PREDATION-ASSOCIATED DIFFERENCES IN SEX LINKAGE OF WILD GUPPY COLORATION

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Evolutionary theory predicts that the sex linkage of sexually selected traits can influence the direction and rate of evolutionary change, and also itself be subject to selection. Theory abounds on how sex-specific selection, mate choice, or other phenomena should favor different types of sex-linked inheritance, yet evidence in nature remains limited. Here, we use hormone assays in Trinidadian guppies to explore the extent to which linkage of male coloration differs among populations adapted to varying predation regimes. Results show there is consistently higher degree of X- and autosomal linkage in body coloration among populations adapted to low-predation environments. More strikingly, analyses of an introduced population of guppies from a high- to a low-predation environment suggest that this difference can change in 50 years or less.

It is a well-established theoretical result that the mode of inheritance of sexually selected characters will influence the outcome of selection, and the maintenance of sexual dimorphism (Fisher 1931; Rice 1984; Charlesworth et al. 1987; Reinhold 1998; Lindholm and Breden 2002; Kirkpatrik and Hall 2004a). For instance, consider a new beneficial autosomal mutation. It will likely be recessive and hence rarely be exposed to selection. If however this new mutation were linked to the sex chromosomes, it would be directly exposed to selection in the hemizygous sex. Given this, one would expect sexually selected secondary male characteristics to be mainly linked to the Y chromosome in a region in which it will not recombine with the X, as it would have a greater chance of spreading to fixation if only transmitted along the male line. Autosomal or X linkage of the trait may be favored due to a variety of mechanisms including: indirect female benefits through the bearing of attractive sons and strong genetic correlations between male attractiveness and female preference (Kirkpatrick and Hall 2004a); greater sex-specific expression when dominance differs from 0.5 (Reinhold 1998); and increased gene dosage of the

sexually selected trait through mechanisms such as increased genetic variance or up-regulation of X-linked genes in males compared to females (Charlesworth et al. 1987: Fairbairn and Roff 2006). Given the above theoretical considerations, the degree and nature of sex linkage should vary among populations in response to sex-specific selection, yet these ideas are challenging to test in wild populations. Here, we have the unique opportunity to use hormone assays of populations of Trinidadian female guppies (*Poecilia reticulata*) to explore the extent to which the sex linkage of male coloration changes between populations known to differ in predation pressure and the intensity of sexual selection on color.

Natural guppy populations can be divided into two ecotypes (Endler 1995; Reznick et al. 1996; Magurran 2005). *High-predation* populations are usually found in the downstream reaches of rivers, in which they coexist with predatory fishes that have strong effects on guppy demographics (Reznick et al. 1996, Rodd and Reznick 1997). *Low-predation* populations are typically found in upstream tributaries above barrier waterfalls, in which most predatory fishes are absent. Guppy coloration is subject to

strong natural and sexual selection in Trinidadian streams. Bright coloration attracts the unwanted attention of predators while at the same time attracting females (Houde 1997). In communities where their predators abound, guppies show less conspicuous body coloration than in low-predation areas where female preference for brighter colors is generally higher (Endler 1980; Houde 1997). Molecular studies show that the low-predation populations represent independent colonizations descended from individuals from high-predation populations that colonized the upstream reaches of each river basin (Alexander et al. 2006; Suk and Neff 2009). During these natural colonizations, guppies evolved more and bigger color spots, which are also preferred by females (Kodric-Brown 1985; Stoner and Breden 1988). This evolution has been shown to be extremely rapid when guppies from a high-predation location were artificially released from predation by transplanting them to a low-predation community (Endler 1980).

Traits under strong sex-specific selection can result in sexual dimorphism (Ellegren and Parsch 2007). This may especially be the case in guppies as males are significantly smaller and exhibit numerous body and tail color patterns, whereas female guppies are larger and typically exhibit a tan background coloration with no distinct spots. The cost-benefit interplay between increased attractiveness and risk of predation has long made guppy coloration be implicated as a sexually antagonistic trait (Fisher 1931; Brooks 2000; Postma et al. 2011). However becuase color genes are not normally expressed in female guppies, the genetics of guppy coloration is likely now driven by male sex specificity or male bias of expression (Fisher 1931) rather than sexual antagonism.

The Y chromosome of guppies has a region of suppressed recombination in the vicinity of the sex-determining gene. At least 20 color pattern genes have been identified in or near this region of suppressed recombination (Winge 1922, 1927; Haskins and Haskins 1951; Haskins et al. 1961; Nayudu 1979; Khoo et al. 2003). These genes are generally inherited as a Y-linked supergene. At least 17 additional genes that recombine between the X and the Y chromosomes have been identified, however, recombination rate is approximately 4% (Lindholm and Breden 2002). Only six genes have been shown to be autosomal, but the majority of these genes are rarely found in wild populations. Most guppy color genes have been found to be codominant.

Haskins et al. (1961) used pedigree experiments to document that, in the Aripo River in Trinidad, at least one color pattern (*sb* or saddleback) was strictly Y-linked in high-predation environments but was linked to both the X and the Y chromosome in a neighboring low-predation population. Haskins et al. also surveyed both populations for the relative abundance of color genes that were either on the X chromosome or autosomes by treating wild-caught females with testosterone. Such treatment causes non-Y-linked color genes, which are normally only expressed in males, to be expressed in females. They found that such non-Y-linked color

was far more abundant in the low-predation population than the high-predation population. These two populations are genetically more similar to each other than either is to populations found outside the Aripo drainage (Suk and Neff 2009), suggesting that the difference in abundance of X-linked and potentially autosomal color elements evolved recently within the Aripo River and may be related to predation.

For the above reasons, guppies are well suited and unique for addressing questions regarding the evolution of sex linkage. Here we may focus solely on the X/Y chromosomal sex-determining system, but several studies suggest that other sex chromosome systems have very similar properties, and that much of the information known about the X and Y chromosomes can be generalized to include them (Bull 1983; Charlesworth and Charlesworth 2005).

In this article, we evaluate the generality of Haskins et al.'s latter finding using female hormonal manipulations. We test for associations between predation and the abundance of color genes that are not linked to the Y chromosome. We include multiple streams in Trinidad, which contain populations of closely related guppies that occupy either high- or low-predation habitats. Moreover, we test whether a population that was translocated from a high- to a low-predation environment 52 years ago has diverged in its degree of sex linkage from its ancestors. Evidence for such consistent associations between predation and sex linkage places the interaction of gene location of sexually selected traits in an ecological genetic context, and could help us understand the factors leading to the preferential linkage of genes to either sex chromosome in specific cases.

Methods

We used hormone assays to evaluate the degree of sex linkage of color loci, as has been used in a variety of organisms including fish (Haskins and Haskins 1951; Hildemann 1954; Haskins et al. 1961; Koger et al. 2000). Females express very few autosomal and X-linked color traits naturally. However, when exposed to a male hormone mimic they reveal male characteristics that are X-linked or autosomal but not those that are strictly Y-linked (because females do not have a Y chromosome). Results from testosterone manipulations have been shown to correlate well with more extensive pedigree assessments of the degree of sex linkage in guppies (Haskins and Haskins 1951; Haskins et al. 1961). We used the proportion of females that developed color under testosterone as an integrative measure of the degree of non-Y-linked coloration in multiple populations of wild guppies. Given that most body color patterns in guppies are known to be sex-linked, with few autosomal loci (reviewed in Lindholm and Breden 2002), our measure is likely to be strongly correlated with the degree of X linkage.

COLLECTION AND HORMONE TRIALS

We collected experimental female fish from natural populations from five streams: two in the Northern slope (Yarra and Paria), and three in the Southern slope (Aripo, Guanapo, and Quare) of the Northern Range mountains of the island of Trinidad. Each collection included fish from high- and low-predation areas separated by a barrier waterfall, except for the Paria river in which the high-predation locality is restricted to a short stretch of river between the ocean and a barrier waterfall; high-predation guppies are rare and may only represent transient migrants from the extensive, predator-free reach upstream of the barrier waterfall. Therefore, we studied only nine populations from these five streams. Each high–low predation pair represents an independent event of phenotypic and genotypic divergence (Alexander et al. 2006; Suk and Neff 2009). We also collected fish from the low-predation environments in the Turure River, which did not contain guppies until 1957, when C. P. Haskins introduced fish from the high-predation section of the Guanapo River (Shaw et al. 1992). Finally, we evaluated fish from the high-predation environment in the Turure, which have been shown to be dominated by descendants of the introduced fish (Shaw et al. 1992; Magurran et al. 1996). A total of 178 females were used for the experiment from both high predation (HP) and low predation (LP) environments (23 Aripo HP and 19 Aripo LP; 20 Guanapo HP and 20 Guanapo LP; 12 Yarra HP and 11 Yarra LP; 21 Paria LP; 10 Quare HP and 5 Quare LP; and 19 Turure HP and 18 Turure LP). All fish were adult, mature, wild-caught females that were over 20 mm in standard body length.

Each wild fish was housed individually in two-gallon glass aquaria in our laboratory at the University of California, Riverside. We treated each tank with 100 µl of a 1 mg/mL dilution of alpha methyl-testosterone in 95% ethanol. This addition was repeated every three days. Each aquarium was cleaned with water changes every 15 days before the addition of the treatment. Digital photographs were taken of each fish under a light source that closely mimicked the spectrum of natural sunlight before, during, and after the treatment (BlueMax™ full spectrum bulb, Jackson, MI, USA). Every three days, we recorded the presence or absence of either melanistic (black, fuzzy black) or xanthophore (orange, yellow) coloration on the body of the fish visually for comparison with the photographs. Structural coloration is not readily seen in photographs (Kemp et al. 2009) and hence was not included in analyses. These distinctions of coloration follow the typical separation of color types in previous studies exploring guppy coloration (Endler 1980; Schwartz and Hendry 2010). It has also been shown in guppies that orange spots are highly preferred by females and black spots are used as a color enhancer and hence both groups are important in terms of fitness (Houde 1997). The final coloration was assessed from the digital pictures by one individual (S. P. Gordon), using a color standard that was present in all photographs to better standardize the assessment of coloration on guppies. Presence or absence was used because of the vast difference between the high-predation guppies that have little to no color on their bodies after treatment with the low-predation guppies that are very colorful. Each trial ran for a minimum of 60 days, with a high proportion lasting as long as 90 days (129/178 fish) to ensure no coloration was missed because of delayed expression.

STATISTICAL ANALYSIS

We first analyzed the nine natural populations for an association between predatory community and stream and the abundance of either melanistic or xanthophore color in females, as revealed by testosterone treatment. We built generalized linear models in which the response variables were binomial (presence or absence of melanistic or xanthophore color) and followed logit linear functions of predation level (HP vs. LP), stream of origin, and their interaction. We tested for significance using likelihood ratio analyses of variance. The interaction term was removed if the χ^2 statistic was lower than 1 and/or the *P*-value higher than 0.5.

We wanted to ensure that lack of color could be interpreted as an absence of color rather than a delayed response cut short by the end of the experiment. To do so, we plotted the cumulative probability of color appearance through time, estimated as the complementary of the Kaplan–Meier survival estimator with predation as a group factor (Kaplan and Meier 1958).

To assess whether sex linkage evolved in the introduced Turure low-predation population after translocation from a high-predation locality, we compared ancestral (Guanapo highpredation) and derived (Turure low predation) populations via a logistic generalized linear model (GLM) as described above. Moreover, to assess how this degree of evolutionary change compares to general predictions of high- and low-predation populations, we used the data from the nine natural populations to create a bivariate logistic model of the treatment effect probabilities for both predation types. Jointly modeling the two response variables (effect on melanistic and xanthophore colors) allows us to account for their covariation by modeling the odds ratio of both events. Models were fit using functions glm and vgam in program R version 2.9.2.

Results

Female guppies from high-predation environments expressed consistently less coloration than their counterparts from low-predation environments in response to testosterone treatment (see Supporting Information for picture examples). Figures 1A and 1B show the results for the proportion of fish showing a testosterone effect on melanistic and xanthophore color, respectively. Strikingly, most high-predation fish show no coloration at all
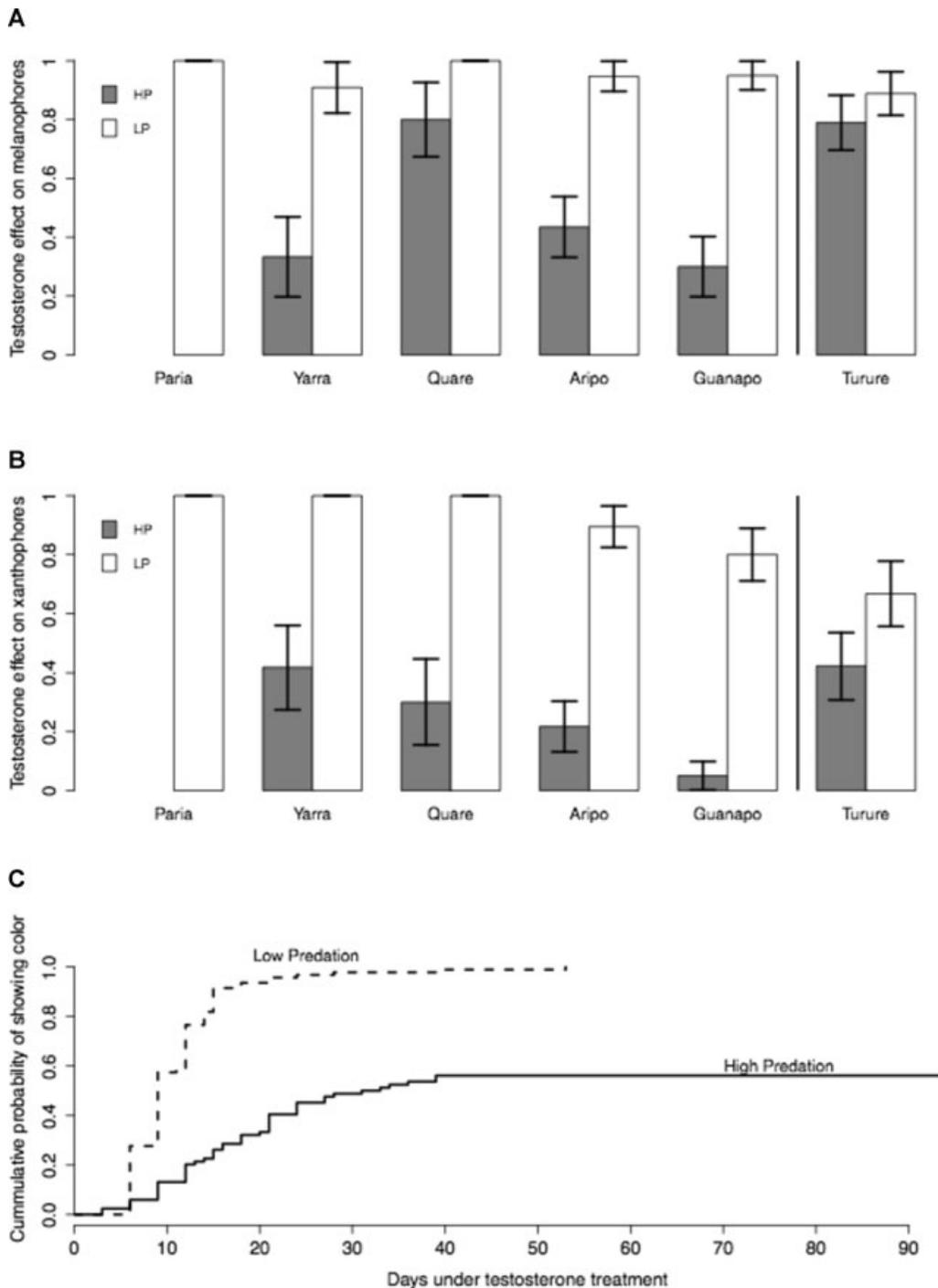
Figure 1. Results of the testosterone trials: proportion of females showing coloration in response to treatment. This is used as a proxy for the amount of X- and autosomal linkage in each population. (A) Binomial trials, separated by predation and stream, for the effect on melanistic coloration and (B) for xanthophore pigmentation. The solid vertical bar separates natural populations from the Turure populations (introduced from Guanapo HP). Error bars represent standard errors. (C) The cumulative proportion of fish showing color throughout the duration of the experiment. Note that no fish shows an increase in treatment effect beyond day 40.

after treatment even though male fish in those populations usually have both types of coloration. No fish showed continued color change after day 40 of the experiment, even though we kept some for over 90 days (Fig. 1C), so the duration of our experiment was sufficient to reveal all color genes that were present. Interaction terms between predation and stream were nonsignificant in both the model for melanistic color ($\chi^2 = 0.36$, $P = 0.95$) and the one for xanthophore colors ($\chi^2 = 1.39$, $P = 0.71$),

Table 1. Analyis of variance results for the binomial models of the testosterone trials.

Effect	LR χ^2	df	*P*-value
Melanistic color model			
Predation	44.91	1	<0.001
Stream	10.08	4	0.039
Xanthophore model			
Predation	66.93	1	<0.001
Stream	14.97	4	0.005

LR, likelihood ratio.

so they were deleted from subsequent analyses. Both predation and stream showed highly significant effects on the proportion of females showing color after testosterone treatments (Table 1). The results indicate a consistently higher degree of X- or autosomal linkage for color among low-predation fish (Fig. 1).

Females from the Turure low-predation population, which descend entirely from Guanapo high-predation fish introduced just over 50 years before the current study, respond to testosterone more similarly to low-predation populations than to high-predation populations (Fig. 2). The response levels of the Turure high-predation population that is largely comprised by descendants of the upstream introduced fish (yet mixed with some native fish, Shaw et al. 1992) lie between both predictions (Fig. 2). This demonstrates that there has been a significant increase in the extent of non-Y-linkage of this sex-specific male trait. Simple comparisons between the Turure introduced fish and its ancestors also show significant differences in both melanophore ($\chi^2 = 14.73$, $P = 0.0001$) and xanthophore occurrence ($\chi^2 = 17.96$, $P < 0.0001$).

Discussion

To our knowledge, this is the first assessment of a consistent association between some feature of the environment and intraspecific variation in the degree of trait sex linkage. Our results show a consistent increase of non-Y-linked genes as fish invade the low-predation reaches of each river. We find the same pattern in a population experimentally introduced from a high- to a low-predation environment indicating that sex linkage can *significantly* and *rapidly* change in response to ecological factors. Below, we discuss possible explanations for these results at two different levels: mechanistic and selective.

With respect to mechanistic explanations, our results can represent two nonmutually exclusive scenarios. First, the results could be due to the appearance of non-Y-linked coloration independently of the amount of Y-linked coloration. In this case, it is likely that all of the increased X-linked or autosomal color that we see in the low-predation populations was either standing
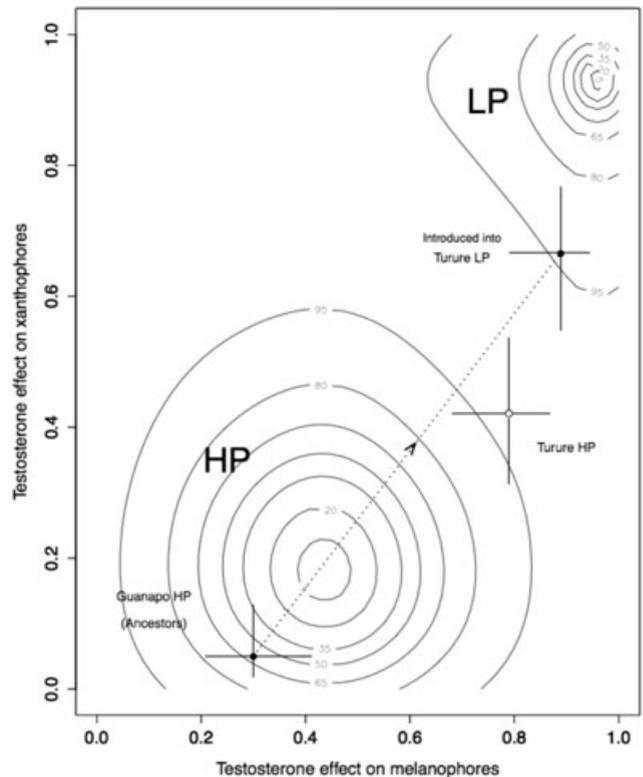

Figure 2. Evolution of Turure fish from HP to LP testosterone response. The plot shows bivariate predictions on testosterone effects on melanistic and xanthophore-based color for HP and LP populations. Contour lines delimit prediction boundaries of natural HP and LP populations up to the 95% quantile. The three compared populations are shown as points with bivariate error bars. The Turure LP population derives from Guanapo HP fish introduced in 1957. The dotted line connects the ancestral Guanapo HP population to the derived (introduced) Turure LP population (black circles) showing the shift in the degree of non-Y-linkage of male coloration to now lie within the natural LP population contour lines. The Turure HP population (open circle) is a mix of introduced and native fish but demonstrated to be dominated by descendants of that introduction, and lie between the HP and LP contour lines.

genetic variation present at low frequencies (or suppressed) in the ancestral high-predation populations, or arose via mutation in which a new gene would produce a novel color pattern. Selection then favors this non-Y-linked coloration as guppies invade the low-predation reaches of the stream.

Second, the increase in X- and autosomal linkage could be associated with an increase in recombination rates between genes on the X and Y chromosomes as guppies invade the low-predation environments. We do not directly test this in our experiment; however, if we assume that the number of distinct loci of color genes, and not necessarily the frequency of alleles, is the same for high- and low-predation populations in the same river (an assumption particularly suitable for the Turure introduction, separated

from its ancestral population for only 50 years), then the differences among populations reflect different ratios of Y-linked and non-Y-linked alleles. Haskins et al. (1961) pedigree studies of guppies from the same high- and low-predation sites on the Aripo River as used in this study support this conclusion for one gene. They found that the "sb" gene is solely Y-linked in the high-predation population, but links to both the X and Y chromosome in the low-predation population in the same river.

Regardless of the cause of our results, they clearly demonstrate an increase in the amount of non-Y-linked coloration in low-predation populations compared to high-predation populations. Although our data cannot reveal the mechanistic scenarios behind such predator-associated differences in sex linkage, it does invoke the importance of selective forces in shaping it. We now discuss two potential forms of selection proposed in prior theoretical studies that are consistent with both mechanistic scenarios of our results.

First, changes in sex linkage may be related to selection for gene dosage. Farr (1983) proposed that when the genes that cause a given color pattern are linked to both the X and Y chromosomes, they can act additively to produce a more conspicuous color pattern. Selection in favor of enhanced male coloration in low-predation environments could favor variants with two allele copies, that is, autosomal and X-linked, rather than Y-linked, genes. In this scenario, selection on sex linkage is an indirect consequence of selection for stronger coloration.

The second selective scenario involves the stronger sexual selection under low-predation than high-predation environments (Kelly and Godin 2001). Characters inherited through both paternal and maternal lines can achieve higher levels of phenotypic diversity, which may be selected for in a system in which female preference for rare color phenotypes has been suggested (Houde 1997; Hughes et al. 1999). Additionally, stronger sexual selection in low-predation environments could favor the evolution of a genetic correlation between female preference and attractive male traits (Lande 1981). Predominant linkage of male attractive traits to the Y chromosome is not conducive to this coupling because daughters will not inherit the Y-linked genes (Kirkpatrick and Hall 2004b).

At this moment, we can offer little more than speculation regarding the importance of the aforementioned molecular or selective scenarios in explaining our results. Sex chromosomes and autosomes are subject to different sex-specific selection pressures (Fisher 1931; Rice 1984), which alter rates of molecular evolution, and presumably patterns of linkage. Theory also states we should have varying rates of evolution if sexually selected traits are linked to the sex chromosomes rather than the autosomes, and if these traits are Y-linked compared to X-linked (Kirkpatrik and Hall 2004a). Nonrandom accumulation of these traits on the sex chromosome, coupled with lowered rates of recombination can

hence lead to faster rates of divergence, a phenomenon that has been seen as important for ecological speciation in recent years (see review by Qvarnstrm and Bailey 2009). Coupled with previous results and theory, our study suggests a rapid restructuring of linkage patterns in guppies via selection, leading to different accumulation of genes on the sex chromosomes. These features showcase the unique and important opportunity to use this system to examine how and how fast changes in sex-linked genes can affect the process of divergence of varying taxa over time.

The findings from this study are also of general significance to those examining how temporal variations in sex linkage affect the mechanisms of sex chromosome evolution, and those examining variations in gene movement between chromosomes, a topic that has recently been tackled in various *Drosophila* species (Singh and Petrov 2007). Clearly, more detailed pedigree and molecular work is needed to fully understand the nature of the differences in linkage reported here. Nevertheless, the consistent correlation between linkage and predation, including the documented case of contemporary evolution in the Turure, provides empirical support for theories suggesting that genetic parameters such as sex linkage may be subject to selection.

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Associate Editor: J. Mank

Supporting Information

The following supporting information is available for this article:

Figure S1. Example photograph of normal male and female, and eight testosterone females (four each randomly chosen from same high and low predation population).

Supporting Information may be found in the online version of this article.

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