor or adenosine 3',5'-monophosphate induction of neurites from PC12 cells is accompanied by increased GAP-43 expression. GAP-43 RNA is easily detectable, although at diminished levels, in the adult rat nervous system. This regulation of GAP-43 is concordant with a role in growth-related processes of the neuron, processes that may continue in the mature animal.

THE TIP OF THE GROWING NEURITE is the growth cone. This motile structure, of a neuron otherwise fixed in space, is responsible for reaching and identifying appropriate postsynaptic targets. The molecular constituents of the growth cone are synthesized in the soma and transported down the axon, and constitute a limited subgroup of the total cellular proteins. Because growth-cone proteins presumably play an important role in generating patterns of specific connectivity in the nervous system, we (*1*) and others (*2*) have developed methods to enrich for such molecules and have identified several as candidates for involvement in either target recognition (*1*) or axon growth (*3*). GAP-43 is one of the latter group, which consists of proteins expressed or transported at higher rates in nerves of young animals or nerves undergoing regeneration (*3*). It is similar or identical to B-50, a phosphoprotein described by Gispen in both growing and adult nerves as a prominent substrate for protein kinase C, its phosphorylation being modulated by neurotransmitters and peptides (*4, 5*). Antibodies localize GAP-43 to the growth cone and B-50 to growing neurites and to the presynaptic membrane of adult nerves (*6, 7*). The regulation of GAP-43 and its cellular localization suggest that it has an important function in growth cones. We report here the complete sequence of GAP-43 obtained by complementary DNA (cDNA) cloning and present evidence for its regulation at the level of gene expression during neurite growth.

L. R. Karns, S.-C. Ng, M. C. Fishman, Howard Hughes Medical Institute and Medical Services, Massachusetts General Hospital, and Harvard Medical School, Boston, MA 02114.
J. A. Freeman, Department of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232.

*To whom correspondence should be addressed.

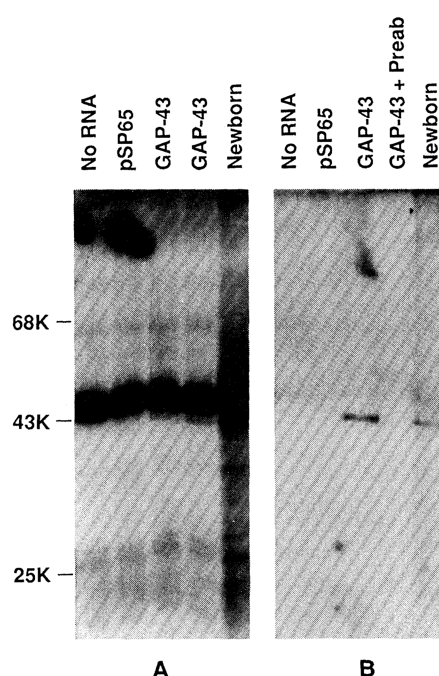

Fig. 1. Hybrid-selected translation of GAP-43 cDNA. The Eco RI insert, GAP43-2, was used to select mRNA by the procedure of Ricciardi *et al.* (*21*). In brief, 0.5 μg of the GAP43-2 insert, or equivalent amounts of nonspecific DNA, the bacterial plasmid pSP65, were spotted onto nitrocellulose and hybridized with 17.5 μg of newborn rat brain polyadenylated [poly(A)$^+$] RNA in a solution with 65% formamide, 400 m*M* NaCl, 10 m*M* 1,4-piperazine diethanesulfonic acid (Pipes) *p*H 6.4 at 42°C for 16 hours. After being washed in standard saline citrate (SSC) (X1), 0.5% SDS at 65°C, the filter was boiled, and the RNA was precipitated with ethanol and translated with rabbit reticulocyte lysate, and the proteins were labeled with [^{35}S]methionine (*22*). Translation products, or products immunoprecipitated with the antibody to GAP-43, were separated on a 12% SDS-polyacrylamide gel. (**A**) In vitro translation products with (i) no exogenous RNA, (ii) pSP65-selected RNA, (iii and iv) GAP43-2–selected RNA, and (v) poly(A)$^+$ newborn brain RNA (newborn). (**B**) Immunoprecipitations by antibody to GAP-43 of the translation products of (A), as described for (A) except for the fourth lane which shows immunoprecipitation of the translation product after having preabsorbed the GAP-43 antibody with GAP-43 protein, prepared as in (*9*).

A cDNA library was generated from RNA of rat dorsal root ganglia from embryonic day 17 and cloned into the λgt11 expression vector (*8*). Three presumptive GAP-43 clones were identified with the antibody to GAP-43 described by Snipes *et al.* (*9*). The identity of the longest clone, GAP43-2, was confirmed by hybrid-selected translation (Fig. 1). GAP43-2 selected by hybridization a messenger RNA (mRNA) that directed the translation of a polypeptide that migrated in SDS-polyacrylamide gels with the expected mobility of native GAP-43, that is, a molecular size of about 43 kD. This in vitro translation product was selectively immunoprecipitated by antibody to GAP-43. The specificity of the immunoprecipitation was demonstrated by competition with unlabeled, purified GAP-43. For additional confirmation we sequenced a peptide prepared by cyanogen bromide cleavage of purified GAP-43. The sequence, Arg-X-Lys-Gln-Val-Glu-Lys-Asn-Asp-Glu-Asp-Gln-Lys-Ile, is completely included within the predicted open reading frame of GAP43-2. (The X represents a cycle of sequencing at which the identity of the amino acid could not be determined with certainty.)

The complete nucleotide sequence of GAP43-2 and the predicted amino acid sequence are shown in Fig. 2. The reading frame includes the peptide fragment that was sequenced and is in the same reading frame as the β-galactosidase gene of λgt11