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Evolution of Male Coloration in The Wild: The Role of Sex Linkage and Selection

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by

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ABSTRACT OF THE DISSERTATION

Evolution of Male Coloration in The Wild: The Role of Sex Linkage and Selection

by

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Male secondary sexual characters can be quite distinct, striking, and elaborate in nature. Despite many advances in the field of sexual selection, much remains to be discovered regarding why some organisms evolve these features more than others in a variety of taxa including plants (e.g. Geber, Dawson, and Delph 1998), insects (e.g. Stubblefield and Seger 1994), fish (e.g. Basolo and Trainor 2002), birds (e.g. Hill and McGraw 2006), and reptiles (e.g. Schulte-Hostedde and Schank 2009). Since Darwin published *On the Origin of Species* in 1859, many studies have measured the strength of natural selection in the wild showing that it is often strong and rapid (Both and Visser 2001; Pelletier et al. 2007; Kinnison et al. 2008; reviews: Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Stockwell et al. 2003; Strauss et al. 2008; reviews: Endler 1986; Kingsolver et al. 2001; Hairston Jr. et al. 2005). Despite all these examples, a recent

review has shown that very few of the traits measured in these studies involve secondary selected traits (Svensson and Gosden 2007). This is unfortunate because sexual selection is often stronger than natural selection and should be able to drive rapid evolution of particular traits. Moreover, the evolution of one these particular traits in nature, male guppy coloration, represents one of our best examples of rapid evolution (Endler 1980).

Adaptation requires both inheritance and selection, however most studies in rapid evolution either ignore heritability and concentrate on selective pressures or assume a particular mode of inheritance. Theoretical models have long established the importance of genetic architecture through sex linkage in sex-limited or sexually selected traits. However, empirical research in this topic is rare. In this thesis, I present an experimental evaluation of the manner in which ecology and genetics interact to drive the rapid evolution of a sexually antagonistic, sex-limited trait in the wild.

First, I use a standard multivariate animal model to evaluate the heritability of two sub-traits of male coloration known to be linked to male fitness; orange and black body coloration. I also partition phenotypic variance of two introduced populations of guppies *Poecilia reticulata*) into its environmental and genetic components. The genetic components are then further partitioned into Y-linked versus non-Y-linked variance to test the idea that sexually selected male traits are generally linked to the Y-chromosome where evolution is presumed to be faster as established by theory. I also studied genetic correlations among the two color patterns, and use all findings to predict the future trend of evolutionary change in this novel introduction. Using a quantitative genetics approach in this manner can help extract the genetic parameters affecting evolutionary change, and to

my knowledge this is the first study that separates Y-linked from non-Y-linked quantitative genetic variance using a wild pedigree. Results show high proportion of Y-linked to non-Y-linked genetic variance and that overall variation in Y-linkage accounts for most of the phenotypic variation in both introduction sites. Both sub-traits are also highly heritable and so combined with the abrupt change in selection pressure I predict evolutionary change to be rapid in both of these introduced populations.

Second, here I directly track changes in adaptive divergence in both introduction sites bimonthly for one year post-introduction to see if prior predictions in Chapter one were sound. My goal in this chapter was to investigate how variation in different selective pressures, such as predation and stream canopy cover, affect rates of divergence in a sexually selected polymorphic trait. Guppies were introduced from environments where they coexist with predators to two novel environments where there are no predators. In addition to the abrupt change in predation pressure, I also manipulated the canopy cover in one introduction site, hence doubling productivity in that environment. Results show rapid phenotypic and genotypic divergence in male coloration as expected, to date the fastest measure of change in wild guppies. Results also demonstrate that abrupt changes in habitat as well as predation-mediated mortality rates affect variation in rates of evolution of secondary sexual characters, an idea previously proposed but never formally tested.

In the third Chapter I test the idea that microgeographic variation in sex-linkage occurs in multiple high- versus low-predation guppy populations, the first step needed to test theory regarding interactions between sex-linkage and selection. I examine a hypothesis that high-predation guppies have mainly Y-linkage of color patterns whereas

low-predation guppies have color patterns linked to both the X- and Y-chromosome. I examine multiple high- and low-predation natural population using hormone assays in female guppies (which normally do not show coloration, and do not have a Y-chromosome) to test for differences in X-/autosomal linkage. I presume that changes in the amount of non-Y-linked inheritance are combined with changes in Y-linkage. I also examine three introduction populations (from high- introduced into low predation sites), to see if these differences in linkage relationship respond rapidly to selection pressure. Results show that indeed low-predation guppies show a significantly higher amount of non-Y-linked color patterns compared to high-predation guppies, and that this variation in linkage relationship can evolve in a matter of few guppy generations.

Together, these results point to the importance of understanding both sex-specific selection and genetic forces when understanding the rapid evolution of sexually selected traits. More over, they suggest that not only the phenotype, but also the genetic parameters behind it, such as sex-linkage, may be subject to selection.

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Introduction

Since Darwin published *On the Origin of Species* in 1859, many studies have measured the strength of natural selection in the wild and have shown that it is often strong (reviews: Endler 1986; Kingsolver et al. 2001; Hairston Jr. et al. 2005). Many populations appear to have undergone adaptive contemporary evolution of certain traits, demonstrating that organisms can rapidly adapt to changing environments (Both and Visser 2001; Pelletier et al. 2007; Kinnison et al. 2008; reviews: Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Stockwell et al. 2003; Strauss et al. 2008).

Very few of the rapidly evolving traits measured in these studies have been of a sexually-selected nature though (Svensson and Gosden 2007). This is unfortunate because sexually selected traits often represent the most complex, elaborate traits in a variety of taxa from plants (e.g. Geber, Dawson, and Delph 1998), insects (e.g. Stubblefield and Seger 1994), fish (e.g. Basolo and Trainor 2002), birds (e.g. Hill and McGraw 2006), and reptiles (e.g. Schulte-Hostedde and Schank 2009). The lack of emphasis on sexually selected traits in studies on rapid evolution contrasts with the fact that one of the first, and fastest, examples of contemporary evolution involves a secondary sexual trait: male guppy coloration (Endler 1980). In that study, Endler (1980) introduced Trinidadian guppies (*Poecilia reticulata*) that were adapted to one high-predation environment into a low-predation environment in the same river that contained no guppies. Within only two years of their transplant into the new environment the

introduced high-predation guppies had changed conspicuously in size and number of color spots (resembling a low-predation phenotype), and this change had a genetic basis.

Despite many advances in the field of contemporary evolution we still have relatively little understanding of the factors driving or maintaining variation in the adaptive potential of populations. Adaptation requires both inheritance and selection. Most studies in rapid evolution, however, either ignore heritability and concentrate on selective pressures or assume a particular mode of inheritance. Little attention is paid to variation in the mode of inheritance itself (Fig. I1). This gap in literature inspires the question: How important is genetic architecture in the evolution of traits?

Models of sexual selection have already shown the importance of underlying genetic architecture in facilitating or constraining evolutionary processes (Rice 1984; Charlesworth et al. 1987; Reinhold 1998; Lindholm and Breden 2002; Kirkpatrik and Hall 2004a). Theory suggest that one way in which genetic architecture can influence the maintenance of genes in a particular environment is through the sex-linkage of sexually-selected traits. Theory also predicts that selection will be more efficient in the fixation of sex-linked genes rather than autosomally linked ones (Rice 1984; Lindholm and Breden 2002; Kirkpatrik and Hall 2004a; Mank et al. 2007). For instance, consider a new beneficial autosomal mutation. It will likely be recessive and hence it will be obscured by the ancestral alleles, and only rarely be exposed to selection. If however this new beneficial recessive mutation were linked to the sex chromosomes, it would be directly exposed to selection in the hemizygous sex. Thus, selection would be expected to act faster on the sex chromosomes than autosomes (Rice 1984; Charlesworth et al. 1987).

Sex-linkage can influence the rate of evolution in various ways (reviewed in Kirkpatrik and Hall 2004b). First, if mutations vary in dominance then the mode of inheritance of the sexually-selected trait can affect substitution rates (Rice 1984; Charlesworth et al. 1987; Kirkpatrik and Hall 2004b). Second, differences in mutation rates between males and females (Hedrick 2007) will cause variation in the evolution of autosomal versus sex chromosome linked genes (Kirkpatrik and Hall 2004b).

Sexually-selected traits are suggested to be primarily Y-linked rather than X-linked (Table I1; Lindholm and Breden 2002). This is particularly important for sexually antagonistic traits, which offer a fitness advantage to one gender but are detrimental when expressed in the other (Rice 1984). A male-beneficial sexually antagonistic mutation, for example, would not increase in the population if linked to an autosome unless the benefit to males vastly outweighs the disadvantage to females (Ellegren and Parsch 2007). If this gene were linked however to the Y-chromosome in a region where it will not recombine with the X, it would have a greater chance of spreading to fixation as it would only be transmitted to males and would not affect the female line.

Given the above, one should expect few male beneficial sexually selected traits or secondary sexual characters to be X-linked. However, if costs to females are relaxed, 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 as one sex is hemizygous for the X-chromosome (Reinhold 1998), or increased gene dosage of the

sexually selected trait in males (Charlesworth et al. 1987: Fairbairn and Roff 2006). Additionally, a by-product of suppressed recombination on the Y-chromosome is that it will degrade over generations and genes are eventually lost. Hence, the evolution of any male-beneficial mutations may be favorably linked to the X-chromosome.

Given the above theoretical considerations, the degree and nature of sex-linkage should vary among populations in response to sex-specific selection, and may influence evolutionary rates of sexually selected traits. These ideas are challenging to test in wild populations for various reasons. In my dissertation *I use an integrative approach to empirically examine, in nature, the ecological versus genetic (linkage patterns) factors that contribute to the maintenance and evolution of guppy male coloration, a rapidly evolving, sexually selected, and sex limited trait.*

Study System

Three remarkable features make guppies an ideal study system for exploring this topic. First, the system display sexual dimorphisms for various traits, some of which like coloration have been implicated as sexually antagonistic. For example, male guppies are significantly smaller and exhibit numerous body and tail color patterns, whereas female guppies are larger and few exhibit any color patterns. Female preference for more colorful males has been shown to cause the evolution of extreme male secondary sexual characteristics including male coloration and display, however strong predation cause this to be less pronounced in certain environments.

Second, the species is considered to be in the early stages of Y-chromosome evolution, since it has very similar X and Y chromosomes (except for a small non-recombination region near the sex-determining region (see Box 1), and the Y-chromosome is not very degraded. Moreover, it shows microgeographic variation in the formation of sex linkage of color genes in at least one population. Haskins et al. (1961) found that at least one color pattern (the 'sb' or saddleback gene) was solely Y- linked in high predation environments but linked to both the X- and Y-chromosome in low predation environments. This discovery, if general, places the study of variation of sex-specific linkage in an ecological genetic context. Additionally, since male coloration is known to rapidly evolve in numerous transplants, examining differences in the amount or type of sex-linkage in populations over time may allow us to make predictions regarding rates of evolution of sexually selected traits.

Third, populations of guppies separated by only a few meters vary greatly in fitness related traits. Natural guppy populations can be roughly divided into two types (Endler 1995; Reznick et al. 1996a; Rodd and Reznick 1997; Magurran 2005). *High-predation* populations are usually found in the downstream reaches of rivers, where they coexist with predatory fishes that have strong effects on guppy demographics. *Low-predation* populations are typically found in upstream tributaries above barrier waterfalls, where strong predatory fishes are absent. This broad contrast in predation regime has driven the evolution of many adaptive differences in morphology, behavior, and life history (reviews: Endler 1995; Houde 1997; Magurran 2005). For example, high-predation females mature at an earlier age and have more but smaller offspring than do low-

predation females (Reznick et al. 1996b). Additionally, high-predation males are significantly less colorful and significantly better at predator avoidance behavior than low-predation males (Endler 1980; Houde 1997). The color polymorphism is presumably because crypsis reduces predation in the high-predation environment (Endler 1980), but in the low-predation environment females prefer more colorful males (Houde 1997; Evans and Magurran 1999). Moreover, color patterns themselves are also highly variable within predation regimes. All these differences between the two ecotypes of guppies have evolved independently in many different watersheds (Reznick et al. 1996b; Alexander et al. 2006), thus providing convenient replication and allowing robust evolutionary predictions (Magurran 2005). Additionally transplants of guppies from high to low predation environments show that various morphological, life history, and behavior traits can rapidly evolve in the wild (Magurram 2005).

Dissertation Chapters

In Chapter one, I use a multivariate animal model approach to partition phenotypic variance of male coloration in introduced populations of guppies (from a high to low predation environment) into its genetic and environmental components. I further partition additive genetic variance of male coloration into Y-linked and non-Y-linked (X- and autosomal) components to examine the proportion of genotypic variation due to Y-linkage. The aim of the chapter is to examine whether indeed much of genetic variation in male coloration is sex-linked, mainly to the Y-chromosome, even in organisms with artificial or laboratory fixed levels of genetic diversity, and to establish predictions on the

evolution of color in the introduced populations. This study represents the first application of the animal model to partition genetic variation into its sex-linked components in any wild population. The results show that Y-linked variance explains most of the additive genetic variance in both introduction streams. This suggests that male coloration (orange and black patterns) is highly Y-linked, as expected from past theoretical and laboratory-based studies (see Box 1). Results also show that coloration is highly heritable and thus expected to respond strongly to the shift of selection pressures in the new environments.

In Chapter two, I explore how environmental differences influence the adaptive divergence of male coloration in wild guppies by following the experimental transplant used in the first chapter for one year. Guppies were taken from an environment where they co-exist with predators and introduced into two low-predation streams. I use bimonthly censuses of color measurement in the introduced populations to measure the temporal divergence between the ancestral and derived fish. Common garden assays performed one year post-introduction allow me to test if any changes I found in the wild have a genetic basis. Results show rapid divergence of male coloration in the wild, however change was different in the two component groups: melanistic (black) and carotenoid (orange) coloration. Common garden results show that these changes are likely a combination of genetic and plastic effects. Manipulation of the typically closed canopy in one introduction site showed little effect on the divergence of male coloration.

In my third chapter, I use hormone assays to compare the amount of X-/autosomal linkage between wild high- versus low-predation adapted guppies. I found

microgeographic variation in sex-linkage associated with predation risk. High-predation populations consistently have a significantly lower degree of X-/autosomal linkage of coloration compared to natural low-predation populations. I also show that an introduction population of a high-predation population (Guanapo river) into the low predation reaches of the Turure river show signs of rapid evolution of sex-linkage after less than 54 years. I interpret these results as suggesting population differences in Y-linkage, yet I discuss alternative interpretations in the chapter.

Finally, also in the third chapter I attempt to bring all three chapters together by including the introduction populations studied in Chapter 1 and 2 to test whether the change in linkage can occur after a year since introduction, and to assess whether ecological factors other than predation may affect changes in linkage relationships. Previous results have shown that coloration has diverged rapidly in these populations (both phenotypically and genetically) and that canopy cover manipulation or other habitat features correlated with predation can have some effect on adaptive divergence. Can they also affect changes in the sex-linkage of coloration? My findings indicate that in the span of only one year, linkage relationships have begun to shift towards that typical of natural low-predation populations, and that ecological factors such as light or resource availability may also be important.

Overall, these chapters show that when examining the evolution or maintenance of secondary sexual selected traits it is important to consider, not only the selection pressures on the traits, but also their degree of sex-linkage and the selection pressures associated with that. This dissertation has evaluated the evolution of male coloration in

the wild. I have shown for the first time using quantitative genetic methods on a wild pedigree that Y-linkage represents an important portion of the genetic architecture of male beneficial sexually antagonistic traits. I have also shown that male coloration, especially orange spots, have high heritability, and this should lead to a strong response when that trait is under selection. This rapid change relates to environmental factors beyond predation, such as canopy cover. Finally, I have demonstrated for the first time consistent microgeographic variation in sex-linkage of male coloration in both natural and introduced populations of guppies. This variation strongly correlates with the degree of predation, suggesting that it is malleable to selection pressures. This result has important implications, since it means that the selection pressures on the traits as well as on their genetic parameters must be accounted for when studying the evolution of sexually selected characters.

Box 1: Background Information on Color Genetics in Guppies

The guppy has been the subject of genetic analysis for almost a century. Research has shown that the X- and Y-chromosomes in guppies are similar in cytogenetic structure and do recombine with each other, indicating that they are in the very early stage of sex chromosome evolution (Nanda *et al.* 1990, 1992). However, current research does show that recombination is greatly reduced in the pairing region of the chromosomes (Traut and Winking 2001). A hypothesis as to why this occurs is that several male-specific genes have accumulated in the sex-determining region of the Y-chromosome selecting for suppressed recombination.

This hypothesis agrees with the information garnered from pedigree analyses in the species (reviewed in Lindholm and Breden 2002) showing that many male secondary characters are inherited in a sex-linked fashion. In terms of color genetics, the work of Winge, Haskins, Nayudu, and colleagues (see references) make it clear that a large number of the polymorphic color patterns behave as though linked to the Y chromosome (with only a few that seem to be linked to both the X- and the Y- chromosome). Over 80 years of breeding designs by various researchers have shown the consistency of these color patterns in mode of inheritance (Table I2; Winge 1922a, 1922b, 1923, 1927; Winge and Ditlevsen 1947, Haskins and Haskins 1951; Haskins et al. 1961; Nayudu 1979; Nayudu and Hunter 1979; Khoo et al. 2003).

Many guppy color patterns are sex-limited or sex-influenced to males and can only be brought out in certain females through the use of hormones. Winge (1927) proposed the first linkage map of the location of 18 color genes on the sex chromosomes in the guppy. This linkage map has since then been revised and updated by various laboratories throughout the years (Fernando and Phang 1990; Khoo et al. 1999, 2003). From what we now know, at least 20 color pattern genes have been identified on or near the sex-determining, non-recombining portion of the Y-chromosome, and these genes are generally inherited as a Y-chromosome supergene (closely

linked together) (Table I2; Van Oosterhout et al. 2003; Winge 1922, 1927, 1934; Haskins 1961; Yamamoto 1975; Lindholm and Breden 2002). At least 17 loci have been identified that recombine between the X- and the Y-chromosomes (recombination rate is approximately 4%), and at least 5 have been shown to be linked to the autosomes (these generally control body color rather than particular color spots). Most of these color genes have been found to be dominants (two recessive patterns can be studied in the pedigree analysis) and when a gene is linked to both the X- and the Y- chromosome, an additive effect is observed.

An interesting phenomenon was found in guppies by Haskins et al. (1961); geographic variation of sex-linkage patterns. Using hormone manipulation and breeding tests, the authors were able to show that for one color pattern (X- and Y-linked 'sb' pattern) linkage to the Y-chromosome was comparable in both high-and low-predation environments. However, linkage of this pattern to the X-chromosome was only shown in the low-predation environments and was non-existent in the high-predation environment. The authors hypothesize that there is a strong selective process at work confining the pattern to the Y-chromosome in high-predation environments and that this process could be natural selection by predation. Yet, amazingly even today it has not been tested whether this variation in linkage patterns extends to other color patterns and other portions of Trinidadian streams. Female preference for certain color patterns could maintain this variation in linkage patterns of color traits but has also yet to be tested.

Timeline of Quantitative Genetic Studies in Guppies:

-In the first recorded quantitative genetic study of the inheritance of male color patterns Houde (1992) demonstrated that the area of orange could be studied as a quantitative trait and that it was inherited largely from the sire, suggesting Y linkage.

-In the second recorded study, Brooks (2000) showed that sexual attractiveness of male guppies is inherited largely from the sire, a pattern consistent with Y-linked inheritance.

-Later models were done by Brooks and Endler 2001; Hughes et al. 2005, and later by Postma et al. 2011 using lab-reared populations showing once again that carotenoid coloration demonstrates high genetic variation mainly attributed to Y-linkage and that there is higher heritabilities of carotenoid rather than black coloration.

-All the quantitative genetic studies as explained by Hughes et al. 2005 and Postma et al. 2011 were performed on lab-reared natural populations derived from wild stock and hence provide a measurement of genetic parameters in natural populations, but no study has of yet focused on wild populations or populations experiencing environmental changes.

Figure I1. Figure showing the influence of genetic architecture in causing adaptive divergence. Many studies only look at the ecological/environmental contrasts causing varying selective pressures on traits leading to adaptive divergence between populations. Very few studies, however, focus on: (1) how genetic architecture may constrain, maintain, or facilitate this divergence over time; and (2) how varying selective pressures may influence the prevalence of a particular underlying genetic architecture in different environments contributing to adaptive divergence between populations.

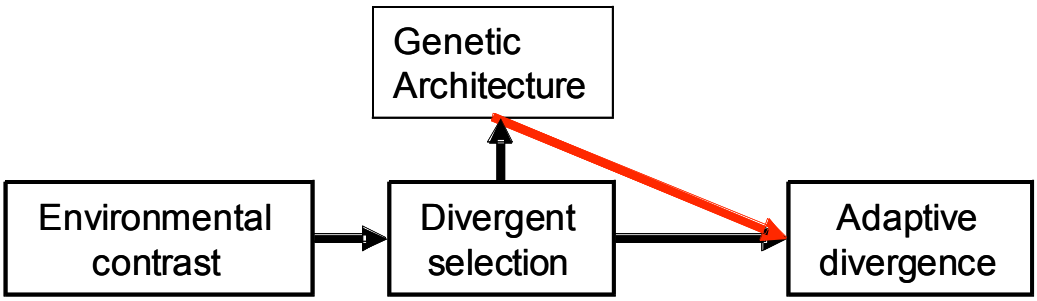

Table I1: Reported sex-linkage for sexually-selected traits

Species	X or Y Linked	Trait	Citation
Poecilia reticulata (guppy)	Both	Male color	Winge 1922, 1927; Haskins *et al.* 1961; Lahn *et al.* 2001; Brooks and Endler 2001
Poecilia reticulata (guppy)	Y	Male sexual and aggressive behavior traits; body size and gonopodium length	Farr 1983; Karino and Haijima 2001
Xiphophorus maculatus	Y	Male pigment	Kallmann 1970
Poecilia parae	Y	Male Color	Lindholm, Brooks, and Breden 2004
Mus musculus (mice)	Y	Male aggressive, sexual, and social behavior	De Vries *et al.* 2002; Selmanoff *et al.* 1975
Drosophila melanogastor (fruit fly)	X	Adult fitness components	Chippindale and Rice 2001; Gibson *et al.* 2002;
Silene latifolia (White campion)	Y	Flower number and size	Scotti and Delph 2006
Cyrtodiopsis dalmanni (Stalk-eyed flies)	X	Male eye span	Wolfenbarger and Wilkinson 2001; Wilkinson *et al.* 2005

Table I2: Sample list of linkage of known guppy color patterns, * indicates color patterns that both females and males naturally express. For example, the guppy pictured for the color pattern 'Blond' is female. All trait information garnered from the following studies (Winge 1922, 1927 1934; Winge and Ditlevsen 1938; Haskins and Haskins 1951; Haskins et al. 1961; Nayudu 1979; Phang et al. 1999; Brooks and Endler 2001; and Khoo et al. 2003)

Trait	Linkage
A) Maculatus (red)	Y
B) Armatus	Y
C) Pauper	Y
D) Aureus	Y
E) Ferrugineus	Y
F) Sanguineus	Y
G) Oculatus	Y
H) Iridescens	Y
Orange area/Black area/Mean Brightness	Y
Sb	X and Y
I) Elongatus	X and Y
J) Vitellinus	X and Y
K) Coccineus	X and Y
L) Cinnamomeus	X and Y
M) Nigrocaudatus I and II	X and Y*
N) Lineatus	X
O) Zebrinus	Autosomal*
P) Blond	Autosomal*
Bar	Autosomal*

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Chapter 1

Genetic and Environmental Determinants of a Rapidly Evolving Secondary Sexual Trait

Abstract

Evolutionary theory predicts that sexually antagonistic or sex limited traits linked to the Y chromosome may experience faster rates of evolution than non-Y linked traits. However, evidence of an association between sexually selected traits and Y-linkage in rapidly evolving natural populations is limited. Here, I estimate the contribution of Y-linked and non Y-linked quantitative genetic variation to two sub-traits of coloration, orange and black body patterns in male guppies (*Poecilia reticulata*) experiencing abrupt changes in their environment. These sub-traits, previously implicated as important for male fitness, are sexually antagonistic and prior research has indicated rapid evolution of certain aspects of male coloration in transplant experiments. My findings indicate that a high proportion of additive genetic variance of male coloration in wild guppies is Y-linked rather than non Y-linked, and show the evolution of Y-linkage may be important for sexually antagonistic male traits. The heritabilities of these color elements are also high suggesting these traits may have strong evolutionary responses to shifts in selection pressure. Generally, the data reveal that sex linkage may be important to consider when examining the genetics of secondary sexual traits, and may have important implications for understanding their evolution.

Introduction

Understanding how organisms adapt to abrupt changes in their environment is an increasingly important research topic. Theoretical models have shown the value of understanding the genetic architecture through sex linkage in these processes for sexually selected traits (Rice 1984; Reinhold 1999; Kirkpatrik and Hall 2004a), but empirical studies have been limited. Theory predicts that selection will be more efficient in the fixation of sex-linked genes rather than autosomally linked ones (Rice 1984; Lindholm and Breden 2002; Kirkpatrik and Hall 2004a; Mank et al. 2007). For instance, consider a new beneficial autosomal mutation. It will likely be recessive and hence it will be obscured by the ancestral alleles, and only rarely be exposed to selection. If, however, this new beneficial recessive mutation were linked to the sex chromosomes it would be directly exposed to selection in the hemizygous sex. Thus, selection would be expected to act faster on the sex chromosomes than autosomes (Charlesworth et al. 1987). Theory also suggests that any sexually antagonistic trait that specifically benefits males should accumulate on the Y-chromosome because they are inherited haploidly (Roldan and Gomendio 1999). This haploid-like inheritance of Y-linked traits is predicted to cause a faster rate of evolution of those traits in response to environmental change (Kirkpatrick and Hall 2004a). These theoretical findings suggest that beneficial male traits should have a high proportion of Y-linkage and that one should see an association between the amount of Y-linkage and high rates of evolution in these traits.

On the other hand, it is predicted that the degenerate nature of the Y chromosome make it less likely that functional genes (even those of a sexual antagonistic nature)

would be linked to it (Rice 1996; Lindholm and Breden 2002; Fairbairn and Roff 2006). It is likely that male-beneficial sexually antagonistic mutations are recessive. Therefore, these genes would have a good chance of spreading to fixation in a new environment if linked to the highly functional X chromosome as they would mostly be expressed in the males (XY), and less so in the females who need the mutation to be on both X's for the gene to be expressed (Charlesworth *et al.* 1987; Rice 1996; Reinhold 1998; Gibson *et al.* 2002; Clark 2003).

Laboratory studies have often demonstrated Y-linkage for secondary sexual characters (Table I1 in Introduction), and a consequently high degree of heritability (Postma et al. 2011). This suggests that the selective mechanisms countering the reduction of Y-linked variation are often strong. However, genetic variances in laboratory experiments tend to be higher due to the constancy of the environment, and no one has yet to evaluate the magnitude of Y-linked genetic variation in a wild population using a quantitative genetic approach. This is important because recent research has emphasized that heritability can change depending on the quality of the environment (Hoffmann and Merila 1999; Garant et al. 2004; Wilson et al. 2006). For example, Wilson et al. (2006) found that the heritability of offspring size in Soay sheep is high in environmentally favorable years, when selection is weak, and low under harsh environments, when selection is strong. Hence, a lab-based setting with constant environmental features may not explain results from field-based studies.

Male guppy (*Poecilia reticulata*) coloration, a sexually antagonistic trait, has been shown to be strongly Y-linked in laboratory studies (Box 1 Introduction; Winge et al.

1927, Haskins et al. 1961; Brooks and Endler 2001; Hughes et al. 2005; Postma et al. 2011). For example, in their quantitative genetic analyses Postma et al (2011) used a six-generation breeding design on laboratory guppies descended from a wild stock to show that male coloration is mainly Y-linked in undisturbed stable populations. However, it is unknown how ecological effects influence this pattern in wild populations (natural or manipulated). The only study comparing the inheritance of color across different environments (Haskins *et al.* 1961) found that certain color patterns can be linked to either the X or the Y chromosome depending on which part of the river the population originates from. Guppies inhabiting the downstream portion of the rivers are exposed to large predatory fish (which prey on more colorful guppies) and show a higher degree of Y-linkage for color traits (Haskins et al. 1961). Guppies in the upstream portions of the river, which typically have barrier waterfalls preventing the upstream migration of large predators, are both X and Y linked for color traits (Haskins et al. 1961; Chapter 3). Guppy coloration has also been shown to have one of the fastest rates of evolution in the wild when guppies are transplanted from a high to a low predation environment (Endler 1980). Guppies therefore represent a good model system to examine associations between amounts of Y-linked genetic variation and mechanisms of evolution in sexually selected traits.

Here, I use a quantitative genetics approach to ask the following questions: (1) *How much genetic variation of a rapidly evolving secondary sexual male trait is Y-linked in wild introduced populations?*; and (2) *How does the degree of Y-linkage affect the heritability, and thus evolutionary potential of the trait in these populations?* As

heritability is tightly correlated to the extent at which traits can respond to selection, I use my results to make predictions regarding what changes I expect to find in male coloration as high-predation guppies adapt to novel low predation environments over time. I use data derived from a pedigreed population of wild high-predation adapted guppies introduced into two low-predation streams (hence relaxing normal predation selective pressures). One stream has been left natural, with closed canopy cover. In the other stream, the canopy has been thinned to elicit higher productivity. Since a previous study has found that altering the canopy in this manner does not affect male coloration (Schwartz and Hendry 2010) I here frame the two introduction sites as replicates, however future studies are undergoing which will replicate this entire design in two more introduction sites. This chapter is the initial step in an experimental design following the fate of Y-linked variation of a sexually selected trait under selection, and I aim to perform future analyses of these two populations (as well as the upcoming two novel introduction sites) longitudinally as each population adapts to their novel environments.

To estimate quantitative genetic parameters I use the *animal model*, which enables the use of arbitrarily complex pedigrees and does not require designed laboratory crossings (Kruuk 2004; Wilson et al. 2006). In the wild the animal model has been applied primarily to mammals and birds, for which there are long-term mark-recapture studies that provide large data sets on pedigreed populations (see Kruuk et al. 2000; Merila and Sheldon 2000; Milner et al. 2000; Reale et al. 2003; Postma and van Noordwijk 2005). These methods have rarely been applied to other organisms such as fish (Naish and Hard 2008). This is unfortunate because an advantage of fish is that they have short generation

times and include some of our clearest examples of rapid evolution (Morrissey 2010). Classical examples of rapid evolution include fish species such as guppies, *Poecilia reticulata* (Reznick et al. 1997); Hawaiian mosquito fish, *Gambusia affinis* (Stearns 1983); sticklebacks, *Gasterosteus aculeatus* (Barrett 2010); and numerous species of cichlid in Lake Victoria (Johnson et al. 1996).

Given theoretical predictions on the sex-linkage of sexual antagonistic traits and the observed speed of color evolution in previous studies, I predict a high degree of Y-linkage in my populations, resulting in high heritabilities. To my knowledge this is the first time Y-linked versus non-Y-linked genetic variance is partitioned in this manner in a wild population.

Methods

Study system

Guppies are of interest for this type of study for various reasons. First, adaptation to differences in predation regime has caused there to be two general different ecotypes of guppies: high- and low-predation. Male guppies from high-predation environments have fewer color spots and smaller spots on average than do their counterparts from low-predation environments (Endler 1980; Houde 1997). The expression of virtually all coloration is sex-limited, so females lack coloration. Females typically choose more colorful males (especially in orange coloration) as an indication of male quality, however the nature of the color that is preferred varies among populations (Endler and Houde 1995; Houde 1997). Endler (1980) interprets the differences in male coloration in high

versus low predation environments as reflecting the combination of selection for crypsis to reduce predation risk in the high predation environment and female preference for more colorful males. Female preference dominates selection in the low predation environment (Houde 1997; Evans and Magurran 1999). These differences between the two populations of guppies have evolved independently in many different watersheds (Alexander et al. 2006), thus providing natural replication and allowing robust evolutionary predictions (Magurran 2005). Transplant experiments have shown that if guppies are moved from a high predation to a previously guppy-free low predation environment, they quickly evolve brighter color patterns (Endler 1980; Kemp et al. 2009).

Study populations

This study capitalizes on an introduction experiment carried on in 2008 as part of a larger research program I am a part of studying eco-evolutionary interactions in guppies. In March 2008, members of our team collected 150 juvenile guppies from a high-predation environment in the Guanapo River where they cohabit with large predators and brought them back to the lab. There, the guppies were reared to maturity in a laboratory, then mated in groups of ten (five males per five females) in one tank. The collected guppies were then reintroduced into two separate low-predation tributaries in the same river, both of which previously had no resident guppies. The males and females from each mating group were introduced into a different stream to avoid a loss of genetic diversity in each site typically associated with field studies where the wild organisms are

kept in confined conditions prior to introduction. Introduced females contained the sperm from one group of male guppies, but were introduced with a different group of male guppies. Before introduction, we photographed each guppy with a color standard for future quantification of color, individually marked it with subcutaneous injections of elastomer paint, and removed three scales, which were dried and stored for future genetic analyses and pedigree reconstruction.

Each month, most guppies in each stream were recaptured, measured, and photographed. All new recruits were individually marked, and scales sampled for adding to the reconstruction of the pedigree. Recapture probabilities varied among months and streams but were on average high (approximately 90 percent) and consequently, the probability of missing an individual for its entire lifetime was low. I quantified male coloration from the photographs using the program ImageJ (Figure 1.1). With ImageJ, I can quantify the body area of each fish, and identify and measure the area of each colored spots. The colors observed were categorized into two sub-traits: black and orange as both colors components have been shown to be important for male fitness. Preference for orange coloration has been shown in female guppies of all types (Houde 1987; Van Oosterhout et al. 2003), and orange pigmentation is linked to male quality (Grether 2000). Brooks (1996) found that black coloration may function as a signal amplifier for other colors, enhancing the ability of females to discriminate among males based on coloration. Black and orange coloration were also the only patterns that can reliably be estimated from digital photographs (Kemp et al. 2009). The total area for each color on the fish were summed to obtain total color area. In order to control for body area in the

analyses, I used relative rather than absolute color area by dividing total area by body area.

One of the tributaries was further manipulated by trimming the canopy above the stream by approximately 50 percent (as measured with a densitometer). This resulted in a significant increase of stream productivity and resource biomass (Chapter 2; Kohler 2010). Canopies were rethinned on subsequent occasions (by D.N. Reznick and colleagues) to maintain these differences in light level and productivity between the two introduction streams. A recent paper (Schwartz and Hendry 2010) has shown that trimming the canopy had little effect on male coloration over time and so I expect similar results in my analyses of color variation, although I may see slight differences in heritability due to the different levels of productivity between each stream.

Pedigree reconstruction

The pedigree (Figure 1.2) was reconstructed by genotyping all individuals at 12 tetranucleotide microsattelite loci that had an average of 20 alleles each at the beginning of the introduction (P. Bentzen et al. Unpublished results). Pedigrees were reconstructed with the program *CERVUS.* After one year, the mother and father of each individual could be assigned with greater than a 90 percent level of confidence. After one year (or three to five generations), there were 1467 marked and pedigreed individuals in the mark-recapture dataset, and approximately 700 measurement data points of male coloration over the year. Pedigree data indicated that the introduced individuals showed an average level of heterozygosity of 0.77 for the genotyped loci. This is very close to the average

heterozygosity for these loci in multiple wild populations (Paterson et al. 2005). The loss of heterozygosity was minimal for the subsequent cohorts in the introduced population (0.75 and 0.76 in the closed and open canopy respectively, P. Bentzen et al. Unpublished). This suggests that the level of neutral genetic variation in the introduced populations was representative of natural conditions. Pedigree data also indicated that most of the founding males contributed to offspring in both introduction sites (P. Bentzen et al. Unpublished).

Animal model

For each stream I fitted an independent model using a Bayesian framework implemented via package MCMCglmm (Hadfield 2010), in program R. I included both proportion of orange and proportion of black coloration as my response-variable traits in a same bivariate model. The model included as fixed effects only the population mean of the trait for the stream. The following variance components were modeled through random effects: non-Y linked additive genetic variance, Y-linked variance, maternal effects (genetic and environmental), and temporal variation. Coloration of individual males was measured multiple times throughout their life and therefore individual identity was included as a random effect to account for the repeated measure structure of the data. My complete model thus reads:

$$Y = \mu + Za + e$$

Where Y is the matrix of phenotypic values of each trait; μ is vector of trait means; *a* is the vector of random effects; e is the residual error; and Z are the design

matrices relating the random effects to each individual as seen in (Garant et al. 2004; Kruuk 2004; Morissey 2010).

Additive genetic variance was decomposed into Y-linked and non-Y-linked (i.e. autosomal or X-linked). Y-linked variance was defined as that associated to the patriline (i.e. founding sire) to which the individual belonged. The rest of the additive genetic variance is whatever is associated to the relatedness matrix when both effects (patriline and relatedness) are included (Postma et al. 2011). Heritability is the amount of additive genetic variance (Y and non-Y) divided by the total phenotypic variance.

Maternal effects were included as the proportion of variance explained by the mother's identity. Maternal effects occur when the phenotypes of offspring are affected by the phenotype of the mothers irrespective of their genotype (Kruuk 2004; Wilson et al. 2006), and may be environmental or genetic. Both theoretical and empirical studies have shown that maternal effects have the capacity to affect evolutionary responses of traits (discussed in Kruuk et al. 2004), however many times they are not included in quantitative genetic models. This can bias estimates of additive genetic variance (Falconer and Mckay 1996). In the animal model maternal effects are included as random effects and there is no easy way to decipher genetic from environmental influences.

Temporal fluctuations of the environment can cause changes in the trends of breeding values (Morissey 2010). I have, therefore, included temporal changes across month in each environment as a random effect. The repeated measures structure was accounted for by including individual identity as another random effect. I calculated the repeatability of male coloration as the ratio of within individual variance to the total phenotypic variance

(Becker 1992). Individual effect was included in the model so that I could ascertain this. This way, the unexplained variance gets parsed into differences between individuals and variation within individuals (i.e. residual variance).

The full model allowed for non-zero covariances between the two traits in all random effects. Correlations due to either component were thus calculated as the trait covariance due to that component divided by the product of the trait standard deviations due to that component. I tested whether correlations were statistically different from zero by comparing the full model with a series of alternative models lacking some or all of the correlations. Because models were fit within a Bayesian framework, I used the deviance information criterion (DIC) as a measure of model parsimony for model comparison (Wilson et al. 2010).

Finally, the significance of variance factors was tested statistically via model comparisons where each variance component was removed from the model and its DIC compared to the best model garnered from above. Individual and residual variances were not removed because they are considered necessary to account for the repeated measures nature of the data.

Results

Variance components with standard errors for orange coloration are seen in Figure 1.3 (first panel). Heritability of male orange coloration is moderate in both streams: 0.34 in the closed canopy and 0.21 in the open canopy. The genetic component of phenotypic variation is explained mainly by Y-linkage for orange coloration (Figure 1.3). More

specifically, 74% of the additive genetic variation in orange coloration in the closed canopy and 62% in the open canopy attributed to Y-linkage (meaning 26% and 38% respectively are due to non-Y linked additive genetic variance). Y-linkage also explains 26% in the closed canopy and 13% in the open canopy of overall total phenotypic variation. Non-Y-linked additive genetic effects account for less than ten percent of total phenotypic variation in both streams (9% and 8% in the closed and open canopy streams respectively). The rest of the total phenotypic variance is attributed to environmental factors, divided into individual fish measurements, month, maternal effects, and residual errors. Maternal effect accounts for little of the total phenotypic variation (4% closed canopy, 10% open canopy), but it is not known what proportion of this can be attributed to genetic or environmental factors. In both introduction streams the temporal (month) effect on the variation in male coloration was similar (13% in closed and 11% in the open). This indicates that temporal variations explained by measuring coloration in different months are similar between the two streams. In the open canopy individuals account for a significantly larger part of phenotypic variation than in the closed canopy stream, a striking result (Figure 1.3). Residual errors (i.e. within individual differences in color in repeated measurements that is classified as error variance) also account for a large proportion of variation (40% in the closed canopy and 26% in the open canopy).

Variance components for black coloration are summarized in Figure 1.3 (second panel). Black also shows a strong Y-linked effect. Y-linkage accounts for 17% and 26% of the total phenotypic variance in the closed and open canopy streams (70% and 74% of the total genetic variance). This compares to 7% and 9% percent non-Y linked additive

genetic variance. Heritability was 0.24 and 0.35 for the closed and open canopy streams respectively. Environmental components show similar results to what was found for orange coloration (month effects account for 14% and 11% and maternal effects for 7% and 6% respectively), except the residual error was larger (50% and 41%), and there were no large individual effects (5% and 6%).

Table 1.1 shows the model selection results for the model covariance structures. The best model seems to be the full model with all variance components but without the correlations. The table however shows no clear evidence for or against Y-linked or environmental correlations between orange and black, given that both the full model including all correlations and the no-correlation model perform very similarly in both streams (both models differ by a DIC value less than 2). This therefore may suggest that my genetic and environmental variance correlations between sub-traits (orange and black coloration) in both streams are not significant.

When different variance components are removed to assess their significance and its DIC compared to the best model (ie full model without correlations) results show support for the inclusion of Y-linkage and month (particularly in the closed canopy), but weak or no support for the inclusion of maternal effects and non-Y-linked variance (Table 1.2). This suggests the importance of Y-linkage and month components in the model but indicate maternal effects and non-Y-linked variance components may not be as important.

Discussion

My intention in conducting this study was to describe the phenotypic variation in male coloration of wild guppies introduced to new environments and to describe if there is strong Y-linkage, which I have indeed found. Here I directly showed that the proportion of Y-linkage to non-Y-linked additive genetic variation in wild populations experiencing a shift in selective regimes remains large. This highlights the importance of accounting for sex-linkage in the study of secondary sexual trait evolution (Rice 1984; Fairbairn and Roff 2006).

I find little difference in variance components between the open and closed canopy stream except for a high within-individual variance for orange coloration in the open canopy population compared to the closed canopy population. At this moment I can only offer speculation why I find such different within-individual variance in one stream and not the other, and more research is needed in this regard to truly uncover the answer. The result essentially means that for the open canopy, differences between individuals are more important than within individuals for orange coloration. This can be due to stronger intra-individual differences in ontogeny, for example. As male guppies achieve sexual maturity they develop coloration however full coloration is fully expressed about a month after sexual maturity (Houde 1992). All of my guppies were measured once they receive sexual maturity (when fleshy hood on the male organ is larger than the gonopodium itself). Therefore, the same individual measured in its first month may have slightly different measurements in its second month until full coloration is expressed. However it is unknown why the difference in canopy treatment would affect the ontogeny of

coloration between the guppy populations. They can also be due to individual differences in susceptibility to seasonality or environment. Brooks and Endler 2001 did show that genetic factors which may cause differences in the synthesis of carotenoid (orange) coloration in the same fish, for instance, will appear to contribute to environmental variance, thus affecting both repeatability and heritability estimates. The difference in availability of carotenoid (in the diet) between open and closed streams (more in the open canopy) may hence also have contributed to the large difference in individual variance estimates between the two streams since carotenoid coloration is particularly sensitive to changes in diet (Kodric-Brown 1989; Grether et al 1999).

In general, I find moderate heritabilities for both sub-traits (orange and black coloration) in both streams, but a high degree of Y-linkage. These levels of heritability and Y-linkage should, according to theory, indicate a high evolutionary potential in response to selection. In fact, color has been shown to evolve extremely rapidly in guppy populations released from predators (Endler 1980). Theoretical models predict faster rates of evolution when sexually-selected dimorphic traits are linked to the sex chromosomes versus the autosomes, (Rice 1984; Fairbairn and Roff 2006), and linked to the Y- versus the X-chromosome (Kirkpatrik and Hall 2004). This level of Y-linkage and heritability should therefore be accompanied by rapid change in coloration as these guppies adapt to their new environment. Indeed results of actual phenotypic and genetic divergence of male coloration in the introduction sites seem to show this (see chapter 2).

There are some limitations to my use of the animal model in this experiment and I discuss them now. First, this experiment was performed on a wild population subject to

novel selection regimes and an assumption of the animal model is that selection is not occurring. Therefore, changes in selection may indeed be affecting my estimates of phenotypic variation in principle. Nevertheless, the animal model is often used in populations under change (For example, Garant et al. 2004; Charmantier et al. 2006; Wilson et al. 2006) with the assumption that selection is not strong enough to affect the results in early generations. Artificial selection experiments have indeed shown that quantitative genetic analyses are robust predictors of evolutionary response in the first 10-15 generations (Roff 2007). In other cases the animal model is used directly to accommodate or predict how traits will evolve under selection, inbreeding, or assortative mating by evaluating how the estimated variance components predicted from the wild pedigrees change from generation to generation (see reviews: Kruuk 2004; Postma and Charmantier 2007).

Since this experiment is still in its early stages of the transplant I decided that I could study both populations who: a) presumably have not had enough time to strongly change in coloration in response to the relaxation of predation pressure relative to an increase in female choice (ie sexual selection); and b) haven't had enough time to have a canopy effect in coloration (if any will be seen at all, see Schwartz and Hendry 2010). It should also be noted that research has now demonstrated that even in the presence of directional sexual selection there are many ways in which additive genetic variation can be maintained and not erode (Iwasa and Pomiankowski 1991; Pomiankowski et al. 1991; Rowe and Houle 1996; Gray and Cade 1999; Hughes et al. 1999). In any case, the fact that my results are in accordance with lab research in equilibrium, both in a mendelian

framework (Winge 1927) and a quantitative genetics one (Hughes et al 2005; Postma et al 2011; see Box 1 for timeline of quantitative genetic studies involving stable populations of guppies), suggest that my results have biological validity. I aim to repeat analyses in the following years so that I may track changes in phenotypic variance (especially Y-linkage) as the guppies adapt to their novel environments.

Second, this experiment is as of now only a two-group comparison and therefore does not allow me to make causal inferences about any differences between populations (open versus closed canopy). For example, recent research has suggested that heritability can change depending on the quality of the environment (Hoffmann and Merila 1999; Garant et al. 2004; Wilson et al. 2006). This implies that considering both the environmental and genetic components of phenotypic variation are important in determining the evolutionary potential of organisms facing environmental changes. Here I find no differences in the heritability of coloration between the two introduction sites even though they differ in levels of productivity (Chapter 2) and hence the two introduction sites can be framed as replicates. However, the lack of differences between the two streams could be due to the fact that the differences in productivity may not be strong enough to have caused massive changes in environment quality, or that there has not been enough time for the treatment to affect heritabilities of certain traits. In order to explore this issue further this entire experiment itself is currently being replicated in two more treatment streams.

Finally, since our design attempted to avoid a loss of genetic diversity by artificially breeding the introduced fish in the lab, there is a small chance we may have inadvertently

produced an overestimation of genetic variation (including levels of Y-linkage) in our populations to begin with. Lab studies often suffer from inbreeding or artificial levels of disequilibrium while field introductions can also artificially increase mating variance by temporarily placing subjects in confined conditions. Van Oosterhout et al 2003 showed that inbreeding depression which could occur in guppies in confined conditions could cause declines in total area of coloration after only 3 generations of inbreeding and that number of black and orange spots specifically were reduced 25.1% and 19.2% respectively. Since guppy females carry sperm and have litters with multiple paternity it would have been problematic to reconstruct the pedigree of populations comprised of wild reproductive females. By mating the juvenile, virgin females in the lab and ensuring that every male provided offspring to both environments we made our estimates of genetic diversity more biologically realistic to what we would see in the wild in the guppy system. Indeed estimates of heterozygosity levels are similar to what we find in natural wild populations (see Methods). The fact that guppy populations have been previously found to have high levels of Y-linkage for coloration supports the relevance of my findings here. My study aimed at examining the phenotypic variation (including Y-linkage) of populations under selection since it has previously been done for undisturbed populations (e.g. Postma et al 2011). This is the first step in showing that artificial field designs can assess levels of phenotypic variation in wild populations and the next step will be to examine this variation as the populations adapt to their new environments compared to their starting point levels.

Conclusions

Teasing apart genetic from environmental influences on phenotypic change has come a long way from parent-offspring regression. Despite advances in quantitative genetics, exercises to understand the genetic architecture of phenotypic traits in natural populations are rare for organisms such as fish, even more so for sexually selected traits. Here I apply the animal model to data from pedigreed fish from wild populations to partition Y-linked from non-Y-linked genetic variance and to assess measures of heritability. Both Y-linkage and heritability can be used to estimate the extent to which a trait can evolve in response to environmental change.

With their cytologically similar X and Y chromosome indicating an early stage of decay and differentiation of the sex chromosomes, these results show that organism such as guppies may indeed have a prevalence of Y-linked sexually antagonistic male genes as predicted by sexual antagonism theory. The level of differentiation between the two sex chromosomes varies in various organisms (Brooks and Endler 2001; Marshall Graves 2006; Innocenti and Morrow 2010; Kaiser and Bachtrog 2010). For instance you have sex chromosome systems cytologically similar to each other (scuttle fly; guppies), to having the heterogametic chromosome be much smaller than the homogametic chromosome (humans, butterflies, birds), to having the heterogametic chromosome be completely degraded then lost (grasshoppers, cockroaches, nematodes). Since guppies have early sex chromosomes one may use these findings of high levels of Y-linkage of certain traits to generalize what may have occurred in organisms such as some insects and mammals who

have lost much (or all) of the heterogametic chromosome therefore barring us from inferring initial aspects of sex chromosome evolution from their evolutionary history.

This study provides estimates of genetic variation in colonizing or recently introduced wild populations of guppies which provide similarly high levels of Y-linkage as seen in stable or natural guppy populations (see Postma et al. 2011). These applications can hence provide an opportunity for predicting evolutionary potential in industries such as fisheries decimated by over- or selective fishing and control of invasive or rare species.

Figure Legends

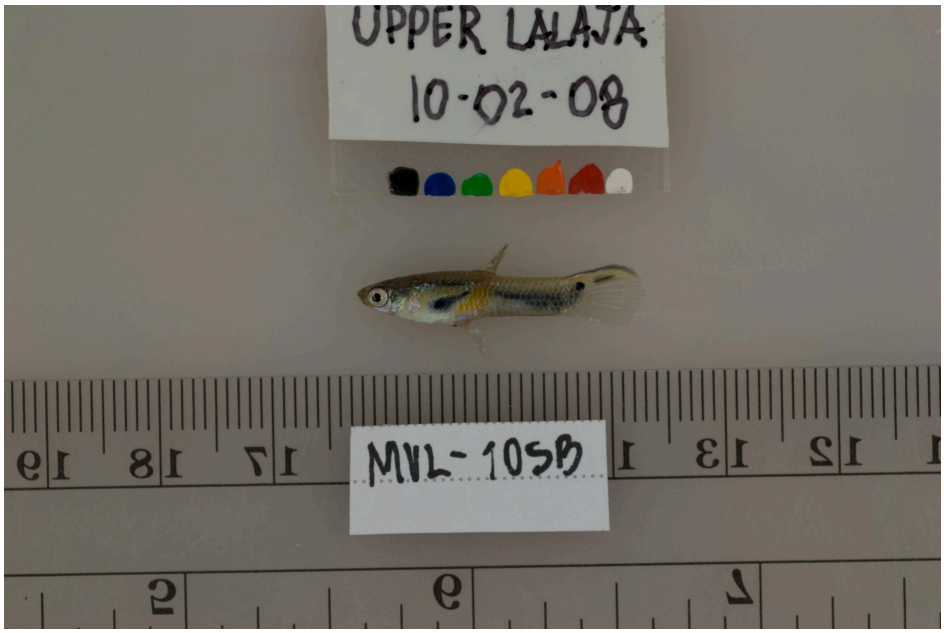
Figure 1.1: First panel is picture of sample male guppy collected from Trinidad showing location captured, date, and fish individual identification (garnered from subcutaneous elastomer paint marks on two locations on the body). Second panel is ImageJ picture measurement of male guppy with circled orange (carotenoid) spot, and measurements collected from ImageJ to excel sheet. All guppies measured blind as to location, month, or ID by same individual**.**

Figure 1.2: Pedigree reconstruction for the first 12 months for both introduction streams. The individual "u's" at the bottom of the figure represent the beginning of the fifth generation under the open canopy stream. Note that there are actually broadly overlapping generations, so being a member of the fifth generation just means that at least one of the parents was from the fourth generation. The blue lines correspond to the fathers of each individual while the red lines lead to the mothers. The founder populations are distributed along the top row with the closed canopy on the left and the open canopy on the right. Since the female founders were mated with a different group of males than the ones they were introduced with, you can see evidence of stored sperm in the form of blue lines that cross from one population to the other. Most of the founding males sired offspring in both streams.

Figure 1.3: Proportion of total phenotypic variance partitioned into additive non-Y and Y-linked genetic variation, and random maternal, individual, month, and residual error

effects of male coloration in the open and closed canopy streams. Panel 1 shows variance components for orange coloration, Panel 2 shows the same components but for black coloration. The black bars indicate the closed canopy results and the white bars indicate the open canopy results. Error bars indicate standard errors.

Figure 1.1


UPPER LALAJA
10-02-08
MVL-105B

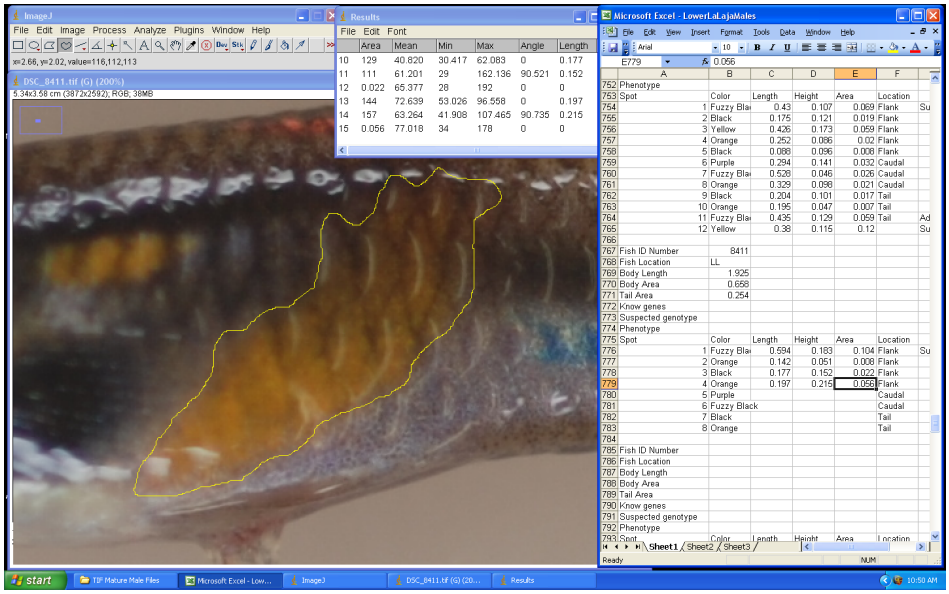

ImageJ
Results
Microsoft Excel - LowerLaLajaMales

Figure 1.2

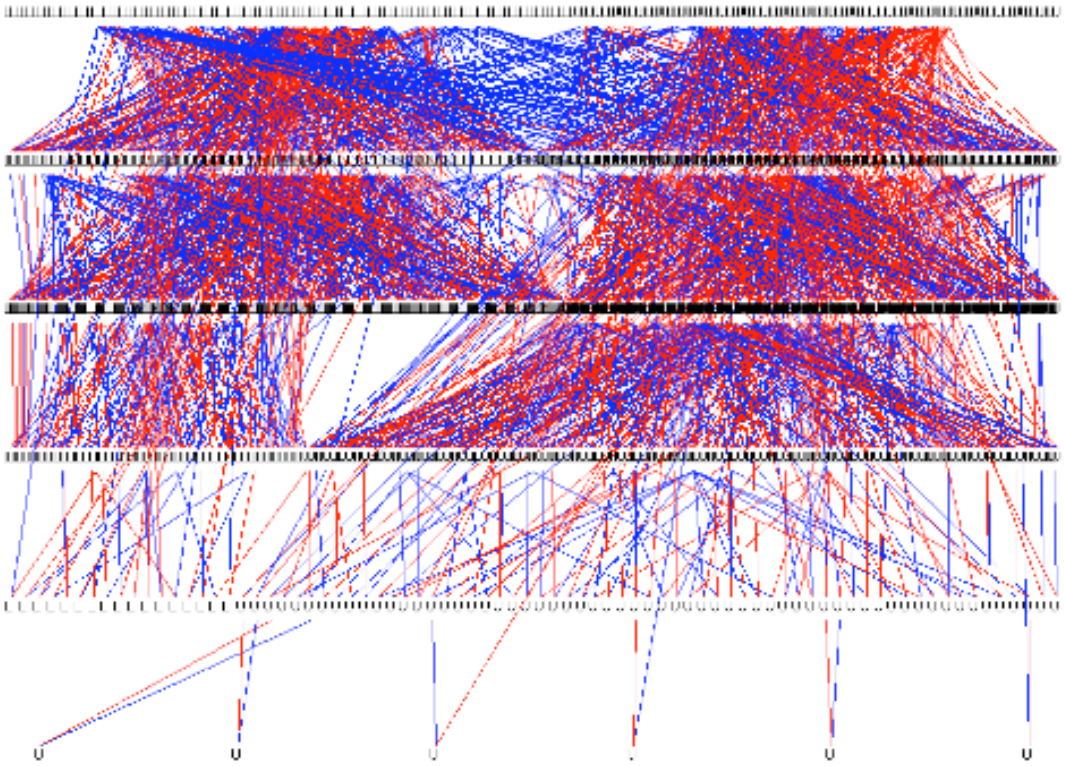


Figure 1.3

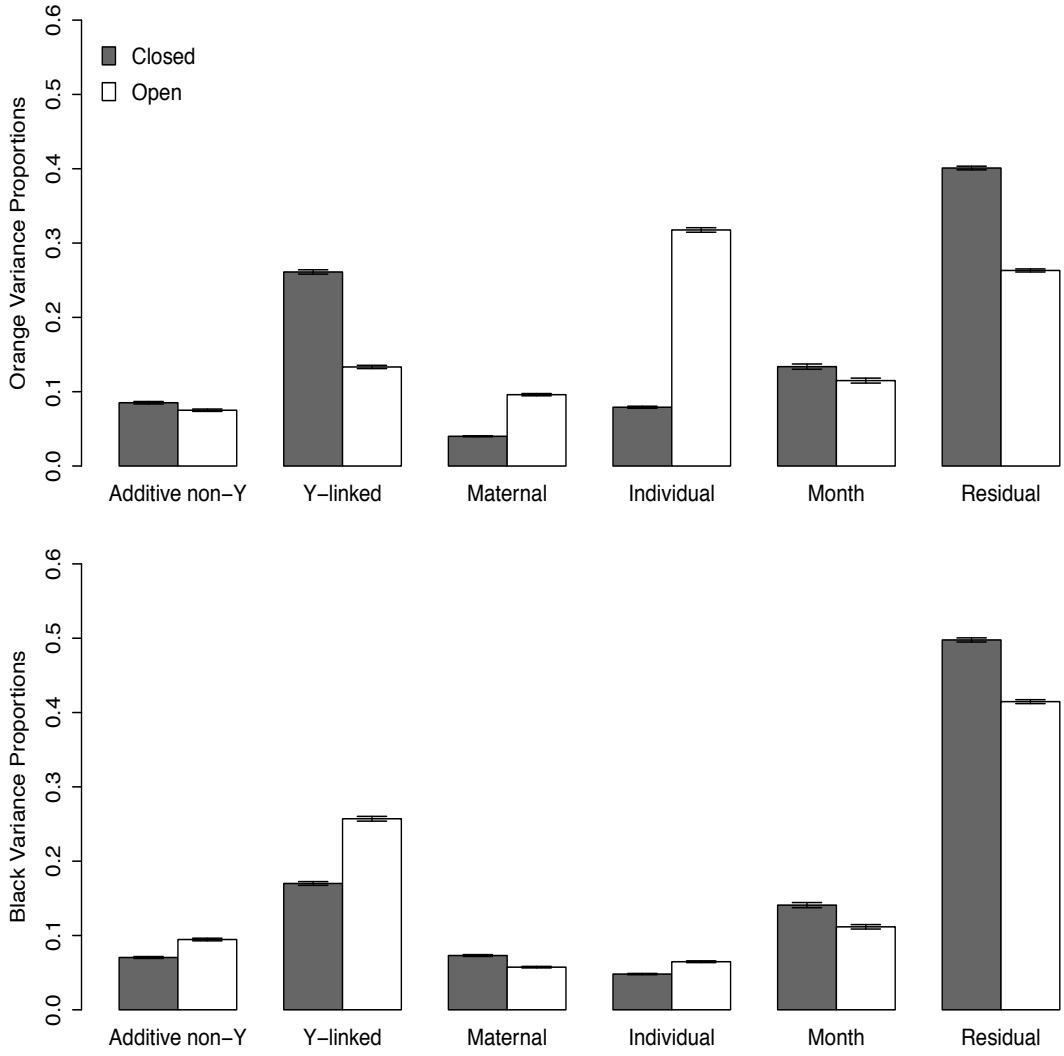

Closed
Open
Orange Variance Proportions
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Additive non−Y
Y−linked
Maternal
Individual
Month
Residual
Black Variance Proportions
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Additive non−Y
Y−linked
Maternal
Individual
Month
Residual

Table 1.1. Model comparison for different covariance structures.

	Closed Canopy		Open Canopy	
Model	DIC	ΔDIC	DIC	ΔDIC
Full	-2139	1	-1987	1
No correlations	-2140	0	-1988	0
Only Y-linked covariance	-2136	4	-1981	7
Y-linked and residual covariance	-2136	4	-1982	6

Table 1.2. Increases in DIC when the variance factor in question is removed from the model. Larger values indicate stronger support for the variable (i.e. its exclusion worsens model fit)

	Closed Canopy	Open Canopy
Non-Y additive genetic	0	2
Y-linked variance	19	6
Maternal	2	2
Month	40	4

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Chapter 2

Rapid adaptive changes in a sexually selected trait in two wild introduced populations of guppies

Abstract

Many studies show that rapid evolution can occur on ecological timescales, but fine scale examinations of evolutionary change in replicated experiments in the wild are rare. Here I present the results of a longitudinal individual-based study of male color evolution in Trinidadian guppies. The studied translocations represent a manipulation of a key environmental feature known to affect population fitness: predation. Guppies were taken from an environment where they co-exist with predators and introduced into two tributaries in the same river but that have barrier waterfalls that excluded guppies and predators. In one of the introduction tributaries the canopy above the river is thinned, thereby increasing light and primary productivity. I use monthly censuses of the guppies to measure the temporal divergence of male coloration between the ancestral and derived fish. I then use selection analyses to evaluate the correlation between male coloration and fitness. Common garden assays performed one year post-introduction allow me to test if any changes I find in the wild have a genetic basis. Male melanistic and carotenoid coloration diverged between the ancestral and derived populations in only one year (< 3 guppy generations). Light and productivity may play a small but important role governing the rate of adaptive divergence in wild guppies suggesting that fluctuating environmental components of phenotypic variation should also be considered when evaluating the evolution of sexually selected traits.

Introduction

Darwin envisioned evolution as a process too slow to be observable within the lifetime of the investigator. The last two decades of evolutionary research, however, show that organisms have the potential to adapt to environmental changes in less than ten generations, a phenomenon often referred to as contemporary or rapid evolution (Thompson 1998; Stockwell et al. 2003; Hendry and Kinnison 1999; Svensson and Gosden 2007; Schoener 2011). For example, Grant and Grant (1995) studied the adaptation of a population of medium ground finches (*Geospiza fortis*) to environmental changes in their habitat brought on by a severe drought or the heavy rainfall associated with El Niño events. The drought caused size-selective mortality as a result of changes in the food supply: larger birds with deeper beaks survived better (i.e. had higher fitness) than smaller birds because they were able to eat the larger and harder seeds that were available after the drought. El Niño events caused evolution in the opposite direction. Rapid evolution of life history and morphology have also been demonstrated in introduced species such as the mosquitofish *Gambusia affinis* in Hawaii (Stearns 1983), and rabbits *Oryctolagus cuniculus* in Australia (Williams and Moore 1989). Endler (1980) and Reznick et al. (1990; 1997) obtained similar results in an experiment executed on natural populations of guppies; they introduced guppies (*Poecilia reticulata*) that were adapted to a high predation environment into a novel low predation environment. The introduced populations were then allowed to adapt for approximately 2-11 years (5-40 generations). Subsequent sampling showed that the introduced guppies had evolved adaptive differences (e.g. more and larger spots on males, increased size and older age at

maturity) that paralleled those generally seen in natural low-predation populations (Reznick et al. 1996).

Despite clear examples documenting how ecological change can trigger rapid evolution, we still have relatively little understanding of the factors driving variation in the adaptive potential of populations. Evolutionary analyses of population introductions (experimental or accidental) have been important in demonstrating the strength of selection and speed of evolution because they subject organisms to abrupt environmental changes that represent new selective pressures. However, in order to gain mechanistic understanding, introductions should be combined with factorial manipulations of the introduction site. One can then compare adaptive divergence of organisms subject to different ecological treatments and test predictions regarding what particular traits or set of ecological factors make organisms better able to adapt to changes in their surroundings. These experiments can yield important information in the control of invasive species as well as the protection of rare species affected by anthropogenic perturbations. However, introduction experiments remain rare in nature (Reznick and Ghalambor 2005; Kemp et al. 2009), as it is generally difficult to find a system where significant adaptive divergence occurs shortly after introduction, and where adaptively meaningful ecological variables can be both manipulated and measured in the wild. Here, I use a replicated introduction of Trinidadian guppies from one predation regime to the next to examine the main environmental drivers affecting adaptive divergence of male coloration, a secondary sexual trait with extreme polymorphisms both within and among populations.

There is added challenge to understand the adaptive divergence of secondary sexual characters in response to environmental change. A balance between natural and sexual selection usually drives the evolution and maintenance of secondary sexual characters. Sexual selection generally favors conspicuous traits preferred by one sex, which at the same time are disfavored because of the energetic costs to production, increased conspicuousness to predators or other ecological risks (Fisher 1930; Zuk and Kolluru 1998; Endler 1980; Schwartz and Hendry 2007). For example, brightly colored male guppies are preferred by female guppies, but are more heavily preyed upon by predators. In another classic example, Andersson (1982) found that female African widowbirds *Euplectes progne* were attracted to males with longer tails. However, increased tail length inhibits flight especially during heavy rain (Savalli 1995), and has been suggested, but not proven, to put them at greater risk of predation. This relationship between viability and fecundity selection complicates predictions regarding evolutionary rates in organisms and is one reason why studies examining rapid evolution of secondary sexual characters are limited (reviewed by Svensson and Gosden 2007).

Svensson and Gosden (2007) note there is robust evidence that sexual selection is often stronger than natural selection and should be able to drive rapid evolution of particular traits. Indeed, one of the first and clearest examples of rapid evolution on secondary sexual characters in wild organisms involves an introduction experiment in guppies (Endler 1980). Endler introduced 200 high-predation guppies from the Aripo River in Trinidad into a low predation environment in the same river that previously had no guppies (Endler 1980). Females in low predation environments generally prefer

greater body coloration causing high sexual selection pressures in those environments, whereas natural selection via predation leads to reduced coloration in high predation environments. This distinction leads to distinct differences in body coloration between high- and low-predation males. Within two years Endler found significant increases in number and area of carotenoid and melanistic spots once predation pressure was relaxed, one of the fastest rates of evolution ever recorded for any trait (Svensson and Gosden 2007).

Later studies on similar guppy translocations, however, could not repeat this result (Karim et al. 2007; Kemp et al. 2009). For instance, a release from predation resulted in the evolution of more brightly colored males, but in a different way from that seen by Endler in his experiment in the Aripo River (Kemp et al. 2009). Kemp et al. (2009) found a decrease in carotenoid and melanin-based spots but an increase in structural coloration in the El Cedro River. Likewise, Karim et al. (2007) analysed an introduction to the Damier River after eight years and found only slight decreases in melanin-based spots but no significant differences in carotenoid. Their methods did not allow them to address the evolution of structural coloration. It remains unclear whether changes in coloration are a sole result of predator-driven differences in natural and sexual selection, an issue I hope to better address in this chapter.

I can think of three explanations for the differences in trends seen in the examples above. First, other factors besides predation may intervene in selecting for different colors, as seen with the costs in heavy rain for increased length of African widowbird tails. High predation guppy environments are typically larger in size, more productive,

and have more open canopies when compared to low predation sites (Grether et al. 2001), but variation exists and some high versus low predation environments can be quite similar (Gordon et al. 2009). In particular, canopy openness and light availability can vary significantly between low predation environments (Grether et al. 2001). The evolution of coloration can be sensitive to small environmental differences such as these (Gray and Mckinnon 2007). Light availability can affect perception of color as well as stream productivity, both of which can directly affect selection on coloration. For example, artificial selection from simulating different light conditions elicited strong evolutionary responses to the preferred color (Endler et al. 2001). Streams with more light also have larger standing crops of unicellular algae (i.e. more productivity or resources). These algae are a major source of carotenoids, a known limited environmental resource in the diet of guppies that brightens the color saturation or chroma of the orange and yellow spots. Variation in diet has been shown to affect the costs and benefits of carotenoid (i.e. orange) production in guppies (Grether et al. 2005). These findings point to light availability being an important factor in the maintenance of color variation in the wild; however, a recent study revealed no phenotypic response of carotenoid or melanin-based guppy body coloration to anthropogenic disturbance following an increase in canopy openness (Schwartz and Hendry 2010).

Second, temporal fluctuations in environment and temporal differences in evolution could explain differences in evolutionary rates among populations (Svensson and Gosden 2007; Gingerich 1983). Kemp et al. (2009) and Karim et al. (2007) analyzed their introduction after 28 and 8 years post-introduction, whereas the Endler introduction was

analyzed after only two years. Multiple studies have shown that the strength and direction of selection can significantly change between years (Siepielski et al. 2009; Kingsolver and Diamond 2011). Fluctuations in environmental quality can tip the balance between natural and sexual selection in different directions. This was seen in Soay Sheep (*Ovis aries*), where sexual selection for horn size is stronger in benign years, whereas mortality selection against horn size is greater in harsh years (Robinson et al. 2008). Fluctuating evolution can also come in the form of frequency dependent cycles, such as in the side-blotched lizard *Uta stansburiana* (Sinervo and Lively 1996). In guppies, it has been hypothesized that frequency dependent cycles can be caused by female preference for rare color patterns (Hughes et al. 1999; Eakley and Houde 2004), or by predator search-image recognition (Olendorf et al. 2006). Density dependent-selection can also favor different phenotypes at different stages of the new populations colonization to its new environment (Kokko and Rankin 2006). Moreover, it has been shown that life-history differences can affect sexual selection (Gustafsson et al. 1995), implying that different stages in the evolution of guppy life-histories could potentially affect the evolution of secondary sexual characters.

Finally, studies may have found different evolutionary responses due to intrinsic genetic differences between populations. It is generally well established that genetic factors such as genetic variation, sex linkage, and multi-trait genetic correlations can affect the rate and direction of evolution (Lande 1980; Charlesworth et al. 1987; Funk et al. 2005; Gingerich 2009).

In this chapter I present results from a new introduction aimed at addressing the above hypotheses. Individuals from a high-predation population were introduced into four low predation streams (using methods akin to the Endler introduction except for the replication). Two of the four introduction sites are also experimentally manipulated to artificially increase the canopy openness. I then tracked changes in male coloration bimonthly. As noted by Svensson and Gosden (2007), studies such as this that examine the rapid evolution of sexually selected traits in nature are rare, but should be attempted. My study involves a transplant of guppies from one environment where natural selection of male coloration via predation is strong (Kemp et al. 2009), to two others where sexual selection via female choice for highly colorful males should be stronger. Novel aspects of this research include the following. First, the ancestral populations to be introduced in different treatments were previously mixed to minimize genetic founder effects and concentrate on the effect of differential selective pressures. I first will ask the question: *How do large-scale differences in predation (and canopy cover) affect divergence of male coloration*? Second, I follow phenotypic change longitudinally to evaluate fine scale temporal trends in phenotype in order to assess: *How do temporal fluctuations affect phenotypic divergence of sexually selected traits over time?* I next perform a selection analyses to *test statistical correlations between color and fitness (survival and reproductive success)*. Mean changes in coloration are also evaluated via common garden experiments of a subsample of individuals one year after introduction in order to ascertain *whether changes found in the field have a genetic basis and hence represent rapid evolutionary change.*

This chapter represents the first in a series of papers regarding the evolution of male coloration in this new long-term data study. This chapter includes only two of the four introduction streams due to logistic reasons. The two streams were given different treatments of light availability: while one was left untouched and thus had a dense canopy, the other had its canopy trimmed to increase light availability. Due to the lack of replication of the treatment in the sample, I here refrain from making strong causal inferences regarding the effects of light until I analyze the remaining replicate streams.

Methods.

Guppy System

Guppies (*Poecilia reticulata*) are small live-bearing fish native to the Island of Trinidad. In the wild, guppies have relatively short generation times (110-210 days), small body sizes, and are easily captured (Magurran 2005). They are also relatively easy to rear and breed in captivity. Male guppies are significantly smaller and exhibit numerous body and tail color patterns in comparison to females. (Houde 1997). Male guppies are colorless as juveniles but attain full coloration not long after maturity, at approximately 50 days of age (Houde 1997). Female preference has been shown to cause the evolution of male secondary sexual characteristics including coloration and display (Houde 1997).

Guppies inhabit small, typically shallow freshwater rivers in Trinidad that have been described by some as a ‘natural experiment’ (Haskins et al. 1961). In the Northern Range

Mountains of Trinidad, rivers are arranged in parallel, flowing both to the north and south slopes of the mountain range. In each river, guppy habitats are separated by barrier waterfalls that block large predators from getting to the more upstream mountainous environments. Adaptation to differences in predation regime has driven the evolution of many differences in morphology, behavior, and life history between guppies on either side of the waterfalls leading to distinct eco-types of guppies in high versus low predation environments (Endler, 1995; Magurran et al. 2005). For example, low-predation guppies are larger, have later ages of maturity, fewer offspring per litter, but larger individual offspring than their counterparts from high predation environments (Reznick et al. 1996). Phylogenetic relationships of fish populations between rivers suggest that the adaptive divergence between high and low predation guppy populations has proceeded in parallel, providing convenient replication and allowing testable predictions regarding evolutionary change across rivers.

The Experiment

In March 2008, 150 immature guppies were collected from the Guanapo high predation (GH) main stem river in Trinidad and reared in the lab until maturity in single-sex tanks. After maturity, fish were housed in groups of ten fish (five males and five females) and allowed to mate. Before introduction, every fish were anesthetized using MS-222 (tricaine methylsulfonate) and digitally photographed against a light background with standard illumination from full spectrum fluorescent lightbulbs (to closely mimic natural sunlight). They were also individually marked by subcutaneously injecting an

elastomer dye (following methods explained in Gordon et al. 2009). Approximately 75 individuals were introduced into one of two low predation tributaries further upstream from their collection site, which did not have any resident guppies. Males and females from any given mating tank were introduced into different streams, so that the females in each stream were exposed to a different set of males than the ones they had been mated with before introduction. This was done to increase genetic diversity within and genetic homogeneity between streams, since all male genotypes were represented in both streams, either as introduced individuals, or as fertilized eggs and stored sperm. Both introduction sites are bordered on either side by barrier waterfalls that exclude all major predators except killifish (*Rivulus hartii),* which only rarely preys on juvenile guppies that do not yet have color.

One of the tributaries was further manipulated by trimming the canopy above the stream by approximately 50 percent by David Reznick and colleagues (D. Reznick pers. comm.). Differences between the two streams in canopy openness was measured at 15 different points throughout each stream with a hand-held densiometer as the percentage of visible sky. Net primary productivity (NPP) was measured on natural benthic substrate in the two introduction streams in April 2008 and May 2009. The change in the level of dissolved oxygen (DO) was measured with a YSI 85™ device before and after of 30 minutes of incubation in 1.75L chambers covering 104cm^2 of benthic surface (Marshall et al. unpublished). NPP is expressed in mg O_2 m^{-2} h^{-2} (Hauer and Lamberti 2007). Data show the canopy manipulation resulted in a significant increase of stream productivity and resource biomass (see Kohler 2010). Canopy openness differed significantly

between the two streams (effect=12.6±1.7; ANOVA $F_{1,29}$=54.10, p<<0.001). Net primary productivity differed significantly between the two streams (effect=17.9±6.9; ANOVA $F_{1,32}$=6.52, p=0.015) but not between years ($F_{1,32}$=0.94, p=0.339). Canopies were re-thinned at least once per year to maintain these differences in light level and productivity.

Every month every guppy that could be recaptured in both tributaries (open canopy and closed canopy) were recaptured and brought back to the laboratory. Recapture probabilities were calculated for males in each month. After recapture guppies were once again anesthetized using MS-222 then digitally photographed as described above. At the same time all new recruits greater than 14mm standard length were uniquely marked. A year after introduction, the dataset contained 1,467 individually marked fish (664 in the closed canopy treatment and 803 in the open canopy), of which 682 were males (306 and 376 respectively). As females do not show coloration, only males were measured and analyzed bimonthly for changes in male coloration over time between the ancestral and derived populations. The males were analyzed bimonthly because it takes approximately two months for new males to mature.

In February 2009 (11 months post introduction), a subset of juvenile guppies from both low predation introduction tributaries and the ancestral Guanapo high predation environment were collected and brought back to the lab. These fish would have reached maturity in the wild in March 2009, and hence are comparable to the wild data on adults one year after the introduction. The collected guppies were reared under common garden conditions until maturity. Once mature, males and females were mated in pairs so that all wild-caught fish could be equally represented in the first generation of lab-born offspring,

and to avoid inbreeding. Their offspring (the grandchildren of those collected in the field) were again raised and mated under the same common conditions. Second generation mature males were photographed a few months after maturity following the same methods as the wild fish and their coloration scored for number and area of spots. Rearing the fish under common conditions removes environmental effects experienced by the wild-caught juveniles before capture and suggests that any changes between them have a genetic basis.

Color Measurement

The program ImageJ was used to measure coloration on all digital photographs for both wild and lab-reared male guppies. ImageJ can quantify body area of each fish, and identify and measure the area of each colored spots. The colors observed were categorized into melanistic (black, fuzzy black); and carotenoid (orange) following methods in Brooks and Endler (2001) and Kemp et al. (2009). The total area for each color on the fish was summed. To obtain relative color area, I divided the total area of each color group by body area. Analyses using relative area of spots and absolute area using body area as a covariate yielded similar results so only relative area differences are illustrated in the figures. I adjusted for the effects of body size (e.g., so spots are not larger simply because male size is larger). Structural coloration (blue, violet, silver, and green colors) was not included in the analyses because they are not accurately represented in photographs (Endler and Mielke 2005; Kemp et al. 2009). All fish were

measured blind (by one individual) with respect to population and different months were not analyzed in any order.

Statistical analyses

Male capture probabilities were estimated using an open population capture-mark-recapture model that included month-specific recapture probabilities (Amstrup et al. 2005). The model was fit by maximum likelihood using package 'Rcapture' in program R. A different model was fit to each stream. Recapture probabilities varied among months and streams but were on average high (see table 2.1) and consequently, the probability of missing an individual for its entire lifetime was quite low.

To compare the beginning and end points of the introduction as done in the previous guppy transplants simple ANOVA's using body area and relative color area were used to examine differences between the ancestral fish introduced into either the closed or open canopy sites. Phenotypic divergence in male coloration across month from the wild data were analyzed using LMMs (Linear Mixed Models, Rstudio v2.15) which allows a repeated-measures analyses with color area (melanistic and carotenoid separately) as response variable, body area as covariate, month as a discrete explanatory variable and individual as a random effect. Separate analyses were done for both streams, and with both streams in the same analyses.

I next explicitly measured selection: i.e. statistical correlations between color and survival and reproductive success. Survival was modeled as a binomial response variable in a generalized linear model (GLM). Relative carotenoid area and relative melanistic

area were used as explanatory variables. Month was also included as a discrete fixed factor to account for temporal changes in overall survival. Two and three-way interactions were kept in the model only if significant. Separate models were fit for each stream. To measure the effects of color on reproduction I used as a response variable the fraction of recruits (male and females) sired by a given male out of the total number of recruits in the population that given month. I treated it as a binomial proportion (e.g. 3 recruits sired by given male/ 50 new recruits in the population). Month, relative carotenoid area and relative melanistic color areas were included as explanatory variables, using the same criteria as with survival. Note that only recruits that have been marked and pedigreed are included in a male's reproductive success, which excludes all fish <14mm of length (this includes all captured mature offspring).

I tested for genetic divergence in male coloration (i.e. an evolutionary response) using linear models with total area of carotenoid or melanistic coloration separately as the response variables, population as the explanatory factor, and body area as a covariate. As evolution should cause changes in variance, I first tested for unequal variances in my lab-reared data using a Chi-square non-constant variance score test for factor 'Population' (Ancestor GH, closed canopy introduction, and open canopy introduction). If found the variance-covariance matrix of the analyses was corrected using the White Method (White 1980).

Results

Body Area

As expected from previous results male body area increased significantly in both the open and closed canopy when comparing start and end months in the wild data as guppies adapt to the new low-predation environments (ANOVA $F_{1,177}$= 66.32, p<<0.001), but differences were not found between streams in the intercept ($F_{1,177}$= 0.63, p=0.43) or slope (Figure 2.1a; $F_{1,177}$= 2.21, p=0.14). On the other hand common garden data shows a genetic difference in body area only males inhabiting the closed canopy introduction site (Figure 2.1b; estimate=0.048±0.016, t=3.02, p=0.003) but not the open canopy canopy, which showed significantly smaller sizes than the source population (Figure 2.1b; estimate=-0.032±0.016, t=-1.994, p=0.049).

Phenotypic Divergence of Male Coloration Over Time from Wild Data

The beginning and end were analyzed in order to compare with previous studies (Endler 1980, Karim et al. 2007, Kemp et al. 2009). For carotenoids there was a significant effect of month (Figure 2.2; ANOVA $F_{1,177}$= 6.84, p=0.009) but not stream ($F_{1,177}$= 0.79, p=0.37) nor its interaction ($F_{1,177}$= 0.15, p=0.69). For melanistic coloration, there was also a significant effect of month (Figure 2.2; ANOVA $F_{1,177}$= 25.01, p<<0.001) but not stream ($F_{1,177}$= 2.33, p=0.12) nor its interaction ($F_{1,177}$= 0.81, p=0.37).

When both introduction streams were analyzed together across all months there were no difference in fish coloration between the closed and open canopy, and overall there was a non-gradual trend. For that reason, here I only provide the figures for separate

analyses of introduction stream for easier visibility of the results (Figure 2.3). Fish in the closed canopy site did not significantly change in carotenoid coloration over time ($p=0.67$; Figure 2.1). There was, however, a significant decrease in the amount of melanistic coloration for fish in the closed canopy ($p=0.002$; Figure 2.3). Fish in the open canopy site show an almost significant increase in carotenoid coloration over the twelve months of the experiment ($p=0.063$), and a significant decrease in melanistic coloration ($p<0.001$).

Male coloration and fitness in wild data

These results of the selection analyses are presented in table 2.2. They show that male coloration has important fitness consequences. Increases in carotenoid coloration are adaptive because they confer individuals a reproductive advantage. Melanistic coloration shows more complex patterns, with negative effects on survival in both streams but a reproductive trade-off with Carotenoid coloration in the closed canopy. Specifically, selection analysis of survival shows a negative effect of melanistic coloration on fish in both the closed and open canopy, but no effect of carotenoid coloration, and a month effect only in the open canopy. Selection analyses of reproduction reveal a significant positive effect of carotenoids and, marginally, melanistic coloration, as well as a strong negative interaction indicative of a trade-off for male fish in the closed canopy. In the open canopy stream I find only a significant positive effect of Carotenoids. The month the fish is recaptured has a significant effect on reproduction of coloration in both the open and closed canopy (Table 2.2).

Genotypic Divergence of Male Coloration Over Time from Lab Data

The Chi-square non-constant variance score test results show unequal variances for the lab-reared fish populations in carotenoid (Chisquare = 6.16; df =1; p=0.013), but not for melanistic coloration (1.81; df =1; p=0.178). Therefore, I used the White method to correct for the unequal variances. It should be noted that this test was not attempted in the phenotypic data because it was a mixed effects model (however if the random effect is removed the variances are not significantly different). Fish in both introduction sites have divergence significantly in carotenoid coloration compared to the ancestral population (p-value <0.001 in both cases; Figure 2.4), but there were no differences between fish from the open and closed canopy introduction sites (effect= -0.002; t= 0.023, p=0.82). There was, however, no significant difference in fish from either introduction site in melanistic coloration (closed canopy p=0.210; open canopy p=0.226; Figure 2.4) between the ancestral and the two introduction sites. There was, however, a significant difference in melanistic coloration between fish from the closed and open canopy sites (effect = 0.034; t= 3.380; p<0.001; Figure 2.4).

Discussion

Rapid evolution of sexually selected traits is a rarely studied topic in nature (Svensson and Gosden 2007). Introduction experiments can give us the opportunity to study how different selective factors may interact to shape evolution of traits (Reznick et

al. 1997; Kemp et al. 2009), but are rarely applied to secondary sexual characters. Here I present an experimental study of the evolution of a trait associated with sexual selection and assess the role of ecological factors in shaping how sexually selected traits evolve. The results show rapid evolution of male coloration in response to strong natural and sexual selection. I below discuss the results in greater detail.

Phenotypic and Genetic Divergence of Carotenoid Coloration

The rapid increase in male carotenoid coloration over time in the lab-reared and wild fish populations was expected from prior studies (see references below) and may be explained by female preference. This is consistent with the high reproductive advantage conferred by orange (Table 2.2). Differences in female preference between the two streams may explain differences in the strength of this selection component and affect rates of color change. Female preference varies between and among guppy populations, has been shown to strongly affect male coloration, and is generally stronger in low predation environments (Endler and Houde 1995). Preference for carotenoid-based coloration has been shown in most female guppies of all types (Houde 1987; Van Oosterhout et al. 2003), and carotenoid-based (orange) pigmentation has been linked to male quality (Grether 2000), however, not all populations prefer other colors (structural or melanistic). In fact, in one mate choice study, Endler and Houde (1995) show that almost all of the multiple high and low predation populations they tested either preferred orange or had no response to it; whereas for black some preferred it whereas others disliked it. They also found that the Guanapo high-predation fish, who are the ancestral

lineage in this experiment, prefer more orange, less black, and a larger tail (Endler and Houde 1995). This follows the phenotypic trends seen in this introduction (Figure 2.2 and 2.3). Female preference for conspicuous coloration in males is risky in high predation environments because close proximity to a brightly colored male can increase chance of a predatory attack. Additionally, any offspring sired by these females may have lower fitness due to lower survival. These costs of male preference will be reduced when guppies are moved into a predator-free environment and increased preference for bright male coloration may evolve. Indeed my selection analyses showed that males with higher carotenoid coloration confer a reproductive advantage. As expected the selection analyses also showed that in the absence of predation in the new introduction environment there is no survival effect on carotenoid body coloration (Table 2.2).

The two streams, with different light regimes, did not differ in the adaptive divergence of carotenoid coloration during the first year of the experiment. I expected otherwise because increased light should affect the visibility of some colors plus increase the availability of carotenoids in the diet. Prior research revealed that female preference for orange (carotenoid) coloration increases as guppies are transplanted to more open canopy or high-light sites (Long and Houde 1989; Endler 1991). Additionally, the brightness or hue of carotenoid-based coloration, unlike melanistic or structural coloration, is affected by diet (Grether et al. 1999, 2001; Endler 1980). This is seen in various other organisms such as birds where the carotenoid coloration in feathers are also shown to be affected by nutritional condition (Price 2006; McGraw 2006). Therefore I expected that carotenoid coloration would increase more profoundly in fish in the open

canopy introduction site compared to the closed canopy site. There are few possible explanations for these results. First, I only measured areal changes in coloration and not the brightness or hue. A previous study has given evidence that canopy manipulation may not affect areal changes in male coloration (Schwartz and Hendry 2010) and therefore I may not notice changes which may be there in my experimental design. Second, the experiment has been in progress for only one year. More time may be required for all aspects of the evolution of male coloration to appear. Third, this manipulation may have increased stream productivity but may not have been a strong enough manipulation to affect areal changes in coloration. More replicates and longer-term analyses are needed to evaluate whether canopy has an effect on the evolution of male coloration.

In both introduction sites there was a temporal break in the increasing trend of male carotenoid coloration around the month of October. One possible explanation is a potential episode of selection that occurred during a major flood the previous August; 68.75% of the mature males in the thinned canopy site and 38.46% of the mature males in the intact canopy site disappeared during this month (A. López-Sepulcre unpublished results). New mature recruits in October would have been juveniles or newborn offspring and hence colorless at this time, but would have achieved full coloration in October. It is thus possible that the break in the trend I can see in the phenotypic wild data in October or November is in some way related to this strong selection. It is difficult to understand why a natural event such as a flood would cause a change in male coloration, but maybe behavioral differences between colorful and drab males or a tight correlation between morphology (such as tail size or body shape) and body coloration could leave certain

guppies more susceptible in times of flooding. Alternatively, the fluctuations in trends could be due to some other form of seasonal variation (mini dry season also occurs during that time).

Phenotypic and Genetic Divergence of Melanistic Coloration

Male fish in both introduction sites showed a significant decline in melanistic coloration over time. Analyses of the common garden results revealed that this difference in the area of melanistic pigmentation did not have a genetic basis between ancestral and derived fish, and hence was a plastic response to changes in the environment. Despite the lack of significant differences between ancestors and derived populations within each stream, by the end of the year the two populations diverged genetically in their amount of melanistic coloration (higher in the open than the closed canopy, Figure 2.4). This divergence could be due to the negative reproductive tradeoff between melanistic and the increasing carotenoid coloration detected in the in the closed canopy and not the open canopy (Chapter I). Melanin based coloration has been shown to be labile in fish (Sumner 1935), meaning that fish can readily change their coloration depending on the location of pigment granules within the melanocyte cells (i.e. if dispersed the guppy is quite dark, and if concentrated in one area the guppy is paler). Since guppies can adjust its pigment granules in response to environmental features, there may be some adaptive significance to plasticity in this trait.

Brooks (1996) found that black coloration functions as a signal amplifier for other colors, enhancing the ability of females to discriminate among males based on coloration.

Hence, a phenotypically plastic increase in melanistic coloration could be a mechanism by which high-predation guppies rapidly highlight their initially limited amount of orange coloration, when introduced into an environment that lacks strong predators. This was not the case in this study. Kemp et al. (2009) found that black (melanin-based) coloration also decreased in the El Cedro introduction. The El Cedro is a tributary off the main Guanapo River just like the introduction sites in this experiment streams. Since Guanapo guppies prefer less black coloration in mate choice experiments (Endler and Houde 1995), these results could be driven by the predominance of female preference in shaping the evolution of melanistic coloration after the introduction. If this interpretation is correct, then it begs an explanation for why more melanistic coloration is favored in high predation environments.

Melanistic coloration is independent of diet and hence I did not expect to find any differences between the open versus closed canopy low predation introduction sites. This was true for the phenotypic results, however genetically there were significant differences in melanistic coloration between the open (higher melanistic color) and closed canopy introduction sites. These differences are consistent with the genetic results of Chapter 1, where I demonstrate that black shows strong genetic correlations with orange, but in opposite directions in both populations. For example, in the closed canopy, there is a negative correlation and thus, I expect the trend for black to decrease as orange increase. Differences in selection pressures may also explain the different results. If melanistic coloration needs to evolve first to be a signal amplifier it could be that in the open canopy sites melanin-based coloration is changing first so that it may better amplify the more

highly preferred carotenoid coloration in an environment where carotenoid is predicted to be enhanced over time. Indeed, the negative reproductive tradeoff between carotenoid and melanistic coloration found in the closed canopy stream disappears in the open canopy site (Table 2.2).

Conclusion

In this study I have evaluated adaptive divergence of male coloration, a secondary sexual character, in two ways: through fine temporal resolution of changes in mean phenotype in the field, and by an experimental comparison of the derived and ancestral populations in the lab. My results show male melanistic and carotenoid coloration, two sub-components of a sexually selected male trait, can rapidly diverge in populations subject to large-scale changes in predation and canopy cover in less than three generations.

This study mirrored previous ones where guppies are introduced into different predation regimes and their adaptation measured once a specific amount of time has passed. However, unlike the previous designs here I also perform a selection analysis of male coloration. Additionally, I track the changes longitudinally throughout time and manipulated the canopy in a second stream in order to make some inferences regarding the effect of canopy on male coloration. Since the replicated stream of the canopy manipulation is not yet measured I cannot make any causal inferences on the effect of canopy. I can therefore only justify framing this experiment as a repeated introduction experiment under different conditions. Nevertheless, the individual-based nature of the

data makes these introductions an rare mechanistic case-study of the rapid evolution of secondary sexual traits in the wild.

Figure Legends

Figure 2.1: Phenotypic and genetic changes in body area. Figure 2.1a shows phenotypic changes for body area between start (month 0, March 2008) and end (month 12, March 2009) months in both introduction streams using wild males. Figure 2.1b shows the genetic changes in body area between the ancestral and two derived introduction populations using lab-reared males. Error bars represent standard errors.

Figure 2.2: Phenotypic changes of coloration between start (month 0, March 2008) and end (month 12, March 2009) months in both introduction streams. Error bars represent standard errors.

Figure 2.3: Phenotypic changes of male coloration across months using wild data. This figure shows the bimonthly changes in relative areas of carotenoid and melanistic coloration for both introduction streams. Error bars represent standard errors.

Figure 2.4: Genetic changes in relative color area of coloration for lab-reared males from the ancestral and two derived introduction populations. Error bars represent standard errors.

Figure 2.1a

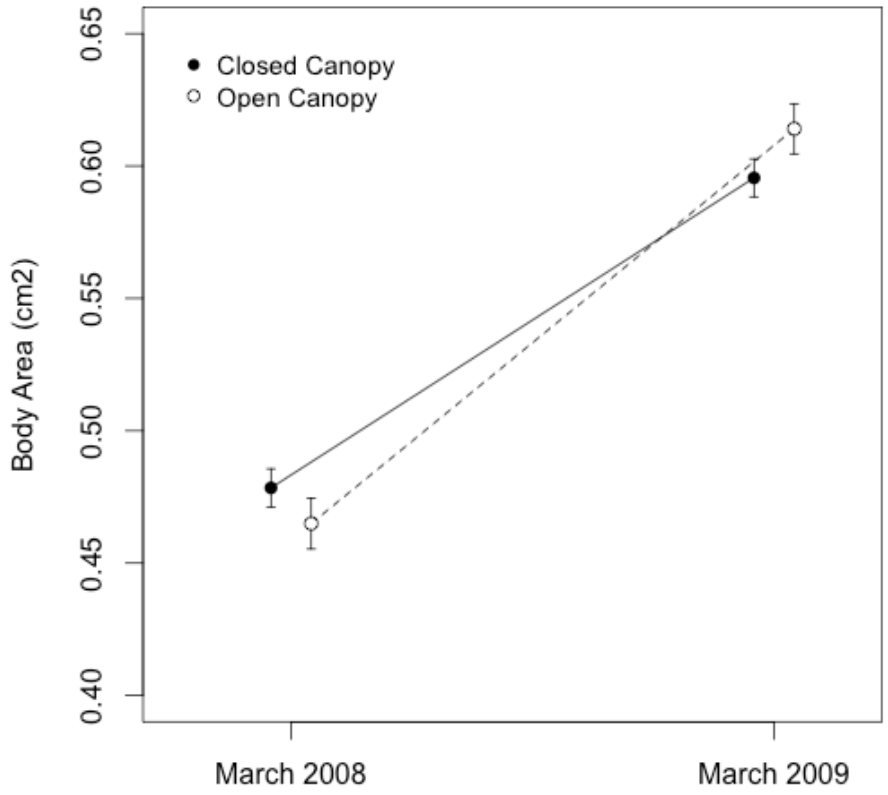
Closed Canopy
Open Canopy
Body Area (cm2)
0.40
0.45
0.50
0.55
0.60
0.65
March 2008
March 2009

Figure 2.1b

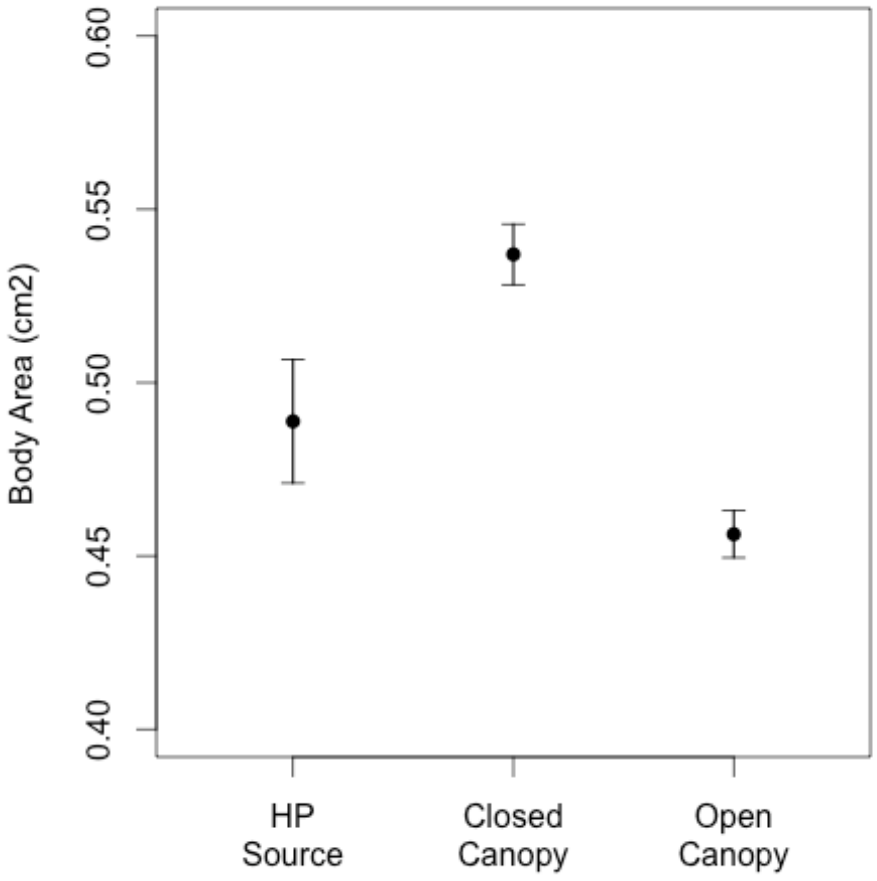
Body Area (cm2)
0.40
0.45
0.50
0.55
0.60
HP Source
Closed Canopy
Open Canopy

Figure 2.2

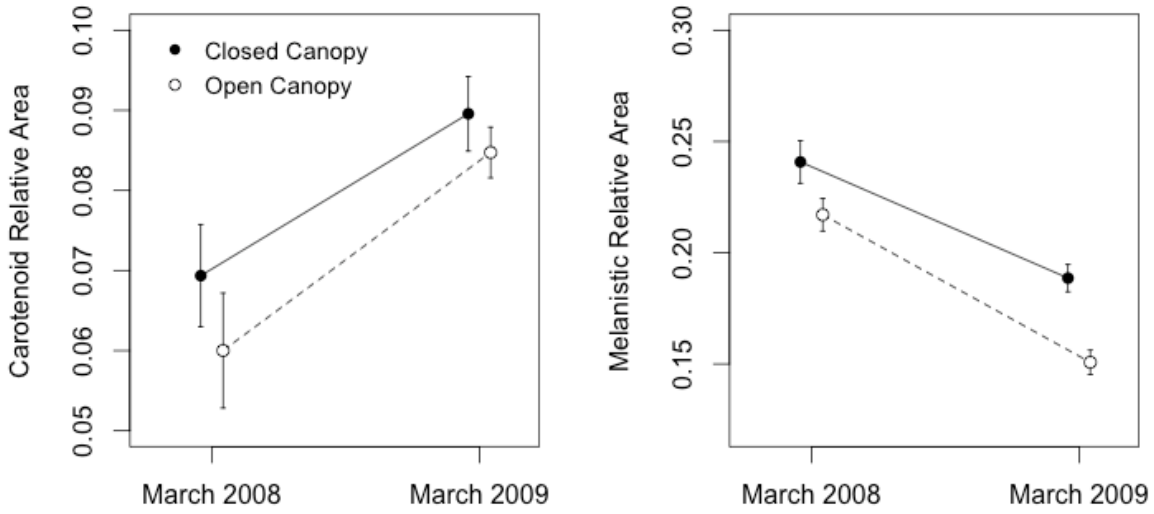
Carotenoid Relative Area
0.05
0.06
0.07
0.08
0.09
0.10
Closed Canopy
Open Canopy
March 2008
March 2009
Melanistic Relative Area
0.15
0.20
0.25
0.30
March 2008
March 2009

Figure 2.3

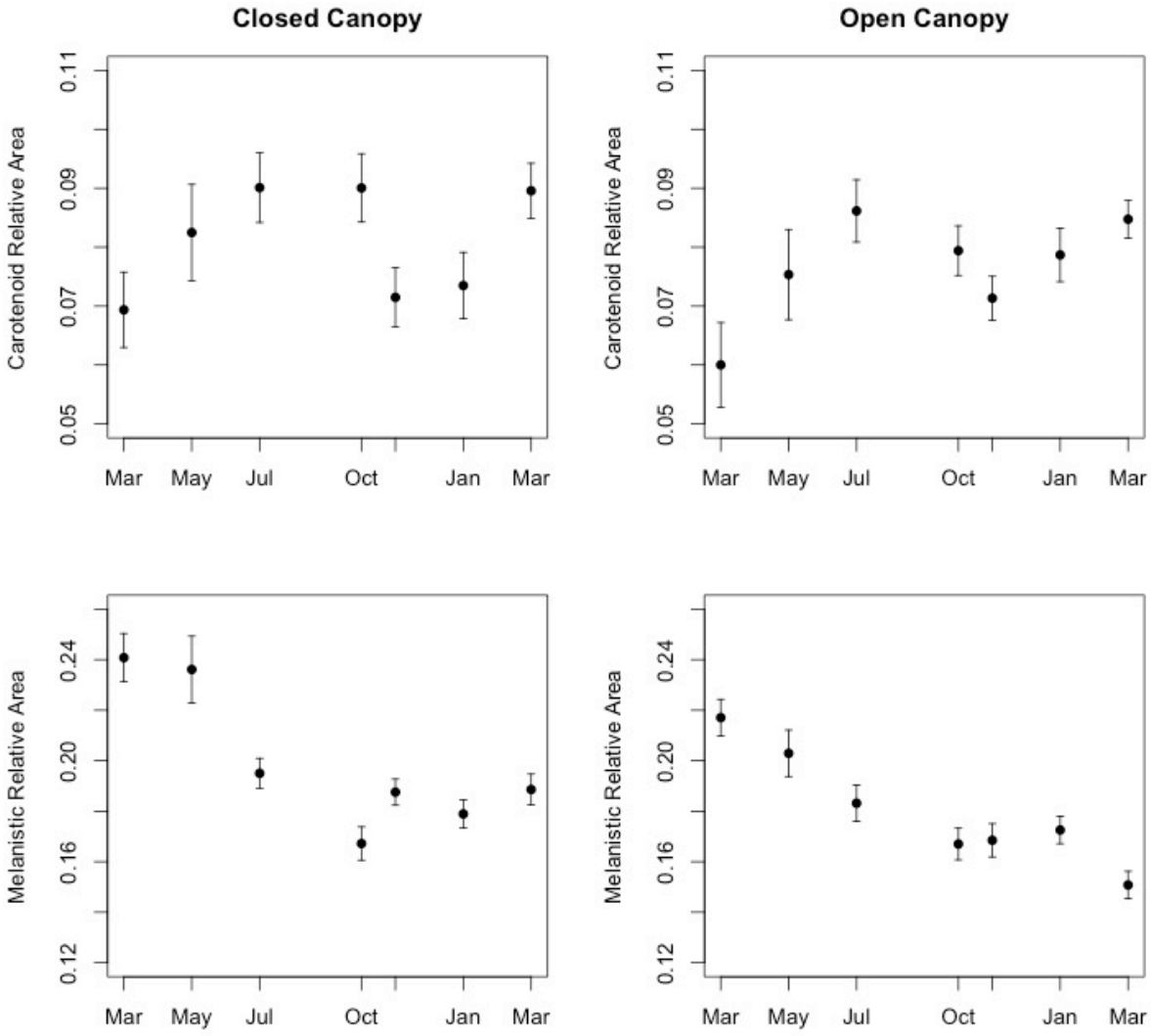
Closed Canopy
Open Canopy
Carotenoid Relative Area
Melanistic Relative Area
0.05
0.07
0.09
0.11
0.12
0.16
0.20
0.24
Mar
May
Jul
Oct
Jan
Mar

Figure 2.4

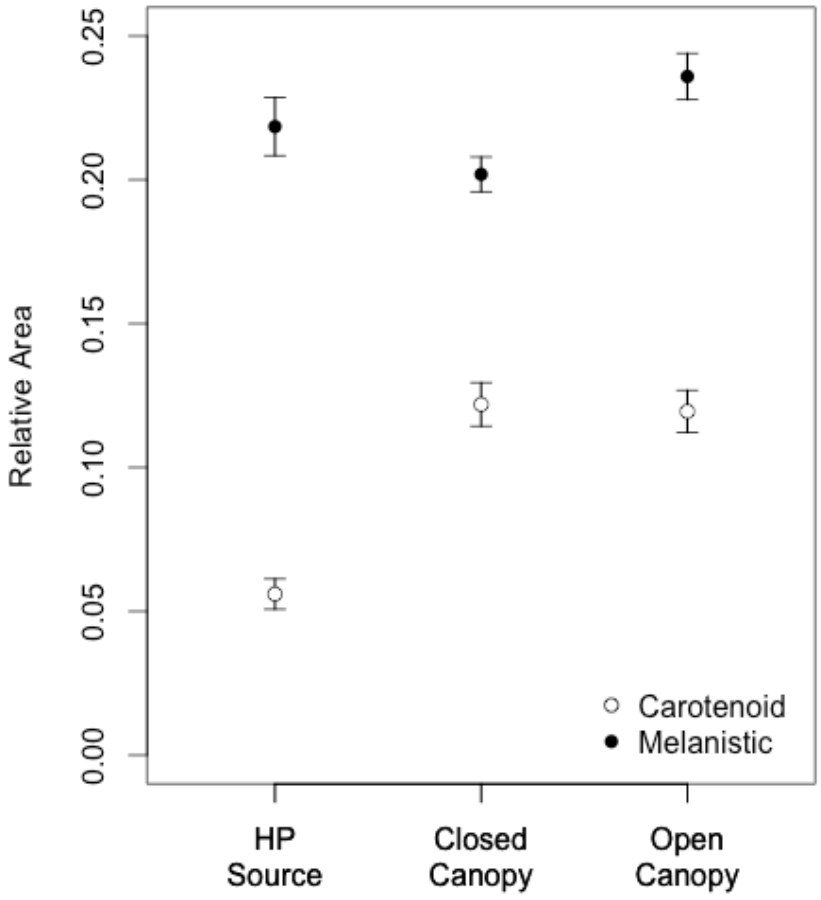
Relative Area
0.00
0.05
0.10
0.15
0.20
0.25
Carotenoid
Melanistic
HP Source
Closed Canopy
Open Canopy

Table 2.1. Maximum likelihood estimates of male monthly recapture probabilities (mean + se)

	Closed Canopy	Open Canopy
April	0.90±0.06	0.96±0.04
May	1.00±0.00	0.84±0.10
June	0.94±0.06	0.86±0.09
July	1.00±0.00	0.87±0.09
August	0.89±0.05	0.72±0.09
September	0.86±0.06	0.88±0.08
October	0.85±0.05	0.87±0.08
November	0.89±0.04	0.75±0.08
December	0.81±0.05	0.77±0.09
January	0.79±0.05	0.81±0.08
February	0.67±0.06	0.57±0.08
March	0.90±0.04	0.80±0.06

Table 2.2. Selection analyses table showing effects of coloration on fitness components: survival and reproduction. (–) denotes removed interaction due to non-significance.

	Closed Canopy				*Open Canopy*			
	estimate	Chi^2	df	p	estimate	Chi^2	df	p
Reproduction								
Carotenoid	14.0±5.9	5.29	1	0.021	5.1±2.5	4.16	1	0.041
Melanistic	5.4±2.8	3.47	1	0.062	1.3±1.7	0.63	1	0.425
Interaction	-79.6±29.6	226.46	1	<0.001	-	-	-	-
Month		7.04	5	0.008		45.79	5	<0.001
Survival								
Carotenoid	-0.3±3.6	0.01	1	0.928	-0.3±4.8	0.003	1	0.951
Melanistic	-5.6±2.7	4.47	1	0.034	-6.6±3.1	4.64	1	0.031
Month		6.04	5	0.302		22.37	5	<0.001
Residual df			294				208	

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Chapter 3

Predation-associated differences in sex-linkage of wild guppy coloration

Abstract

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 I 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.

Introduction

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). This is particularly important for sexually antagonistic traits, which offer a fitness advantage to one gender but are detrimental when expressed in the other (Rice 1984). A male-beneficial sexually antagonistic mutation, for example, would not increase in the population if linked to an autosome unless the benefit to males vastly outweighs the disadvantage to females (Ellegren and Parsch 2007). If this gene were linked however to the Y-chromosome in a region where it will not recombine with the X, it would have a greater chance of spreading to fixation as it would only be transmitted to males and would not affect the female line. Given this one should expect females to bear few sexually antagonistic genes under strong sexual antagonism. However, if costs to females are relaxed, 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 as one sex is hemizygous for the X-chromosome (Reinhold 1998), or increased gene dosage of the sexually selected trait in males (Charlesworth et al. 1987; Fairbairn and Roff 2006). Additionally, a by-product of suppressed recombination on the Y-chromosome is that it will degrade over generations and genes are eventually lost. Hence, again the evolution of any male-

beneficial mutations may be favorably linked to the X-chromosome. 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 I use hormone assays of populations of Trinidadian female guppies 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 colour. Strong sexual dimorphism exists in guppies (Houde 1997). Male guppies are significantly smaller and exhibit numerous body and tail color patterns, whereas female guppies are larger and are typically colorless. However, it has been previously shown in this system that when females are exposed to male hormones they exhibit male traits such as body coloration that are not Y-linked (see Supporting Information for pictures).

Natural guppy populations can typically be divided into two eco-types (Endler 1995; Reznick et al. 1996; Magurran 2005). *High-predation* populations are usually found in the downstream reaches of rivers, where 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, where most predatory fishes are absent. Guppy coloration is subject to strong natural selection (and sexual selection) in Trinidadian streams. Bright coloration attracts the unwanted attention of predators while at the same time attracting females. So, 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). The cost-benefit interplay between increased attractiveness and risk of predation has long made guppy coloration be recognized as a sexually antagonistic trait (Fisher 1931; Brooks 2000; Postma et al. 2011). However, here I am concerned with the sex-linkage of coloration. Since color genes are not normally expressed in female guppies the genetics of guppy coloration is likely driven by male sex-specificity or male-bias of expression (Fisher 1931).

During multiple independent natural colonizations from high to low predation environments throughout their evolutionary history (Alexander et al. 2006; Suk and Neff 2009), guppies have 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 released from predation by artificially transplanting them to a low predation community (Endler 1980).

Guppy coloration has been found to be mostly sex-linked, generally to the Y-chromosome. 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 (Haskins and Haskins 1951; Haskins et al. 1961; Winge 1922, 1927; Nayudu 1979; Khoo et al. 2003). These genes are generally inherited as a Y chromosome supergene (closely linked together). At least 17 additional genes have been identified that recombine between the X and the Y chromosomes, although recombination rate is approximately 4% (Lindholm and Breden 2002). Only 6 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 co-dominant.

Haskins and colleagues (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 all non-Y-linked color genes, which are normally sex-limited in their expression, 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, 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. This discovery, if general, 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.

For the above reasons, guppies are well suited and unique for addressing questions regarding the evolution of sex linkage. Here I focus solely on the X/Y chromosomal sex determining system. However several studies suggest that other sex chromosomes 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 chapter I evaluate the generality of Haskins et al. 's latter finding using female hormonal manipulations. I test for associations between predation and the abundance of color genes that are not linked to the Y-chromosomes. I include multiple streams in Trinidad which contain populations of closely related guppies that occupy either high or low predation habitats. Next, I 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. Finally, I explore this point further by including two more novel transplant populations introduced from high predation into low predation environments, but this time also differing in resource availability and light. Evidence for consistent associations between predation and sex-linkage would create the future opportunity to identify ecological factors which influence the evolution of patterns of sex-linkage.

Methods

I used hormone assays to evaluate the degree of sex-linkage of color loci, as have been used in a variety of organisms including fish (Haskins and Haskins 1951; Haskins et al. 1961; Hildemann 1954; 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

manipulation tests 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). I 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), my measure is likely to be strongly correlated with the degree of X-linkage.

Collection and hormone trials

I collected experimental female fish from natural populations on five unmanipulated streams across Trinidad: 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 where high predation locality is restricted to a short stretch of river between the ocean and a barrier waterfall so high-predation guppies are rare). Each high-low predation pair represents an independent event of phenotypic and genotypic divergence (Alexander et al. 2006; Suk and Neff 2009). I also collected fish from the low-predation environments in the Turure River, which did not contain guppies until 1957, when C. P. Haskins introduced a population of fish from the high-predation section of the Guanapo River (Shaw et al. 1992). I then evaluated fish from the high-predation environment in the Turure, because it has been shown via genetic relationships that the natural high-predation Turure population were decimated by gene

flow of the introduced guppies into their environment and the current population is now 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 (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 females that were all over 20 mm in length.

Finally, I collected fish from the closed and open canopy introduction sites in the Guanapo River. In March 2008, D.N. Reznick and colleagues introduced ~150 guppies from the Guanapo high predation environment (same ancestor as in previous Turure introduction) into two low predation previously guppy-free tributaries in the same river (see Chapters 1 and 2 for more information regarding the specifics of the introduction). One of the tributaries was further manipulated by trimming the canopy above the stream by approximately 50 percent (as measured with a densitometer) by members of our research team. This resulted in a significant increase of stream productivity and resource biomass (Kohler 2010). Canopies were re-thinned at least once per year to maintain these differences in light level and productivity. In February 2009 (11 months post introduction), a subset of juvenile guppies from both low predation introduction tributaries and ancestral Guanapo high predation environment were collected and brought back to the lab. These fish would have reached maturity in the wild in March 2009, and hence are comparable to wild data on adults one year after the introduction. The collected guppies were then reared under common garden conditions until maturity. Once mature these males and females were mated in single pairs so that all wild-caught fish could be

equally represented in the first generation of lab-born offspring. Their offspring (the grandchildren of those collected in the field) were again raised under common conditions. A total of 42 second-generation mature fish were used in this portion of the experiment: 10 Guanapo high-predation lab-reared (GH F2), 16 closed canopy (Closed), and 16 open canopy (Open).

Each wild fish was housed individually in two gallon glass aquaria during the experiment. I treated each tank with 100µl of a 1mg/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). For the natural populations and Turure introduction: every three days, I recorded the presence or absence of either melanistic (black, fuzzy black) or xanthophore (orange, yellow) coloration on the body of the fish (yellow pterin spots are rare on the body and only 12 of 178 fish in my experiment show at least 1 yellow spot) visually so as to compare to digital photographs. For the canopy manipulated introduction: every three days I recorded the presence or absence of either melanistic, carotenoid (only orange), and tail coloration (as no fish showed any yellow coloration). 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). At the end final coloration was assessed from the digital pictures by one individual (S.G.), and using a color standard, which was used in all photographs to better standardize presence or absence of coloration. 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 delayed expression was missed.

Statistical analysis

I first analyzed the 9 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. I built generalized linear models where the response variables were binomial (presence or absence of color) and followed logit-linear functions of predation level (HP vs. LP), stream of origin, and their interaction. I tested for significance using likelihood ratio ANOVAs. The interaction term was removed if the χ^2 statistic was lower than 1 and/or the p-value higher than 0.5. I wanted to ensure that lack of color could be interpreted as lack of a response rather than a delayed response cut short by the end of the experiment. To that effect, I 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 the occurrence of rapid evolution of sex-linkage in the introduced Turure low-predation population after translocation from a high-predation one, I compared ancestor (Guanapo high-predation) and derived (Turure low predation) populations via a logistic GLM as described above. Moreover, to assess how this degree of evolutionary change compares to general predictions of high and low-predation populations, I used the data from the 9 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) allow me to account for their covariation by modeling the odds ratio of both events. In both sections models were fit using functions glm and vgam in program R v.2.9.2.

To assess the occurrence of rapid evolution of sex-linkage in the introduced open and closed canopy low-predation populations after translocation from a high-predation one, I compared five streams: ancestor (both wild natural Guanapo high-predation, common garden reared Guanapo high-predation F2), derived (open and closed canopy low predation) populations, and natural wild Guanapo low-predation population via a logistic GLM as described above where the response variables were again binomial (presence or absence of color).

Results

Results indicate significant differences in testosterone effect between high and low-predation natural populations. Figures 3.1a and 3.1b 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 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, as shown in Figure 3.1c. Interaction terms between predation and stream were non-significant in both the model for melanistic color ($\chi^2 = 0.36$, $p = 0.95$) and the one for xanthophore colors ($\chi^2 = 1.39$, $p = 0.71$), 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 3.1). The results indicate a consistently higher degree of X or autosomal linkage for color among low-predation fish (Figure 3.1).

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 predictions than to high-predation predictions (Figure 3.2). The response levels of the Turure high-predation population, which is largely comprised by descendants of the upstream introduced fish (yet mixed with some native fish, Shaw et al. 1992) lie between both predictions (Figure 3.2). 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 ($\chi^2 = 17.96$, $p < 0.0001$).

Females from the Guanapo high-predation introduction into the two low predation tributaries also show a rapid response to testosterone more similarly to low-predation predictions than to high-predation predictions (Figure 3.3). This is striking considering

these populations have only been adapting to changes in their environment for less than a year. In terms of carotenoid (orange) coloration, the two introduction sites are not significantly different from each other (effect = 0.251; $p = 0.723$) or the natural Guanapo low-predation (GL) wild population (effect = -1.135 and -1.386; $p > 0.14$), but are significantly different from the Guanapo natural wild high-predation ancestors (effect = 2.944 and 3.196; $p < 0.01$) and almost significant from the Guanapo lab-reared high-predation fish (effect = 1.386 and 1.638; $p = 0.065$, probably due to sample size issues). In terms of melanistic coloration the Guanapo high-predation ancestors (GH) are significantly different from all groups (effect > 2.910; $p > 0.005$) except the Guanapo high-predation lab-reared, GH F2 (effect = 1.587; $p = 0.08$). Tail coloration is the only variable that shows a significant difference between the two introduction sites (effect = 2.547; $p = 0.033$), and all groups are significantly different from each other ($p > 0.014$).

Discussion

To my knowledge, this is the first assessment of consistent intraspecific variation in the degree of trait sex-linkage. My results show a consistent increase of non-Y-linked genes as fish invade the low predation reaches of each river. I find the same pattern in populations experimentally introduced from a high to a low predation environment indicating that sex-linkage can *significantly* and *rapidly* change in response to ecological factors.

Natural low-predation populations have an abundance of non-Y-linked genes (supported by a higher proportion of female guppies showing coloration under testosterone treatment) compared to natural high-predation populations. Since high-predation females show little to no coloration but high-predation males show an abundance of coloration, these results seem to suggest that there is a greater proportion of Y-linkage (i.e. amount of Y linkage/total genetic linkage) in these populations (appendix 2). The Turure introduction shows that once fish are transplanted into low-predation from high-predation environments they develop linkage relationships in coloration more similar to typical low-predation populations within 50 years or less and than gene flow of these fish back into the high-predation environment can potentially revert the relationship back to typical high-predation relationships (seen by the Turure high-predation guppies showing a greater proportion of coloration than a typical high-predation population, but significantly less than a normal low-predation population, Figure 3.2). Lastly, the novel introduction streams show that other ecological factors such as canopy manipulations can also affect linkage relationships of male fitness sub-traits such as tail coloration, and that changes to linkage relationship can change in less than one year.

My results can represent two non-mutually exclusive scenarios (Figure 3.4). 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 I see in the low-predation populations was either present at low frequencies (or suppressed) in the ancestral high-predation populations (Figure 3.4,

scenario 1a), or arose via mutation (Figure 3.4, scenario 1b). Selection then favors non-Y-linked coloration as guppies invade the higher reaches of the stream.

Second, the increase in X and autosomal linkage could be associated to a decrease Y-linkage as guppies invade low predation environments (Figure 3.4, scenario 2). I did not directly test this in my experiment but if I assume that the number of color genes is the same for high- and low-predation populations (an assumption particularly suitable for the Turure introduction, diverging only by 50 years from its Guanapo HP ancestors; and the canopy manipulation introduction sites diverging only 1 year from its Guanapo HP ancestors), I can state that differences among populations reflect different ratios of Y-linked and non-Y-linked alleles. The implication in this case is that selection for bright coloration in low predation environments is causing a change in linkage relationships. This could occur via recombination, and it has already been shown that limited recombination does occur in a number of genes between the X- and Y-chromosome in guppies (Lindholm and Breden 2002). In this case, selection favors the translocation of ancestral Y-linked alleles to the X-chromosome in the low-predation environments. Haskin et al. 's (1961) comparison with pedigree studies in the same populations also support this conclusion for specific genes as explained previously where 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. There have also been studies using a quantitative genetics approach to show that coloration, especially orange, is largely Y-linked in guppies (Hughes et al. 2005; Postma et al. 2011). Indeed, I myself have performed quantitative genetic analyses using the animal model showing

high proportions of genetic variation in the canopy manipulated Introduction sites show a high proportion of Y-linkage (see Chapter 1). The novel hormone findings in that same introduction seem to point to this relationship changes quickly and in response to both changes in predation pressure (carotenoid and melanistic spots) and light/resource changes in habitat (tail coloration).

Regardless of the specific scenario, my results clearly suggest an increase in the amount of non-Y-linked coloration in low-predation populations compared to high-predation populations. Although my data cannot reveal the molecular mechanisms behind such predator-associated differences in sex-linkage, it does invoke selective factors. I now discuss three potential ones previously proposed in theoretical studies that encompass both explanations of my results.

First, optimal inheritance rules may play an important role in the sex-linkage divergence of populations that differ in the importance of viability versus sexual selection. In the high predation environment where bright male coloration is strongly selected against (Godin and McDonough 2003) one may expect selection for stronger similarity between fathers and sons: a male that has survived to reproduction is likely to bear a successful color pattern and hence, may be selected to produce offspring which bare the same color pattern (Kirkpatrick and Hall 2004a). It has also been suggested 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 (Farr 1983). The existence of two color gene copies may therefore represent a mechanism by

which guppies acquire more intensive coloration in a low predation environment but which would be selected against in high predation environments.

Second, sexual antagonism theory predicts selection against non-Y-linked male sexually selected traits that incur a survival cost in the female (Bull 1983; Rice 1996). This cost is higher in high-predation populations (Godin and McDonough 2003) and hence could contribute to explaining my results. However, later models also predict that male beneficial sexual antagonism should favor X-linkage when the trait is recessive (Reinhold 1998; Fairbairn and Roff 2006). Given that most non-Y-linked traits found are known to be co-dominant, the former prediction seems more appropriate in my case. Additionally, it has been repeatedly shown that the orange color spots of guppies are predominantly Y-linked (Hughes et al. 1999; Houde 1992; Postma et al. 2011). My study shows that, while this may be true for high-predation populations, low predation populations show a higher occurrence of non-Y-linked xanthic and carotenoid coloration, supporting later sexual antagonism predictions.

A third selective factor to consider is that stronger sexual selection under low predation risk (as reported in Kelly and Godin 2001) could favor 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 I can offer little more than speculation regarding the importance of the aforementioned mechanisms in explaining my 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. Coupled with previous results my study seems to suggest a rapid restructuring of linkage patterns in guppies via selection, leading to different accumulation of genes on the sex chromosomes. This finding is of general significance to those examining 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 here reported. Notwithstanding, the consistent correlation between linkage and predation, including the documented case of contemporary evolution in the introduced populations, provides empirical support for theories suggesting that genetic parameters such as sex-linkage may be subject to selection.

Figure Legends

Figure 3.1. 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. The third panel (c) shows 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.

Figure 3.2. 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 intrioduced 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.

Figure 3.3. Proportion of females in introduction experiment (involving canopy manipulated stream) exhibiting coloration in response to hormone treatment. This is used as a proxy for the amount of X- and autosomal linkage in each population. Populations examined are the natural wild Guanapo high- and low-predation fish (HP, black bar and LP, white bar), Lab-reared F2 Guanapo high-predation fish (HP F2, black bar), and introduced open and closed canopy fish (GH fish introduced into two low predation environments, grey bars). Panels: (a) Binomial trials, separated stream, for the effect on carotenoid (orange) coloration and (b) for melanistic (black) pigmentation. The third panel (c) shows the proportion of fish showing tail coloration. Error bars represent standard errors.

Figure 3.4. Proposed scenarios explaining results of increased non-Y-linkage of coloration once guppies invade the low predation reaches of the streams. Phylogenetic relationships suggest that guppies in low-predation populations descend from high-predation ancestors (Alexander et al. 2006) so these scenarios are likely for both the natural and artificially transplanted movement of guppies from high- to low predation environments over time. In scenario 1a, all of the increased X-linked/autosomal (most likely X-linked as explained in introduction) that I see in the low-predation populations was either present at low frequencies (or X-linked counterpart allele suppressed) in the ancestral high-predation populations. Selection then increases the frequency of these alleles, or the removal of the suppressor once guppies invade the low predation sites. In

scenario 1b, novel mutations of X-linked genes arise and are then selected for in low-predation environments. In scenario 2, increased recombination between the X and Y chromosome within the grey region (recombination zone) in the low predation environments cause solely Y-linked alleles (no X-linked counterpart) to be expressed on the X chromosome.

Figure 3.1

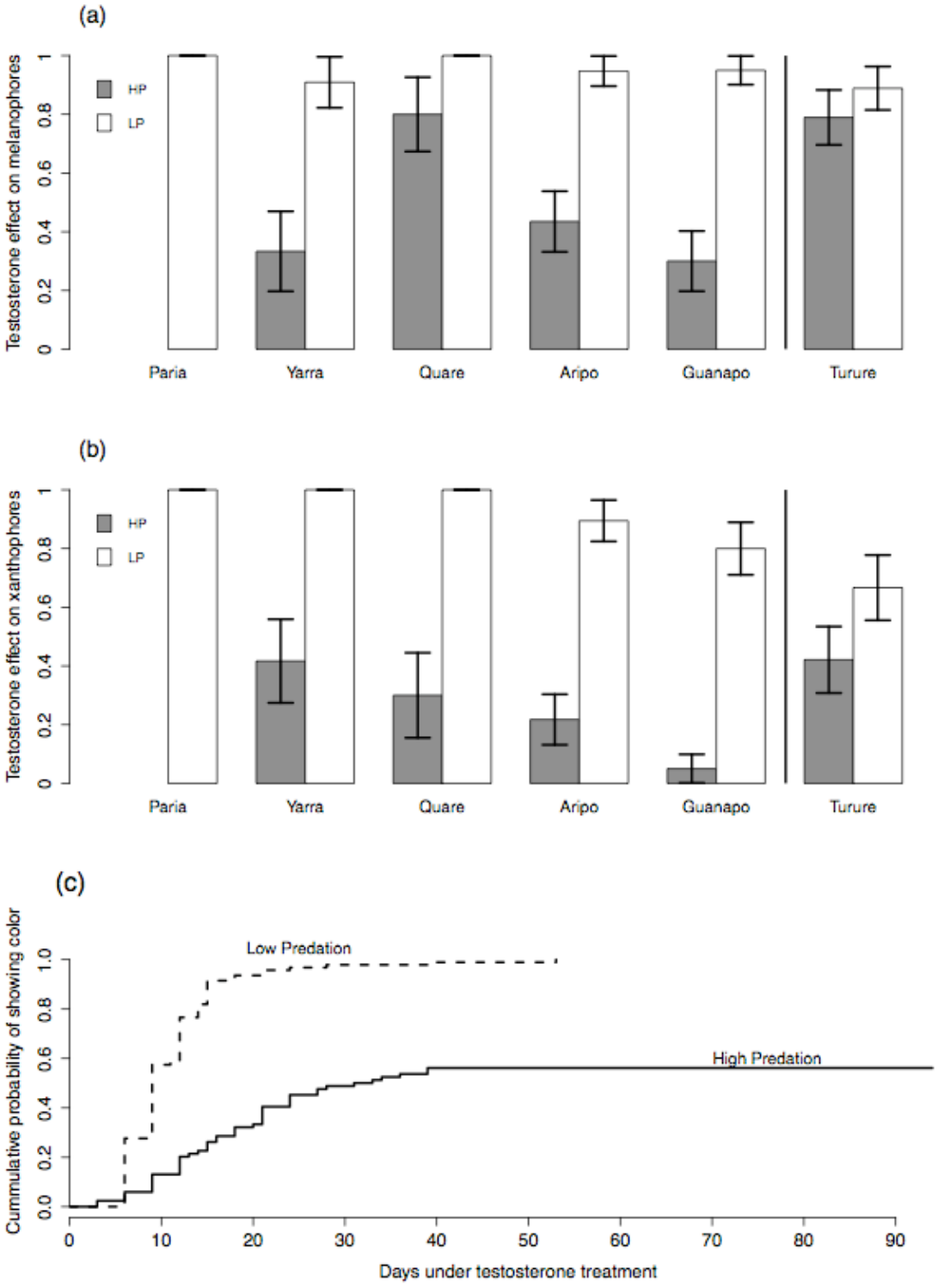
(a)
Testosterone effect on melanophores
HP
LP
Paria
Yarra
Quare
Aripo
Guanapo
Turure
(b)
Testosterone effect on xanthophores
HP
LP
Paria
Yarra
Quare
Aripo
Guanapo
Turure
(c)
Cummulative probability of showing color
Low Predation
High Predation
Days under testosterone treatment

Figure 3.2

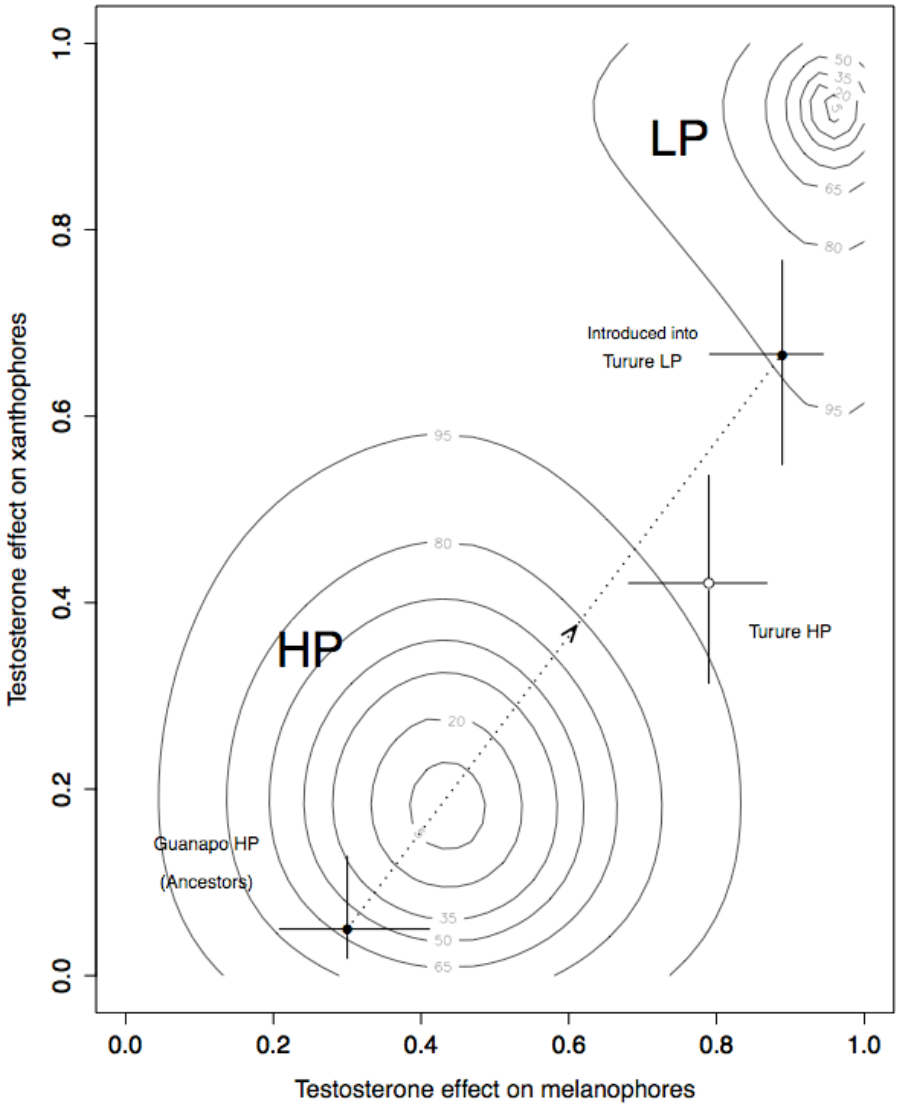
LP
HP
Introduced into
Turure LP
Turure HP
Guanapo HP
(Ancestors)
95
80
65
50
35
20
5
Testosterone effect on xanthophores
Testosterone effect on melanophores
0.0
0.2
0.4
0.6
0.8
1.0

Figure 3.3

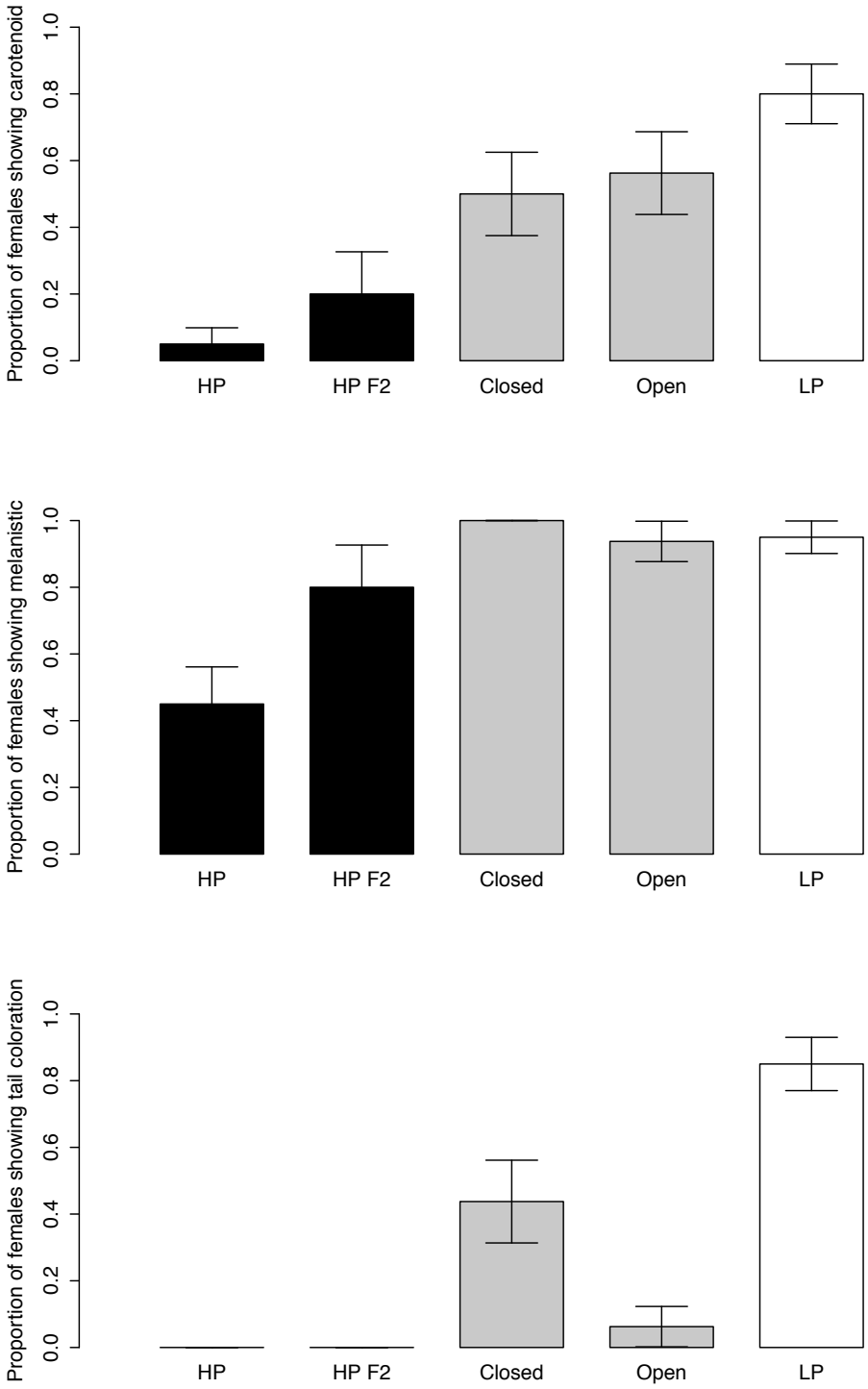
Proportion of females showing carotenoid
0.0
0.2
0.4
0.6
0.8
1.0
HP
HP F2
Closed
Open
LP
Proportion of females showing melanistic
0.0
0.2
0.4
0.6
0.8
1.0
HP
HP F2
Closed
Open
LP
Proportion of females showing tail coloration
0.0
0.2
0.4
0.6
0.8
1.0
HP
HP F2
Closed
Open
LP

Figure 3.4

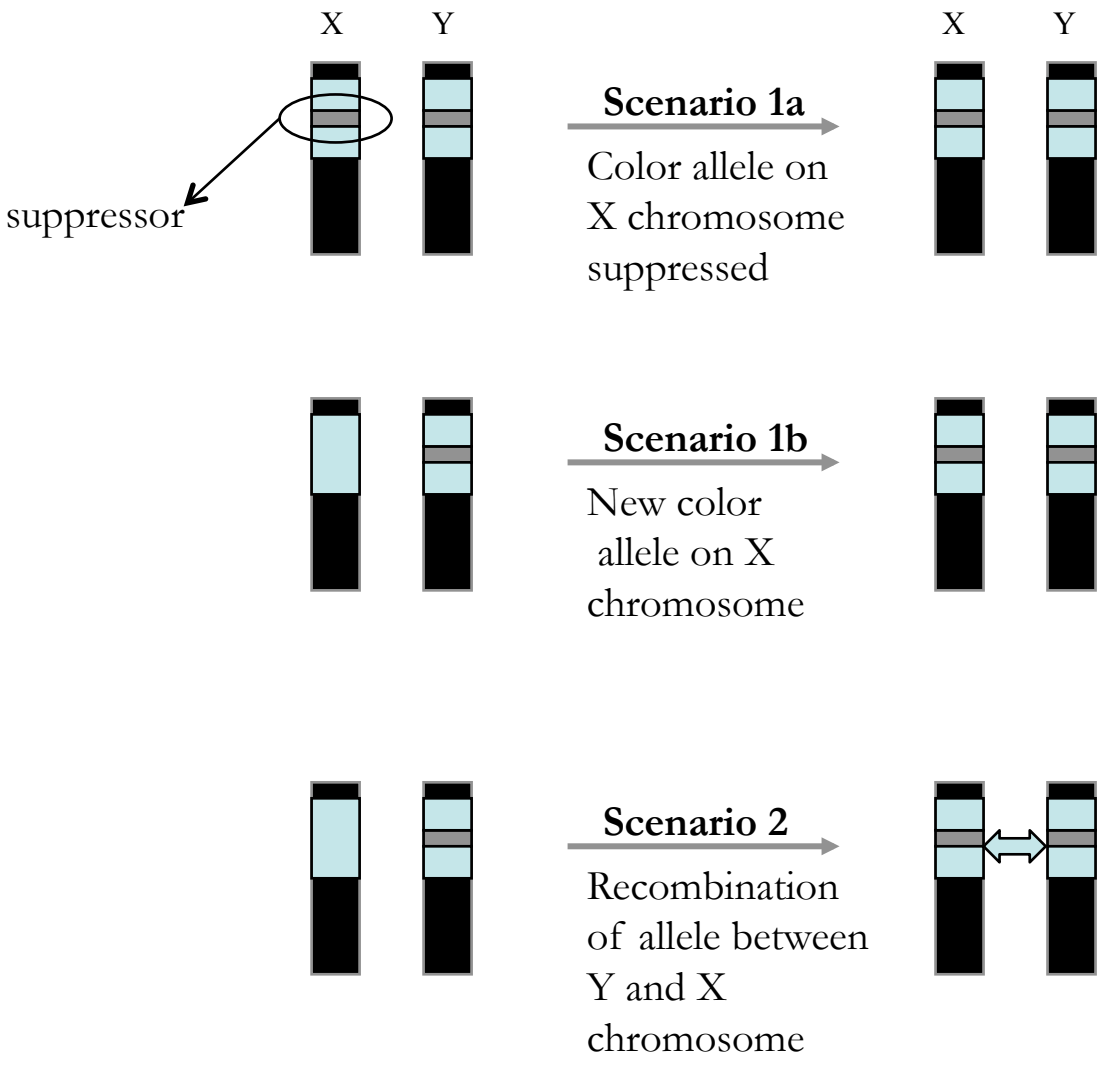
X
Y
suppressor
Scenario 1a
Color allele on X chromosome suppressed
X
Y
Scenario 1b
New color allele on X chromosome
Scenario 2
Recombination of allele between Y and X chromosome

Table 3.1. ANOVA 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

Supplementary Information

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

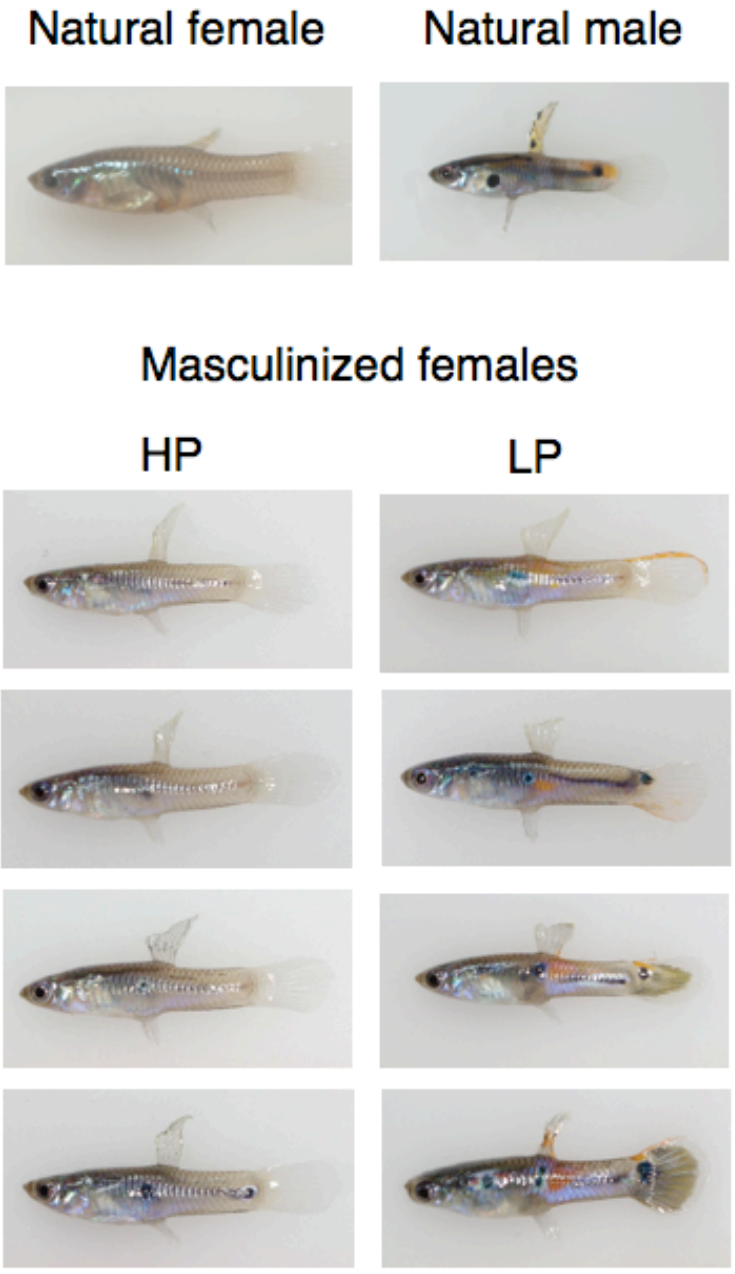

Table S1: I used presence-absence of different colorations as my response variables, assuming that my females would represent a random sample of color patterns shown by wild males, and hence provide an estimate for the amount of those that are not Y linked. All males show coloration (both orange and black spots), and thus in the binary case, they would all represent a '1' which is why I did not include them in my analyses. However, I here show some data of male coloration two streams to establish that in the future I aim to try and estimate the proportion of Y-linked coloration by comparing lab-reared males with testosterone treated females. Identifying specific independently inherited patterns in males is very difficult due to their huge diversity and overlap, and constitutes a field of research in itself. Here I attempt to approximate this by counting the number of spots of different colors in each individual and looking at the ratio of spots in masculinized females to spots in males. I currently only have comparable laboratory pictures of males on two of the study populations: Guanapo and Yarra. Due to the paucity of population replicates, I only include it in the supplementary information. In summary, the ratio of female to male orange (carotenoid) spots (i.e. proportion of non-Y linkage) is larger for both low-predation populations.

	Average Number of Orange Spots		
	Male	Female	Proportion of Female/Male
High-predation Guanapo	2.14	0.10	**0.05**
Low-predation Guanapo	2.45	1.05	**0.43**

	Average Number of Orange Spots		
	Male	Female	Proportion of Female/Male
High-predation Yarra	2.53	0.42	**0.16**
Low-predation Yarra	3.20	1.91	**0.60**

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Concluding Remarks of Dissertation

Since the time of Darwin various studies have documented contemporary or rapid evolution in the wild yet biologists are still puzzled about how and why some organisms are more adaptive than others. Adaptation requires both inheritance and selection, but most studies either ignore heritability and concentrate on selective pressures, or assume a particular mode of inheritance. Sexual selection is often stronger than natural selection and should be able to drive rapid evolution of particular traits, yet there are few studies examining the rapid evolution of sexually selected traits in nature (Svensson and Gosden 2007). Theoretical research has shown the importance of underlying genetic architecture in facilitating or constraining evolutionary processes of secondary sexual characters, so any variation in the inheritance of a particular trait can indeed affect the mechanism of adaptive divergence. This research will for the first time empirically test important implications and ideas from theory regarding the relationship between sex-linkage and selective pressures of male coloration in wild Trinidadian guppies (*Poecilia reticulata*).

Theory predicts that selection will be more efficient in the fixation of sex-linked genes rather than autosomally linked ones (Charlesworth et al. 1987). For instance, consider a new beneficial autosomal mutation. It will likely be recessive and hence it will be obscured by the ancestral alleles, and only rarely be exposed to selection. If, however, this new beneficial recessive mutation is linked to the sex chromosomes it would be directly exposed to selection in the hemizygous sex.

Theory also predicts that traits linked to the Y-chromosome will have a faster evolutionary rate than traits linked to the X chromosome (Kirkpatrik and Hall 2004). This is especially important for sexually selected traits because any trait that specifically benefits males should preferentially accumulate on the Y-chromosome where it would be inherited haploidly, marking the early steps of sex-chromosome evolution. This latter finding from theory is highly debated, because many predict that the tendency of the Y-chromosome to degenerate as it evolves makes it unlikely that functional genes should remain linked to it rather than the accompanying X-chromosome (Postma et al. 2011).

The guppy also has cytologically similar chromosomes indicating they are in early stages of Y-chromosome evolution. I would hence expect them to have male beneficial genes tightly linked to the heterogametic chromosome. In fact guppy coloration, a sexually antagonistic trait, has been shown to be mainly Y-linked in laboratory studies (Box 1 Introduction). However, it is unknown how ecological effects influence this pattern in wild populations, and how the degree of sex-linkage affects the heritability and hence evolutionary potential of these traits in nature.

In the first chapter, I examined the degree of Y-linked and heritability of male coloration in two wild populations of high predation guppies introduced into two low predation environments. The introduction environments differed from each other in that the canopy cover in one site was artificially trimmed thus increasing light and habitat productivity, and allowing me to examine how genetic variation is affected by environmental quality. To my knowledge this was the first time Y-linked versus non-Y-linked genetic variance was partitioned in this manner in a wild population. In both

introduction populations I found that Y-linkage remains the main source of additive genetic variation for male coloration. This resulted in high heritabilities and hence should allow for the fast rates of evolution observed for color in this species. I also found lower heritabilities of coloration in the high-productivity stream, which was consistent with a previous study in Soay sheep where investigators found higher heritabilities under poorer environments (Wilson et al. 2006). Overall my results implied that considering both the environmental and genetic components of phenotypic variation were important in determining the evolutionary potential of organisms facing environmental changes, and that Y-linkage may be important for sexually antagonistic traits.

Finding high heritabilities and high proportion of Y-linked to non-Y-linked variation in male coloration in both introduction sites indicated a high evolutionary potential in response to selection. In my second chapter I actually measured this in both introduced populations. I used bimonthly censuses of the guppies over 12 months post-introduction to measure the temporal divergence of male coloration between the ancestral and derived fish. Common garden assays allowed me to test if any changes I found in the wild had a genetic basis. Male melanistic and carotenoid coloration diverged between the ancestral and derived populations in only one year (< 3 guppy generations).

Carotenoid coloration generally increased over time, but melanistic coloration generally decreased (except for genetic divergence where melanistic coloration increased in the open canopy but decreased in the closed canopy compared to the ancestors). This result was surprising to me based on previous evidence suggesting that all colors should increase in area when predation risk is removed (as more conspicuous coloration is

preferred by females). In Chapter 1 however, I found that the strongest genetic correlation between carotenoid (orange) and melanistic (black) coloration was associated to Y-linkage, which had the largest proportion of additive genetic variation of coloration. I found that this correlation was strongly negative in the closed canopy stream and positive in the open canopy stream. This implied, for example, that strong selection for orange could result in a decrease in black under the closed canopy but an increase under the open canopy. These predicted results from Chapter 1 are in remarkable accordance with the results for the genetic divergence of coloration in the two introduction populations.

In Chapters I find evidence of a high proportion of Y-linkage in a high-predation wild population of guppies introduced into two novel environments, and in Chapter 2 I show that this genetic architecture can result in rapid evolution of the trait in nature under abrupt changes in selection pressures (predation risk). In Chapter 3 I explore the extent to which linkage of male coloration differs among populations adapted to varying predation regimes. Theory abounds on how sexually antagonistic selection, mate choice or other phenomena should favor different types of sex-linked inheritance, yet evidence in nature remains limited. One previous study 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. 1961). This discovery, if general, 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.

In my last thesis chapter I extended these results and used hormone assays to test for associations between predation and the abundance of color genes that are not linked to the Y-chromosomes in multiple natural and introduced populations (including the populations studies in Chapters 1 and 2). To my knowledge, this was the first assessment of consistent intraspecific variation in the degree of trait sex-linkage. My results showed a consistent increase of non Y-linked genes as fish invade the low predation reaches of each river. I found the same pattern in populations experimentally introduced from a high to a low predation environment indicating that sex-linkage can significantly and rapidly change in response to ecological factors.

The overall general significance of this dissertation is intended to create the groundwork for developing a future research program examining the importance of sex-linkage in the evolution of sexually selected traits in nature. Sex chromosomes have evolved repeatedly and independently in plants and a diversity of animals, yet we still have relatively little evidence of the mechanism of sex chromosome evolution in nature (Filatov et al. 2000; Bachtrog 2006; Ming and Moore 2007). Research using organisms such as guppies, which have early sex chromosomes, allow us the opportunity to study the early phases of Y-chromosome evolution, or the linkage of male beneficial or sex-limited genes to the Y-chromosome. Finding microgeographic variation in the amount of non-Y linkage (and presumably Y-linkage) of male coloration allow us to examine the role ecological contexts could play in this linkage relationship of these types of traits.

Additionally, theory has shown us that sex-specific evolutionary rates will be different depending on linkage to the X or Y-chromosome. We can test this by performing transplants of guppies that differ in linkage proportions and following their adaptation rates to novel environments.

I believe that future work on the evolution of sexually selected traits should hence focus on: (1) molecular and genetic characterizations of the extent of genetic (gene linkage) versus environmental (differences in selection) control in the maintenance of trait variation in the wild; (2) comparative studies on how differences in linkage patterns can constrain or facilitate the evolution of sexually-selected traits; (3) selection analyses evaluating how variations in the environment can select for different sex-linkage patterns of sexually selected traits; and (4) further development of the genetic mechanisms behind microgeographic variation in guppy sex-linkage to establish guppies as a model system to experimentally explore questions on the evolution of the Y-chromosome.

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