

# Sex Chromosomes and Sexual Selection in Poeciliid Fishes

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**ABSTRACT:** We propose that the evolution of female preferences can be strongly influenced by linkage of attractive male traits to the Y chromosome and female preferences to the X chromosome in male heterogametic species. Such linkage patterns are predicted by models of the evolution of sexually antagonistic genes. Subsequent recombination of attractive male characters from the Y to the X would create physical linkage between attractive male trait and preference. A literature survey shows that Y linkage of potentially sexually antagonistic traits is common in poeciliid fishes and other species with sex chromosomes that are not well differentiated, but may also occur in taxa with degenerate Y chromosomes. In the guppy, attractive male traits are primarily Y and X linked; a literature review of the inheritance of sex-limited attractive male characters suggests that 16 are Y linked, 24 recombine between the X and Y, two are X linked, and two are autosomal. Crosses and backcrosses between high female preference (Endler's live-bearers) and low female preference (Rio San Miguel) guppy populations show that this character has a strong additive genetic component and that it will be possible to investigate the physical linkage of male and female sexually selected characters in this species through mapping studies.

*Keywords:* sexual selection, sex chromosomes, Y chromosome, Poeciliidae, *Poecilia reticulata*.

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To what extent the genetic architecture of behavior constrains its evolution remains highly controversial. Genomic data and molecular genetic techniques recently developed in model genetic organisms and applied to model behavioral systems will help answer this question. One area in which these approaches may be particularly fruitful will be in understanding how linkage to sex chromosomes affects the process of sexual selection. Fisher noted two ways in which the linkage of traits could affect the dynamics of sexual selection. First, the conditions for the evolution of

a sexually antagonistic trait, a trait that benefits one sex but is detrimental to the other, depend on whether the genes controlling that trait are linked to the sex chromosomes or to autosomes (Fisher 1931). Second, genetic correlations between sexual preferences for attractive traits and the traits themselves can lead to rapid coevolution of these characters (Fisher 1958); such genetic correlations are more easily maintained when there is physical linkage between genes controlling the traits. Determining the interplay between sex chromosomes and the linkage of genes for attractive male traits and female preferences has the potential to answer several long-standing questions in sexual selection.

## Effect of Linkage to Sex Chromosomes on the Dynamics of Sexually Selected Traits

### *Linkage of Sexually Antagonistic Genes to Heterogametic Sex Chromosomes*

Evolutionary theory predicts that sexually antagonistic genes that benefit the heterogametic sex are more likely to increase when linked to the sex-determining locus (Fisher 1931; Bull 1983; Rice 1987*a*; see table 1 for definition of terms). This prediction is a consequence of the fact that sexually antagonistic genes that are linked to the sex-determining locus are rarely expressed in the homogametic sex, providing little or no opportunity for selection against these genes. However, the accumulation of sexually antagonistic genes in linkage with the sex-determining locus favors a reduction in recombination between the sex chromosomes (Rice 1987*a*). This is predicted to lead to the decay of nonrecombining regions, since any genetic load that accumulates through Muller's ratchet, genetic hitchhiking, or other mechanisms cannot be purged by recombination (Charlesworth 1978; Rice 1987*b*). This degeneration in turn may make it less likely that functional genes will be linked to the heterogametic sex chromosome.

We conducted a survey of the literature to estimate the frequency with which phenotypic traits that are potentially sexually antagonistic are presently linked to the heterogametic sex chromosome. We ignored characters involved in sexual differentiation, such as genes controlling spermatogenesis in humans (Lahn and Page 1997) or eggshell

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**Table 1:** Definitions used in sexual selection and sex chromosome studies

Term	Definition
Sex chromosomes	A pair of chromosomes with a locus that determines sex
Heteromorphic sex chromosomes	A pair of sex chromosomes, one of which harbors few functional genes, is usually reduced in size, and exhibits reduced recombination with the other sex chromosome
Heterogametic sex	Sex that is determined by a dominant allele at the sex-determining locus; this is often denoted as XY for male heterogamety and ZW for female heterogamety
Heterogametic sex chromosome	The chromosome that harbors the dominant sex-determining allele (Y in XY systems, W in ZW systems)
Pseudoautosomal region	Region of sex chromosomes with functional genes and recombination rates similar to autosomes
Sex-limited gene	Locus whose alleles are expressed only in one sex; this locus is not necessarily located on the sex chromosomes
Sex-linked gene	Locus physically linked to the sex chromosomes; this locus can be in a recombining (pseudoautosomal) or nonrecombining region
Sexually antagonistic gene	Gene whose expression is beneficial to one sex but detrimental to the other sex

patterning in birds (Gosler et al. 2000), because these can only be expressed in a functional member of one sex and therefore cannot be sexually antagonistic. The results (table 2) show that most cases are found in fishes and especially in poeciliids, a family of live-bearing fishes that varies within and between species in sex-determination system and sex chromosome structure (Kallman 1975; Black and Howell 1979; Angus 1989). Sex-limited color patterns in poeciliids, such as those referred to in table 2, are considered sexually antagonistic traits (Fisher 1931; Endler 1980; Bull 1983).

The large number of cases of Y linkage in fishes is probably related to the fact that teleost fishes are mostly characterized by morphologically similar (homomorphic) sex chromosomes (Ohno 1974), so the X and Y chromosomes are equally represented in the genome. However, Y linkage also occurs in chrysomelid beetles, mice, and humans (table 2), which have heteromorphic sex chromosomes with a degenerate Y (Smith and Virkki 1978; Segarra and Petit-pierre 1990; Graves 1995). Several of the cases of linkage to the heterogametic sex chromosome in table 2 involve poorly studied species, and this implies that there may be many more undiscovered cases of such linkage. Furthermore, taxa that presently have few genes linked to the heterogametic sex chromosome, such as birds and mammals, presumably passed through a period in their evolution during which many genes were linked to the evolving sex chromosomes (Bull 1983). This linkage could have been a driving force for the decay of the Y chromosome (Rice 1987*b*) and also may have affected the evolution of sexually selected traits, even though these genes are not presently linked to the heterogametic sex chromosome. Comparative studies of groups exhibiting homomorphic and heteromorphic sex chromosomes could determine the effect of the evolution of differentiated sex chromosomes on the evolutionary dynamics of sexually selected traits.

#### *Sex Chromosomes and Linkage between Female Preference and Attractive Male Characters*

Traits linked to the heterogametic sex chromosome are often male secondary sex characters important in sexual selection (table 2). Explaining the evolution of female preferences for these characters is one of the most difficult challenges in sexual selection, and we propose that patterns of linkage to sex chromosomes could greatly affect this evolution.

Models for the evolution of female preference can be characterized according to whether there is direct or indirect selection on preference. Indirect models depend on a genetic correlation between female preference and other characters undergoing positive direct selection, such as “good genes” that confer overall higher fitness due to natural selection in offspring or the attractive male characters themselves. Genetic correlations may be caused by either pleiotropy or linkage disequilibrium. It is commonly assumed that the genetic correlation in sexual selection models is due to linkage disequilibrium between genes on different chromosomes caused by nonrandom mating (Lande 1981). Indirect models have been criticized on the basis that linkage disequilibrium caused by nonrandom mating would be difficult to maintain for any length of time because of genetic drift and recombination in finite populations (Nichols and Butlin 1989) and that the force of indirect selection caused by this linkage would be weak (Barton and Turelli 1991; Kirkpatrick and Barton 1997).

Predominant linkage of attractive male traits to the heterogametic sex chromosome may have important implications for these indirect models. Genetic correlations between female preferences and attractive male traits that are exclusively Y linked cannot occur through pleiotropy or physical linkage, since daughters will not inherit Y-linked genes (in male heterogametic taxa). Linkage dis-

**Table 2:** Phenotypic traits unrelated to sexual differentiation that are linked to the Y or W (heterogametic) chromosome

Family and species	Trait	Heterogametic sex	Reference
Chrysomelidae:			
<i>Gonioctena variabilis</i>	Pigmentation	Male	de Zulueta 1925; Galán 1931
<i>Phyllotreta nemorum</i>	Survival on host plant	Male	Segarra and Petitpierre 1990; Nielsen 1997
Cichlidae:			
<i>Neochromis omnicaeruleus</i>	Pigmentation	Male or female <sup>a</sup>	Seehausen et al. 1999
Cyprinodontidae:			
<i>Oryzias latipes</i>	Pigmentation	Male	Matsuda et al. 1998; Wada et al. 1998
Hominidae:			
Human	Height, tooth growth	Male	Alvesalo 1997; Kirsch et al. 2000
Muridae:			
<i>Mus musculus</i>	Aggression	Male	Selmanoff et al. 1975; Sluyter et al. 1994
Papilionidae:			
<i>Papilio glaucus</i>	Pigmentation	Female	Scriber et al. 1996
Poeciliidae:			
<i>Gambusia holbrooki</i>	Pigmentation	Male	Black and Howell 1979; Angus 1989
<i>Limia perugiae</i>	Size	Male	Erbelding-Denk et al. 1994
<i>Poecilia latipinna</i>	Size	Male	Travis 1994
<i>Poecilia parae</i>	Pigmentation	Male	A. Lindholm and F. Breden, unpublished data
<i>Poecilia reticulata</i>	Pigmentation, fin shape and size, courtship, attractiveness	Male	Winge 1927; Farr 1983; Brooks 2000; Brooks and Endler 2001
<i>Xiphophorus andersi</i>	Size	Male	Kallman 1989
<i>Xiphophorus maculatus</i>	Pigmentation, size	Male or female <sup>a</sup>	Kallman 1970; Kallman and Borkoski 1978
<i>Xiphophorus milleri</i>	Pigmentation, size	Male	Kallman and Borowsky 1972
<i>Xiphophorus montezumae</i>	Pigmentation, size	Male	Kallman 1983
<i>Xiphophorus multilineatus</i> <sup>b</sup>	Pigmentation, bar suppressor, size, courtship	Male	Zimmerer and Kallman 1988, 1989; Kallman 1989
<i>Xiphophorus nigrensis</i> <sup>b</sup>	Pigmentation, size	Male	Zander 1968; Kallman 1989
<i>Xiphophorus pygmaeus</i>	Pigmentation, size	Male	Kallman 1989
<i>Xiphophorus variatus</i>	Pigmentation, size	Male	Borowsky 1984, 1987

<sup>a</sup> Multiallelic system.<sup>b</sup> Sensu Rauchenberger et al. 1990.

equilibrium between genes on different chromosomes is thus the only mechanism that could account for a genetic correlation between genes for female preference and genes for male attractiveness that are exclusively Y linked. However, we propose that some recombination between X and Y chromosomes could produce physical linkage between preference and trait when female preference genes are also linked to the X chromosome. X linkage of preference genes could occur by chance; that is, a certain proportion of new mutants for female preference would be expected to be X linked. However, if female preferences are sexually antagonistic and alleles that determine them are dominant, theory predicts that these alleles would be preferentially linked to the X chromosome, because the X chromosome spends two-thirds of its time in the homogametic sex (Rice 1984). Female preferences might be expected to be sexually an-

tagonistic because females obtain whatever benefit is driving the evolution of female preference, but males might only pay the costs (e.g., association with conspicuous conspecifics in an environment with a high risk of predation).

If alleles influencing female preferences are linked to the X chromosome, and an allele for female preference on the X crosses over to the Y chromosome, it is no longer exposed to selection for choosiness. However, if many attractive male traits are linked to the heterogametic sex chromosome in a region that occasionally recombines with the X (as observed in guppies; Winge 1934), and if an allele for an attractive male trait recombines from the Y to the X, then alleles for the attractive trait and alleles for the preference will be physically linked. Reduced recombination between the X and Y chromosomes in such regions implies that alleles for the attractive male traits

would not often recombine from the Y to the X, but when they do, they would be closely linked to alleles for preference on the X chromosome. This physical linkage could greatly reduce recombination between genes for male trait and female preference and facilitate the evolution of female preferences.

If indeed female preferences are preferentially linked to the X chromosome, then many factors would determine the potential for the establishment and maintenance of genetic correlations caused by this linkage. These would include recombination rates between the X and Y and between homologous X chromosomes within females, selection on associated attractive characters and preferences, and the pattern of dosage compensation, which affects selection on antagonistic genes (Charlesworth et al. 1987). The interplay between linkage to sex chromosomes and the dynamics of sexual selection is only beginning to be modeled (Lande and Wilkinson 1999), but in general, physical linkage should enhance genetic correlations between preference and attractive character.

In addition to further theoretical work, it is critical to answer several empirical questions concerning linkage patterns of alleles controlling sexually selected characters. What are the patterns of linkage and recombination between attractive male characters in natural populations? Are female preference genes preferentially associated with the sex chromosomes? If so, what are the rates of recombination between genes for preference and genes for attractive male characters linked to these sex chromosomes?

The guppy, *Poecilia reticulata*, is an ideal system with which to examine these questions; it possesses sex chromosomes with many X- and Y-linked male secondary sexual characters (table 3), has male heterogamety, and exhibits genetically determined variation for female preference for these characters. Another poeciliid, *Xiphophorus maculatus*, shows male or female heterogamety and also has X- and Y-linked alleles for pigmentation patterns and body size (Kallman 1975), but genetic variation in female preference for these traits has not yet been demonstrated. This variation in female preference is necessary in order to identify genes underlying preference and to estimate linkage with other genes. The potential for mapping genes controlling variation in attractive male characters and mating preferences and determining their linkage patterns in guppies is greatly enhanced by a genomics project in the closely related poeciliid, *Xiphophorus helleri*. This genomics project is based on a genetic model for melanoma (Kazianis et al. 1998; <http://www.xiphophorus.org>).

#### Linkage of Attractive Male Characters to Sex Chromosomes in Guppies

Guppy males exhibit many elaborate secondary sexual characters, and guppy populations exhibit extreme poly-

morphism for these characters. Several have been shown to be attractive to females: conspicuous coloration, especially bright orange and black spots, large caudal fins, large body size, and high courtship display rate (Farr 1980; Bischoff et al. 1985; Reynolds and Gross 1992; Nicoletto 1993; Endler and Houde 1995; Brooks and Endler 2001). A survey of the literature on the inheritance of these attractive male traits shows that color patterns, caudal fin size and shape, courtship rates, and a composite measure of attractiveness are primarily sex linked in guppies (table 3). An exception is body size, which shows high heritability but has not been shown to be sex linked (Reynolds and Gross 1992; Yamanaka et al. 1995; Brooks and Endler 2001). Only one X-linked gene has been found in guppies that is unlikely to be sexually selected: a low-temperature-resistance gene that is expressed in both males and females (Fujio et al. 1990). Both quantitative genetic and pedigree analyses indicate that most of the attractive male traits are not exclusively Y-linked (table 3; see Winge 1927 or Kirpichnikov 1981 for drawings of many of the named combinations of color patterns and fin morphologies). Many of these traits recombine between the X and Y chromosomes, revealing the homology between guppy sex chromosomes.

It has recently been shown that there is some cytological and molecular differentiation between the X and Y chromosomes in the guppy (Traut and Winking 2001). Only one-half of the Y chromosome pairs with homologous regions of the X in synaptonemal complexes. Furthermore, the orientation of the chromosomes allowed for recombination in only two of 49 synaptonemal complexes observed; this suggests that recombination is also greatly reduced in the pairing, homologous region. Comparative genomic hybridization indicated that a large part of the nonpairing region of the Y chromosome comprises male-specific repetitive DNA (Traut and Winking 2001) and that there is structural variation among Y chromosomes in this region. This agrees with results from an in situ hybridization study showing that Y chromosomes, but not X chromosomes, of some domesticated guppies carry large numbers of simple repetitive sequences (Nanda et al. 1990). However, these male-specific repeats were not observed in recent descendants of wild guppies (Hornaday et al. 1994). Degeneration of the Y chromosome is supported by the observation that inheritance of Y chromosomes bearing alleles for attractive male traits leads to increased mortality (Brooks 2000). The buildup of simple repetitive sequences and deleterious mutations on Y chromosomes that produce male guppies highly attractive to females would provide a mechanism for the result that more attractive males produce sons of lower viability.

This picture of the sex chromosomes concurs with that inferred from pedigree analyses. Some gene complexes

Table 3: Linkage of sexually selected male traits in guppies

	N	Crossover frequency		Reference
		X to Y % (N)	Y to X % (N)	
Y linked: <sup>a</sup>				
<i>Maculatus-red</i>	3,841			Schmidt 1920; Winge 1922a, 1922b, 1927, 1934; Winge and Ditlevsen 1938, 1947; Haskins and Haskins 1951; Haskins et al. 1970
<i>Oculatus</i>	399			Schmidt 1920; Winge 1927
<i>Armatus</i>	1,412			Blacher 1927, 1928; Winge 1927; Haskins et al. 1970
<i>Pauper</i>	636			Winge 1927, 1934; Winge and Ditlevsen 1938, 1947; Haskins et al. 1970
<i>Sanguineus</i>	575			Winge 1927
<i>Iridescens</i>	256			Winge 1922b; Blacher 1928; Winge and Ditlevsen 1947; Dzwillo 1959
<i>Aureus</i>	105			Winge 1927
<i>Variabilis</i>	81			Winge 1927
<i>Ferrugineus</i>				Winge 1927
<i>Bimaculatus</i>	68			Blacher 1927, 1928
<i>Reticulatus</i>				Natali and Natali 1931 (in Kirpichnikov 1981)
<i>Trimaculatus</i>	78			Natali and Natali 1931 (in Kirpichnikov 1981)
<i>Viridis</i>				Natali and Natali 1931 (in Kirpichnikov 1981)
<i>Bipunctatus</i>				Natali and Natali 1931 (in Kirpichnikov 1981); Kirpichnikov 1935
<i>Doppelschwert</i>	609			Dzwillo 1959
<i>Filigran</i>	71			Dzwillo 1959
Orange area <sup>b</sup>				Houde 1992; Brooks and Endler 2001; Karino and Haijima 2001
Black area <sup>b</sup>				Brooks and Endler 2001
Fuzzy black area <sup>b</sup>				Brooks and Endler 2001
Iridescent area <sup>b</sup>				Brooks and Endler 2001
Mean brightness <sup>b</sup>				Brooks and Endler 2001
Brightness contrast <sup>b</sup>				Brooks and Endler 2001
Mean chroma <sup>b</sup>				Brooks and Endler 2001
Attractiveness <sup>b</sup>				Brooks 2000
Tail area <sup>b</sup>				Brooks and Endler 2001
Courtship <sup>b</sup>				Farr 1983
X linked: <sup>a</sup>				
<i>Lineatus</i>	76			Winge 1927, 1934
<i>Nigrocaudatus I</i>	21			Nybelin 1947
X and Y linked:				
<i>Maculatus-black</i>				Winge and Ditlevsen 1947; Haskins et al. 1961
<i>Elongatus</i>		4.31 (348)	6.43 (1,276)	Winge 1922a, 1927
<i>Vitellinus</i>		6.13 (1,321)	3.75 (800)	Winge 1927, 1934; Haskins et al. 1970
<i>Coccineus</i>		.33 (1,198)	.48 (414)	Winge 1927, 1934; Dzwillo 1959
<i>Tigrinus</i>		.21 (938)	2.91 (206)	Winge 1927, 1934
<i>Luteus</i>		.89 (1,012)	3.82 (157)	Winge 1927, 1934
<i>Minutus</i>			2.67 (487)	Winge 1927, 1934
<i>Cinnamomeus</i>				Winge 1927
<i>Solaris</i>			0 (20)	Kirpichnikov 1935
<i>Caudomaculatus</i>				Blacher 1928
<i>Anterior rubra</i>	20 (25)			Blacher 1928
<i>Purpureus</i>			0 (52)	Natali and Natali 1931 (in Kirpichnikov 1981); Kirpichnikov 1935
<i>Lutescens</i>				Natali and Natali 1931 (in Kirpichnikov 1981)
<i>Nigrocaudatus II</i>		0 (74)	4.00 (25)	Dzwillo 1959; Nayudu 1979
<i>Flavus</i>		0 (77)	0 (17)	Winge and Ditlevsen 1947; Nayudu 1979

Table 3 (Continued)

	N	Crossover frequency		Reference
		X to Y % (N)	Y to X % (N)	
<i>Pigmentiert caudalis</i>		5.19 (270)	7.42 (364)	Dzwilllo 1959; Schröder 1969a; Nayudu 1979
<i>Sb</i>				Haskins et al. 1961
Red tail		2.06 (97)	1.64 (548)	Fernando and Phang 1990; Khoo et al. 1999b, 1999c
Blue tail		1.79 (280)		Fernando and Phang 1990; Phang and Fernando 1991
Green tail		0 (312)		Phang et al. 1989a; Phang and Fernando 1991;
				V. P. E. Phang, unpublished data
Snakeskin body			.36 (2,507)	Phang et al. 1989a, 1989b, 1990; Phang and Fernando 1991
Snakeskin tail			.11 (948)	Phang et al. 1989a, 1989b, 1990; Phang and Fernando 1991
Variagated tail		1.03 (679)	3.25 (462)	Khoo et al. 1999a, 1999b
Black caudal peduncle		2.73 (549)	2.56 (260)	Khoo et al. 1999b, 1999c
Autosomal:				
<i>Zebrinus</i>				Winge 1927
<i>Bar</i>				Phang et al. 1999
Blond				Goodrich et al. 1944
Golden				Goodrich et al. 1944
Blue				Dzwilllo 1959
Albino				Haskins and Haskins 1948
Kalymma				Schröder 1969b
Suppressor				Schröder 1969b
Elongated				Horn 1972

Note: N is the number of offspring examined; in some cases, crossing over was detected only outside of controlled crosses.

<sup>a</sup> Not known to recombine.

<sup>b</sup> Denotes quantitative genetic analyses that indicate a Y-linked component.

have never been seen to recombine with the X, while others recombine at a rate of up to 8% (table 3). This rate of recombination is similar to the potential for recombination inferred from the analysis of synaptonemal complexes (2/49, or approximately 4%; Traut and Winking 2001). Crossover frequencies suggest that the recombining regions of the X and Y chromosomes are similar, since the same gene complex recombines from Y to X and from X to Y at a similar frequency (table 3; sign test, NS). Crossover rates have been interpreted in terms of a physical linkage map of the Y chromosome, with tight linkage of those nonrecombining Y-linked genes to a major male sex-determining locus or loci on the Y chromosome (Winge 1927). Recombination is suppressed in this region but increases with increasing distance from the sex-determining region. Suppression of recombination is probably not complete even in the nonhomologous region. Rare crossover events at a frequency of <1/3,800 (table 3) have occurred between the genes for the red and the black elements of the *Maculatus* color pattern, which are believed to be located very close to the sex-determining region (Winge 1934). A recent linkage map based on phenotypic traits suggested that the sex-determining region is flanked on both sides by recombining regions (Khoo et al. 1999b).

The X chromosome is less well understood but is as-

sumed to carry similar genes to the Y, apart from those involved in sex determination of males, since YY males, which have no X chromosome, can be fully viable (Winge and Ditlevsen 1938; Haskins et al. 1970). The X chromosome may have a region homologous to that of the nonrecombining region of the Y, but so far no genes have been shown to be exclusively linked to it. Two genes for color patterns that are on the X but are not known to recombine to the Y (*Lineatus* and *Nigrocaudatus* I; table 3) are candidates for such a region, but the small sample sizes in these studies suggest instead that these genes would be found to recombine with the Y if more crosses were made, as has been the case for many other color genes. X-linked color patterns always have male-limited expression but can be developed in females with testosterone treatment, which allows confirmation of inheritance in females. Only patterns that have never been reported from wild populations show weak expression in females without testosterone treatment (*Nigrocaudatus* I and II, *Flavus*, *Pigmentiert caudalis*, red tail, blue tail, green tail, variegated tail, and black caudal peduncle; references in table 3) and are most likely mutations restricted to domesticated populations.

The autosomes have many fewer genes for pigmentation and fin morphology. *Zebrinus* and *Bar* are similar to the

sex-linked pigmentation traits in that expression is limited to males. The other known autosomal genes are expressed in both males and females (references in table 3).

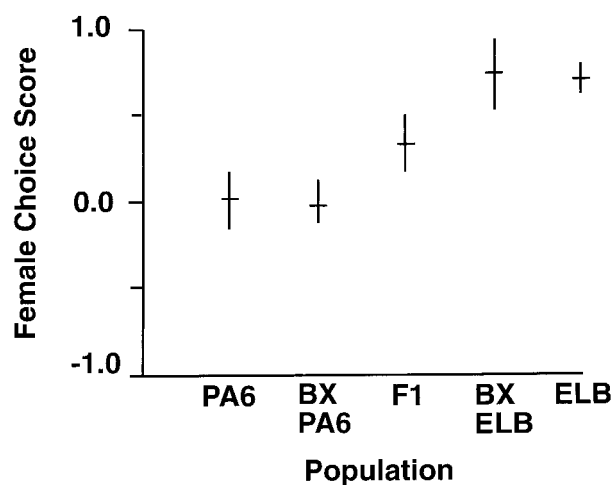
These extensive studies using crossover rates and pedigree analysis of male coloration genes to map guppy sex chromosomes provide only an approximate picture of the linkage patterns of attractive characters in natural populations. First, estimates of sex linkage and rates of recombination in many cases were based on small sample sizes (table 3). More importantly, the extent to which color genes studied in domesticated strains represent genes found in natural populations is unknown.

The sex linkage of genes controlling attractive male characters has been directly measured in a few wild populations. By treating females with testosterone, Haskins et al. (1961) showed that some color patterns that were inherited on the X or the Y chromosome in low-predation populations were exclusively Y linked in high-predation populations. Thus, the low-predation populations that are characterized by higher levels of preference and elevated levels of male coloration are also those that exhibit color genes linked to the X chromosome. This result would be expected under our proposition that physical linkage between female preference genes and male coloration genes, facilitated by linkage of both types of characters to sex chromosomes, helps drive sexual selection in poeciliids.

### Genetics of Female Preference in Guppies

We investigated the genetic inheritance of female preference by crossing and backcrossing populations with highly divergent levels of female preference and male attractive coloration. A low-coloration guppy population (PA6) was collected in 1997 from a coastal, high-predation stream (Rio San Miguel, a tributary of the Rio San Juan) 6 km outside of Caripito, Venezuela. The second parental strain was a population of a distinct and highly colorful form of guppy known to aquarists as Endler's live-bearer (ELB), collected from the coastal town of Cumana, Venezuela. This type has many orange and black patterns not observed in other guppy populations (H. Alexander and F. Breden, unpublished data). A recent survey of this region (H. Alexander and F. Breden, unpublished data) showed that ELB is endemic to only a few canals in Cumana.

Adult females from the two parental populations, female F1 offspring from reciprocal crosses (one with an ELB dam and one with a PA6 dam), and female offspring from backcrosses to the parental populations were tested for preference. Details of methods for measuring choice are given in the caption of figure 1. There was no indication of postmating reproductive incompatibility between these populations, and both F1 males and females produced offspring when backcrossed to the parental populations.



**Figure 1:** Mean and standard error of choice scores from low-coloration population (PA6), Endler's live-bearer population (ELB), F1 offspring from one set of reciprocal crosses, and backcrosses to parental populations. Choice tests were conducted in a 40-L aquarium partitioned by glass into a middle section, with bottom dimensions of 25 cm  $\times$  25 cm, and two end sections, with bottom dimensions of 12.5 cm  $\times$  25 cm. Males were placed in the end partitions, and the test female was placed in the center section. An 8-L dither tank containing female guppies was placed directly behind the center of the tank. The purpose of the dither fish was to calm the female during the acclimation and test periods and to allow her the option of schooling with conspecifics. The female and two males were allowed to equilibrate either for 3–5 h or overnight in the dark. A halogen light placed above the tank was then turned on automatically, and the fish were able to observe each other through the glass for an additional 1-h equilibration period. Following this equilibration period, an observer, facing the side of the aquarium behind a curtain into which a 5  $\times$  15-cm hole had been cut, recorded whether the female was oriented toward a male whenever she was within 5 cm of his side of the tank. Behavior was recorded for 20 min. Females were tested either as virgins or within 48 h postparturition. The stimulus males were one PA6 and one ELB male chosen at random from a set of eight males from each population, matched to within 1 mm standard length; all ELB males were dramatically more colorful than PA6 males. Female preference for the ELB male was calculated using the following equation:  $(X_{\text{ELB}} - X_{\text{PA6}}) / (X_{\text{ELB}} + X_{\text{PA6}})$ , where  $X_{\text{ELB}}$  and  $X_{\text{PA6}}$  are the total time a female was oriented toward the colorful ELB or less colorful PA6 male, respectively. This statistic ranges from  $-1.0$  to  $1.0$ , and positive values indicate a choice for the colorful ELB male.

Choice scores for females from the low-coloration PA6 population ranged from  $-0.83$  to  $0.86$ , and the average choice score was not significantly different from 0 ( $\bar{X} = 0.05$ ,  $N = 11$ ,  $P > .2$ ,  $t$ -test; fig. 1). In contrast, all ELB females preferred the ELB male (choice scores ranged from  $0.20$  to  $1.0$ ), and overall there was a significant choice for the ELB male ( $\bar{X} = 0.66$ ,  $N = 11$ ,  $P < .001$ ). These results support several studies that have shown a correlation between the level of male coloration and female preference in guppy populations (Breden and Stoner 1987; Stoner and Breden 1988; Houde and Endler 1990; Endler and

Houde 1995). The female choice scores for the F1 females showed an intermediate value ( $\bar{X} = 0.36$ ,  $N = 13$ ), and most importantly, the backcross scores showed regression toward the parental population values ( $\bar{X} = -0.07$ ,  $N = 7$ , backcross to PA6;  $\bar{X} = 0.69$ ,  $N = 3$ , backcross to ELB). Because only one set of reciprocal crosses has been tested, and because the sample sizes for each type of F1 and backcross populations are small, it is premature to quantify the results of these crosses in terms of additive and maternal effects. However, these results do show that there is a strong additive genetic component to the difference between these populations and that it will be possible to study the inheritance of guppy female preference in the types of crosses and backcrosses necessary for quantitative trait locus studies. We are currently using microsatellite markers for the guppy anchored on the dense *Xiphophorus* linkage map (S. Kazianis, F. Breden, and R. B. Walter, unpublished data) to test for a large X chromosome effect on female preference in these populations.

In conclusion, organisms with evolving sex chromosomes can show variation in the number of functional genes linked to the sex chromosomes, variation in recombination rates along sex chromosomes, and variation in sex-determination system, such as male or female heterogamety or number of alleles at the sex-determining locus. Such systems provide an ideal opportunity to study the effect of sex linkage on the evolution of behavior and the effect of differences in behavioral strategies between males and females on genomic organization. For example, the forces driving the evolution of sexually antagonistic genes may select for the retention of regions of sex chromosomes with functional genes. Ultimately, modern molecular genetic techniques and genomic data in conjunction with well-studied model behavioral systems will enable us to examine how behavioral evolution is constrained by genetic processes and, in turn, how behavioral processes can constrain the evolution of genomic structure.

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