CONSERVATION OF SYNTENIC RELATIONSHIPS AMONG MICROSATELLITES BETWEEN *POECILIA* AND *XIPHOPHORUS*

by

Martin Earl Brummell
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Name: Martin Earl Brummell

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Examining Committee:

Chair: Dr. R. Nicholson, Associate Professor

Dr. F. Breden, Associate Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. W. Davidson, Professor
Department of Molecular Biology and Biochemistry, S.F.U.

Dr. M. Hart, Associate Professor
Department of Biological Sciences, S.F.U.
Public Examiner

Jan. 27/06
Date Approved

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ABSTRACT

The guppy is unique in the degree to which environmental variables shaping phenotypic variation are known. Genomic resources have been developed in *Xiphophorus* due to their utility in the study of melanoma. If linkage maps for the guppy and *Xiphophorus* are similar, *Xiphophorus* genomic resources will be useful in the guppy. I used an F_2 mapping cross of divergent populations of guppies to construct partial genetic linkage maps incorporating microsatellite markers derived from *Xiphophorus*. Flanking regions for a sample of microsatellites occurring in maps for both taxa were sequenced in guppies and compared to published sequences from *Xiphophorus*. This confirmed that these loci were homologous. The female map comprises sixteen linked markers on six linkage groups and the male map comprises 24 markers on nine linkage groups. Linkage relationships among loci homologous in guppies and *Xiphophorus* primarily show conservation of genetic architecture between species, but several major changes were detected.

Keywords

Poecilia, Microsatellite, Synteny, Linkage map, *Xiphophorus*

DEDICATION

I would like to dedicate my thesis to Christine Pearce. Without her patience, love and support, this work would not have been possible.

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CHAPTER ONE: COMPARATIVE GENOMICS IN POECILIIDAE

Fishes of the family Poeciliidae have long served as model systems for the study of evolution, ecology, behaviour and genomics. Natural populations of the guppy (*Poecilia reticulata*) have been studied for more than fifty years (Haskins and Haskins 1948; Haskins et al. 1961), and the unique aspect of this work is that the selective forces driving phenotypic variation between populations are well known (Houde 1997). *Xiphophorus* species and hybrids have been studied for a similar length of time and, in addition to ecological and evolutionary studies, have been extensively used as a model system for the study of melanoma and genomics (Anders 1991; Kazianis and Walter 2002; Meierjohann et al. 2004).

The extensive work in *Xiphophorus* provides a wealth of genomic and genetic tools that can be used in the guppy to further our understanding of the genetic basis of adaptive evolution and the processes governing the evolution of genomes in this well-characterised evolutionary model organism. Comparison of the genetic linkage maps will predict how useful these tools developed in *Xiphophorus* will be to studies in the guppy.

The goal of this study is to compare the genetic architecture of a well-characterised genetic model, *Xiphophorus*, to the genetic architecture of a well-characterised evolutionary model, the guppy, using microsatellite genetic linkage maps. This will increase the number of available molecular genetic tools for use

in studies of the guppy, which will assist in studying the genetic architecture and evolution of the guppy.

In this chapter, I will review background relevant to the importance of the guppy as a model organism, including studies of sexual selection, behaviour and adaptation in natural and laboratory guppy populations. I will also review the importance of *Xiphophorus* as a model organism, including laboratory studies of melanoma and other neoplasms in interspecies hybrids of this genus as well as studies of the selective forces acting on natural *Xiphophorus* populations. Studies of genetic architecture can elucidate some of the interactions between an organism's selective environment and its genome. I will review some of the background relevant to genetic architecture in the guppy and *Xiphophorus*, and some comparative studies that have included both taxa.

The Guppy as a Model System

The guppy has been used to study sexual selection, natural selection and other evolutionary forces acting on natural populations (Houde 1997). A large number of populations of the guppy distributed across northeastern South America (Rosen and Bailey 1963; Houde 1997) provide a series of replicate treatments varying in population size and density (Farr and Herrnkind 1974), resource availability (Grether et al. 2001b), predation risk (Endler 1980; Godin and Briggs 1996) and other selective forces. Guppies from any two populations yet studied can mate and produce viable offspring (Houde 1997), indicating that guppy populations have not speciated, despite the large interpopulation phenotypic variation in this species.

Variation in population density applies varying selective forces in guppy populations and alters mating behaviour (Farr and Herrnkind 1974; Jirotkul 1999) and reproductive physiology (Dahlgren 1979). Guppies living in high-density populations show more variance in courtship behaviour and female aggression towards males (Farr and Herrnkind 1974), decreased male displays (Jirotkul 1999) and reduced fecundity and fertility in females (Dahlgren 1979). These changes were generally found to be plastic responses, rather than genetic polymorphisms.

Food limitation, particularly of dietary carotenoids, constrains evolution in the guppy. Males must obtain carotenoids from their diet, primarily from algae, to produce their characteristic orange and red skin pigments (Karino and Haijima 2004). Some red pteridine pigments can be synthesized *de novo* by guppies (Grether et al. 2001a), but dietary carotenoids significantly increase male colour and attractiveness to females (Grether 2000), and consequently influence female choice and sexual selection. Additionally, the indicator hypothesis predicts that difficult to acquire resources, such as carotenoids, may be useful as signals of male quality, increasing the role of these chemicals in sexual selection (Zahavi 1975; Kodric-Brown and Brown 1984; Grether 2000).

Where they co-occur with guppies, predatory fish apply natural selection to guppy populations, in a direction opposite to that applied by sexual selection (Haskins et al. 1961; Endler 1995). Predatory fish tend to locate and attack guppies based on visual cues, making colourful males conspicuous to both females and dangerous predators (Haskins et al. 1961; Endler 1980).

This natural selection leads to a balance of selective forces such that drab males and less choosy females become prevalent in high-predation areas (Breden and Stoner 1987; Houde and Endler 1990; Endler and Houde 1995). Other life-history traits such as time to first reproduction and overall reproductive effort vary in guppy populations with predation risk (Reznick et al. 2001). Confounding the selective forces of predation are the selective forces applied by resource availability (Kolluru and Grether 2005). Guppies in upstream, low-predation environments are more severely limited in their resource availability due to extensive rainforest canopy cover over the streams, restricting algae growth (Grether et al. 2001b, Arendt and Reznick 2005).

There are large amounts of phenotypic variation between populations and between individuals (Haskins et al. 1961; Endler 1995; Brooks and Endler 2001). This variation allows the use of guppies in studying directly the effects of natural and sexual selection in natural populations. A series of transplant and introduction experiments, moving guppies between predation and sexual selection regimes and predators into predator-free environments, provides one of the strongest demonstrations of the effects of these selective forces (Reznick and Endler 1982; Reznick and Bryga 1987; Reznick et al. 1990; Houde 1997). High levels of variation within guppy populations would also be expected to contribute to their widespread success in invading new habitats by human introduction (Sakai et al. 2001, Spielman et al. 2004) but this has not been observed for guppies in Australia (Lindholm et al. 2005), where feral guppy populations were found to have relatively low levels of neutral genetic diversity.

Heritable differences are known to underlie much of the phenotypic variation between guppy populations and individuals (Magurran et al. 1996; Reznick 1996; Ghalambor et al. 2003), though the molecular characteristics of the underlying loci are mainly unknown (Shikano and Taniguchi 2003).

Understanding the effects of natural and sexual selection on the genes underlying variable phenotypes is an important goal of evolutionary biology. Given high phenotypic variation in guppies, their suitability to laboratory research, and extensive fieldwork, the guppy is an ideal model for this type of study. A genetic linkage map is an important step in locating and characterising genetic variation underlying adaptive phenotypic variation.

Xiphophorus as a Model System

Like the guppy, *Xiphophorus* fishes have been extensively studied as a model system (Kazianis and Walter 2002). *Xiphophorus* has been used to study sexual and natural selection (Basolo 1990; Baer et al. 1995), sex-determination (Basolo 1994; Traut and Winking 2001; Volff and Schartl 2001) and melanoma (Gordon et al. 1951; Anders and Anders 1978; Anders 1991).

Crossing designs producing offspring with malignant tumours in *Xiphophorus* hybrids were first explored more than 70 years ago (Gordon 1927; Kosswig 1928). The most famous crossing design, termed the "Gordon-Kosswig" cross after its creators, uses a *Xiphophorus maculatus* female mated to a *X. helleri* male, backcrossed to *X. helleri*. The resulting backcross hybrid offspring segregate alleles at two unlinked loci such that one quarter develop

malignant melanoma and die near the age of sexual maturity (Vielkind and Vielkind 1982; Anders 1991; Kazianis et al. 2004).

Other crossing designs using different species or strains of *Xiphophorus* produce offspring with different tumours or with tumours on different tissues (Schartl and Schartl 1996), or with increased susceptibility to melanoma formation after exposure to particular carcinogens (Nairn et al. 2001). Variations in tumor characteristics are under the influence of different oncogenic genotypes. This has increased the utility of *Xiphophorus* as a general model system for the development of neoplasia.

The ease with which *Xiphophorus* that spontaneously or inducibly develop malignant tumours can be produced has resulted in a wealth of genetic information now available including genetic linkage maps (Kazianis et al. 1996; Kazianis et al. 2004; Walter et al. 2004). These high-density linkage maps are an important step in identifying and characterizing genes involved in tumour development and suppression in *Xiphophorus* fishes. Several genes have already been identified, and their linkage relationships with other oncogenes or important phenotypic markers such as sex have been established (Kazianis et al. 2004).

Genetic Architecture and Synteny

Genetic architecture is the study of the composition, arrangements and interactions of genes and regulatory elements in the genome (Weisbrot 1963). Genetic architecture influences evolution by constraining the direction and speed

of evolutionary change, through such mechanisms as epistasis and pleiotropy (Mackay 2001, Wolf et al. 2005). Changes such as gene or chromosome fragment duplication or deletion alter genetic architecture and consequently alter the effects of evolutionary forces such as selection and mutation.

Broad-scale knowledge of genetic architecture allows finer-scale studies of small chromosome regions, eventually leading to chromosome walking, positional cloning and identification of candidate genes involved in quantitative and adaptive phenotypes. Such an approach has proven useful in studying dorsoventral patterning in medaka (Ohtsuka et al. 1999) and in studying human skin pigmentation and homologous genes in zebrafish (Lamason et al. 2005).

Comparative genetic architecture can be studied when genetic relationships, such as syntenic relationships, are known for homologous chromosome segments in two or more taxa. Such comparisons may elucidate the effects of differing selective regimes on genomes (Coghlan et al. 2005). Conserved syntenic relationships, where two loci occur together on one chromosome in two species, suggests conservation of other genetic architecture features, and provides a convenient starting point for further comparative genetic and genomic studies.

Studies of the genetic architecture of Poeciliid fishes have been carried out since the early decades of genetic research. A pioneer in studies of the genetics of sex determination, Winge (1922) demonstrated the guppy XX-XY sex determining system, as well as mapping phenotypic markers (Winge 1927) and establishing the guppy as a model system (Winge 1934).

Genetic architecture is important in the evolution of the guppy. Many body-colour genes are sex-linked in the guppy (Lindholm and Breden 2002), which influences sexual selection by disrupting linkage disequilibrium in females that never carry Y-linked genes. Linkage relationships among body-colour genes change in populations of guppies exposed to differing selection regimes, where some genes are linked only to the Y chromosome in high predation populations, but linked to either the X or the Y chromosome in low predation populations (Haskins et al. 1961).

Genetic architecture has also been important in the evolution of *Xiphophorus*. In eight species, the oncogene X*mrk* is intimately linked to the macromelanophore locus *Mdl* that controls the appearance of black spots on the body and fins (Weis and Schartl 1998). Even in the absence of interspecies hybridization, some cases of malignant melanoma have been documented in natural monospecific populations (Schartl et al. 1995). Which tissues are susceptible to these tumours is mediated by genetic architecture, in that the genes involved in suppression of some tumours in some tissues only function if the unmutated alleles are arranged in *cis* formation (Anders and Anders 1978).

Xiphophorus species are more closely related than many sets of congeners (Anders 1991), and hybridize readily under laboratory conditions. Some species pairs within the genus may hybridize in zones of contact (Hankison and Morris 2002; Rosenthal et al. 2003). The lethal cancers that develop in offspring of some *Xiphophorus* inter-species crosses should select against hybridization in wild populations via reinforcement (Servedio and Noor

2003). The linkage between X*mrk* and *Mdl* may explain the persistence of the apparently dangerous locus X*mrk* by the genetic hitchhiking hypothesis, where selection for *Mdl* by environmental factors prevents loss of X*mrk* (Frank et al. 2001).

Comparative studies of genetic architecture within Poeciliidae have been carried out for more than fifty years (Gordon 1952). Overall conservation of genetic architecture across this family has been found in several studies (Morizot et al. 1977; Leslie 1982). The few observed differences in genetic architecture across this family have been attributed to changes in recombination rate, rather than chromosome rearrangements or changes in syntenic relationships among loci (Morizot and Siciliano 1983).

Studies of genetic architecture in *Xiphophorus* have included the production of a high-density microsatellite genetic linkage map, using microsatellite markers that were also examined in two guppy lineages (Kazianis et al. 2004; Walter et al. 2004). The present study used some of these microsatellites and the existing *Xiphophorus* map to compare the genetic architecture of *Xiphophorus* and the guppy. All of the microsatellites used in the guppy are assumed homologous for both taxa; this was found for four microsatellites by examination of flanking sequences.

Genetic linkage maps developed in *Xiphophorus* will provide important landmarks for mapping in the guppy. Comparisons of maps between species provide clues to the evolutionary history of these organisms and the effects of selective forces on genetic architecture. A guppy linkage map is an important

step towards identification and characterization of genes important in guppy evolution, especially genes involved in the extreme phenotypic variation characteristic of guppy populations.

My study comparing linkage in the guppy and linkage in *Xiphophorus* is an important step in the study of guppy genomics, providing resources for further studies of phenotype, evolution and genetic architecture. Ultimately, understanding the genetic basis underlying the vast amount of phenotypic variation seen in the guppy will be an important advance in the study of the genetics of adaptive variation.

CHAPTER TWO: CONSERVATION OF SYNTENY BETWEEN *POECILIA* AND *XIPHOPHORUS*

Materials and Methods

Mapping cross

A female guppy from laboratory population CCFR was crossed to a male from laboratory population QUL89, producing 11 F_1 offspring. One pair of F_1 offspring was mated; 42 F_2 offspring were analyzed. CCFR is derived from one female from a stream in Cumaná, Venezuela (Alexander and Breden 2004). QUL89 is an inbred line derived from a high-predation population in the lower Quaré river, Trinidad (Kelly et al. 1999), and was collected by Felix Breden in 1989. Both populations have been maintained in the laboratory at Simon Fraser University since collection.

All fish from the cross were euthanised by immersion in ice water for 60-120 seconds, photographed, and preserved in 95% ethanol saturated with EDTA. Carcasses were stored at -20°C. DNA extraction and purification was carried out using standard methods, either following a standard Proteinase K and Phenol/Chloroform method (Fajen and Breden 1992) or using a Genomic DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA), following the manufacturer's instructions.

Concentrations of extracted DNA were measured using a Beckman DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). Samples were stored at 4°C and 40 ng/μL in DNA Hydration solution (Gentra Systems). Shortly before use in Polymerase Chain Reaction (PCR), samples of purified DNA were diluted to 10 ng/μL, to improve precision during handling.

Microsatellites

Primers for microsatellite loci linked to each other on the *Xiphophorus* linkage map (Walter et al. 2004) were synthesized for 61 loci (Invitrogen, Carlsbad, CA, USA). An additional six primer pairs were earlier developed in *Poecilia catemaconis* by Schartl (unpublished), and one more primer pair was developed in the guppy by Taylor (1999). Each primer pair was screened against the F_1 parents for the presence of multiple alleles. PCR optimization for annealing temperature was carried out for loci for which both F_1 individuals are heterozygous. After optimal annealing temperatures had been found, loci were examined in all mapping cross individuals.

One primer for each microsatellite locus was end-labelled with ^{32}P-γ-ATP (Perkin-Elmer, Foster City, CA, USA or Amersham Biosciences, Piscataway, NJ, USA) using T4 Polynucleotide Kinase (New England Biolabs, Ipswich, MA, USA or Invitrogen) following the manufacturer's instructions. PCR reactions consisting of 40 ng of genomic DNA, 5 pmol of forward primer, 3.5 pmol of reverse primer, 0.15 pmol of labelled reverse primer, 1 μL of 10X PCR buffer, 15 μmol of $MgCl_2$, 2 pmol of each dNTP and 0.2 units of Taq DNA-polymerase were carried out in

10 µL volumes in 200 µL microcentrifuge tubes. PCR reagents other than genomic DNA and primers were obtained from Invitrogen or GenScript corporation (Piscataway, NJ, USA).

An Amplitron IIe thermal cycler (Barnstead, Dubuque, IA, USA), a T3 thermocycler (Biometra, Göttingen, Germany) or a T-Gradient thermocycler (Biometra), was used for PCR. Each program started with 5 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at optimum annealing temperature and 60 seconds at 72°C, followed by a final 72°C extension step for 7 minutes and up to 16 hours at 4°C.

Flanking Region Sequences

I obtained sequences for flanking regions of four microsatellite loci (ATG036, CA069, CA114 and TAGA042) from guppies using the same primers that had been used to examine microsatellite allele diversity at these loci. Sequences were short, ranging from 47 to 257 bp. Initial PCR was conducted using a protocol similar to that for radioisotope-labelled microsatellite amplification described above, but lacking any radioisotopes. PCR products were screened for expected fragment sizes and amplicon quantity by running on agarose gels in TBE buffer. The amplification reaction was treated with Shrimp Alkaline Phosphatase and Exonuclease I in buffer (USB corporation, Piscataway, NJ, USA) to remove unincorporated dNTPs and single-stranded DNA that remained and might interfere with the sequencing reaction. One strand was chosen based on expected length of flanking region from published *Xiphophorus*

sequences, and was run in sequencing reaction PCR with the appropriate primer and DYEnamic ET terminator cycle sequencing mix (Amersham). The sequencing reaction PCR involved 10 µL volumes denatured for 5 minutes at 95°C, followed by 35 cycles of 45 seconds at 95°C, 45 seconds at the locus-specific annealing temperature, and 45 seconds at 72°C, then a final extension step of 5 minutes at 72°C and storage indefinitely at 4°C. Temperature changes were limited to 2°C per second.

Sequence reaction products were purified using sodium acetate/EDTA and ethanol precipitation, then suspended in 10 µL volumes of MegaBACE loading solution and analyzed on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were visualized using FINCHTV version 1.3 software (Geospiza, Seattle, WA, USA), edited, and aligned with *Xiphophorus* sequences from GenBank using CLUSTAL W version 1.83 (Thompson et al. 1994).

Substitutions were counted for each sequence, and were considered one change for the purposes of percent divergence. An insertion or deletion (indel) was counted as one change if all inserted or deleted bases were adjacent. Indels were not included in the substitution per site calculation.

Polyacrylamide Gel Electrophoresis: Microsatellite Alleles

Gels 0.3 mm-thick consisting of 8% polyacrylamide, 32% formamide and TBE buffer solution were cast using Sequigen sequencing rigs (Bio-Rad, Hercules, CA, USA). After setting overnight, gels were pre-run in TBE buffer to

warm to 45°C or higher. During pre-run and normal operation, an electric field at 75 watts and 1800 volts was applied to the widest gels, or 40 watts and 1800 volts to the narrower gels.

PCR product was combined with an equal volume of 98% formamide loading buffer (Sambrook et al. 1989), and samples were denatured by heating to 90°C for 5 minutes, and cooled by insertion into a PCR-Cooler (Eppendorf, Hamburg, Germany) at 0 to 7°C. Wells in the acrylamide gels were loaded with 2.5 µL aliquots of each sample and run for 60 to 130 minutes, depending on the expected size of the PCR products.

Gels were removed from the glass plates using large sheets of 3 mm filter paper (Whatman, Maidstone, England) and dried in a ThermoSavant SGD2000 slab gel dryer (Thermo Electron Corporation, Waltham, MA, USA) for 90 minutes, then placed in light-proof metal cassettes with blue-sensitive film (Cole-Parmer, Vernon Hills, IL, USA or Kodak, Rochester, NY, USA). Exposure times for the film varied with the locus and the amount and age of ^{32}P in use, between 24 and 200 hours. Films were developed using a Kodak X-Omat automatic developer and bands were analysed on a portable light table.

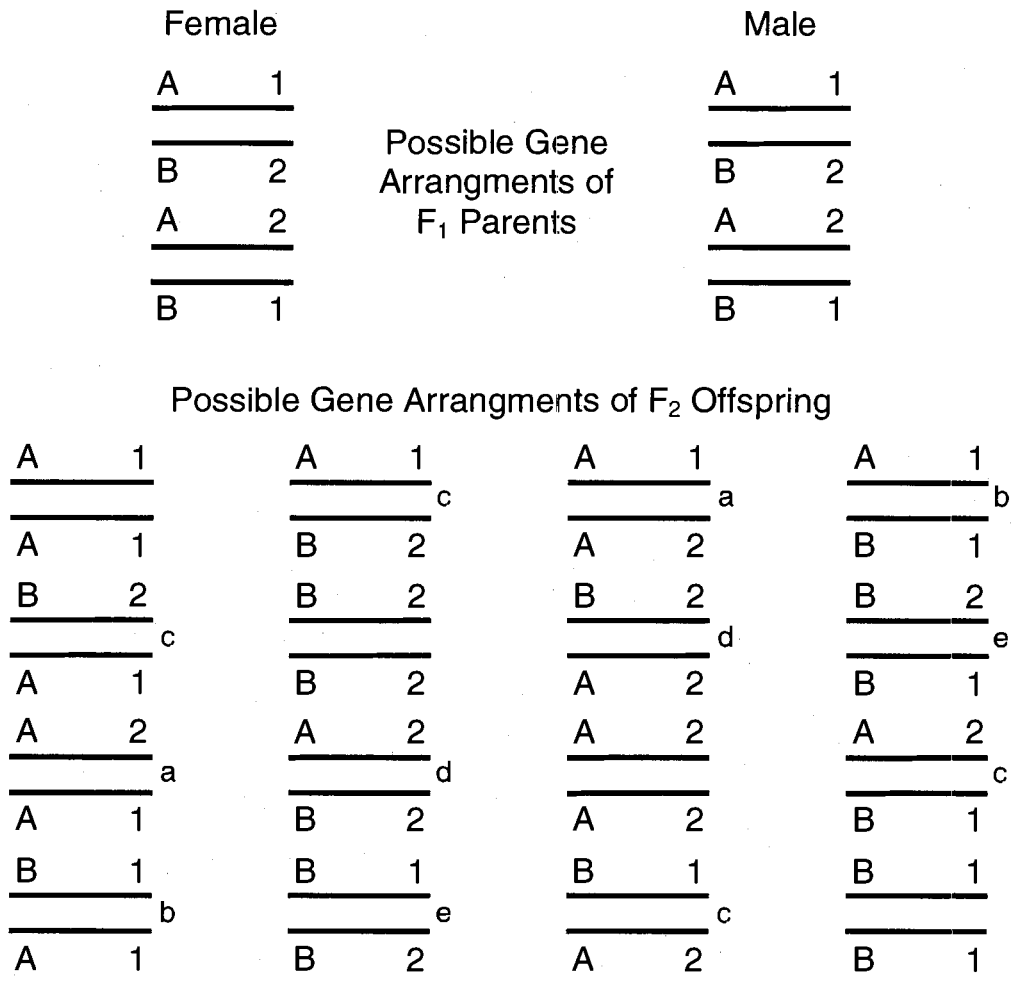
Analysis

Data were entered into a template in MS Excel, error-checked and converted to text files readable by LINKMFEX (Danzmann 2001).

Linkage analysis was performed using a compendium of programs contained in LINKMFEX version 2.0

(http://www.uoguelph.ca/~rdanzman/software/LINKMFEX/). Pair-wise recombination distances were calculated using the program LINKMFEX, and then LINKGRP was used to cluster loci into linkage groups at a logarithm of the odds (LOD) threshold of 3.0. Loci orders within linkage groups were determined using MAPORD, and a text map file was generated by MAPDIS. The text map file was edited for Kosambi corrections as calculated by MAPDIS, and was converted into a graphical representation of linkage groups by a separate program, MAPCHART version 2.1 (Voorrips 2002) (http://www.biometris.nl/uk/Software/MapChart/).

In this type of mapping cross, any co-dominant marker with two alleles in the mapping family, that is, both F_1 parents are heterozygous for the same two alleles, cannot be as reliably mapped as other markers with three or four alleles in the mapping cross. In the F_2 generation, only those individuals that are homozygous for either allele at such a 2-allele locus are informative for recombinations in either F_1 parent, because information regarding phase of alleles is not available. On average, three quarters of the available genotypes will not be useable when comparing two 2-allele loci, severely restricting sample size for analyses of these loci (Figure 1). When comparing a 2-allele locus to a locus with three or four alleles, half of the F2 genotypes will be ambiguous for recombination. LINKMFEX requires heterozygous F_2 individuals at such loci to be coded as missing data.



Observed Genotype	*Occurs in F_2 Offspring*	*Frequency if Unlinked*
AA 11	Unique	1/16
AA 12	a	1/8
AA 22	Unique	1/16
AB 11	b	1/8
AB 12	c	1/4
AB 22	d	1/8
BB 11	Unique	1/16
BB 12	e	1/8
BB 22	Unique	1/16

Figure 1. The problem of detecting linkage between two loci for which both F_1 parents are heterozygous for the same alleles at both loci. Not all F_2 heterozygotes can be unambiguously categorized as either recombinant or non-recombinant for either the female or male meiosis.

Segregation distortion

Segregation of alleles at all polymorphic loci (excluding loci in which both parents were heterozygous for the same two alleles (Woram et al. 2004)) was tested using a log likelihood adjusted X^2 test to determine goodness of fit to the expected 1:1 segregation ratio using the program SEGSORT, included in the LINKMFEX software package. This test is appropriate for sample sizes between 25 and 200 (Sokal and Rohlf 1995). Critical X^2 values were calculated by dividing the alpha (0.05) by the number of linkage groups tested in the female (6) or the male (9).

Results

Sixty-nine pairs of primers for microsatellites were screened in one F_2 mapping cross in guppies for reliable PCR amplification and variation. Of these, 26 did not provide reliable amplification products and a further eleven were not informative for linkage in the mapping cross. Sixteen variable loci segregated to six linkage groups in the female map. These sixteen, plus an additional eight, segregated to nine linkage groups in the male map. A further eight loci were informative in the mapping cross but did not show linkage to any other loci in either sex. The phenotypic marker, sex, was also included in the analysis, as the sex of every individual in the mapping cross was determined. Sex did not show linkage to any other markers using a LOD threshold of 3.0.

In guppies, all of the microsatellites segregating to linkage groups in the female map also appear on linkage groups in the male map (Figure 2). The eight additional loci in the male map occur on three linkage groups unique to the male

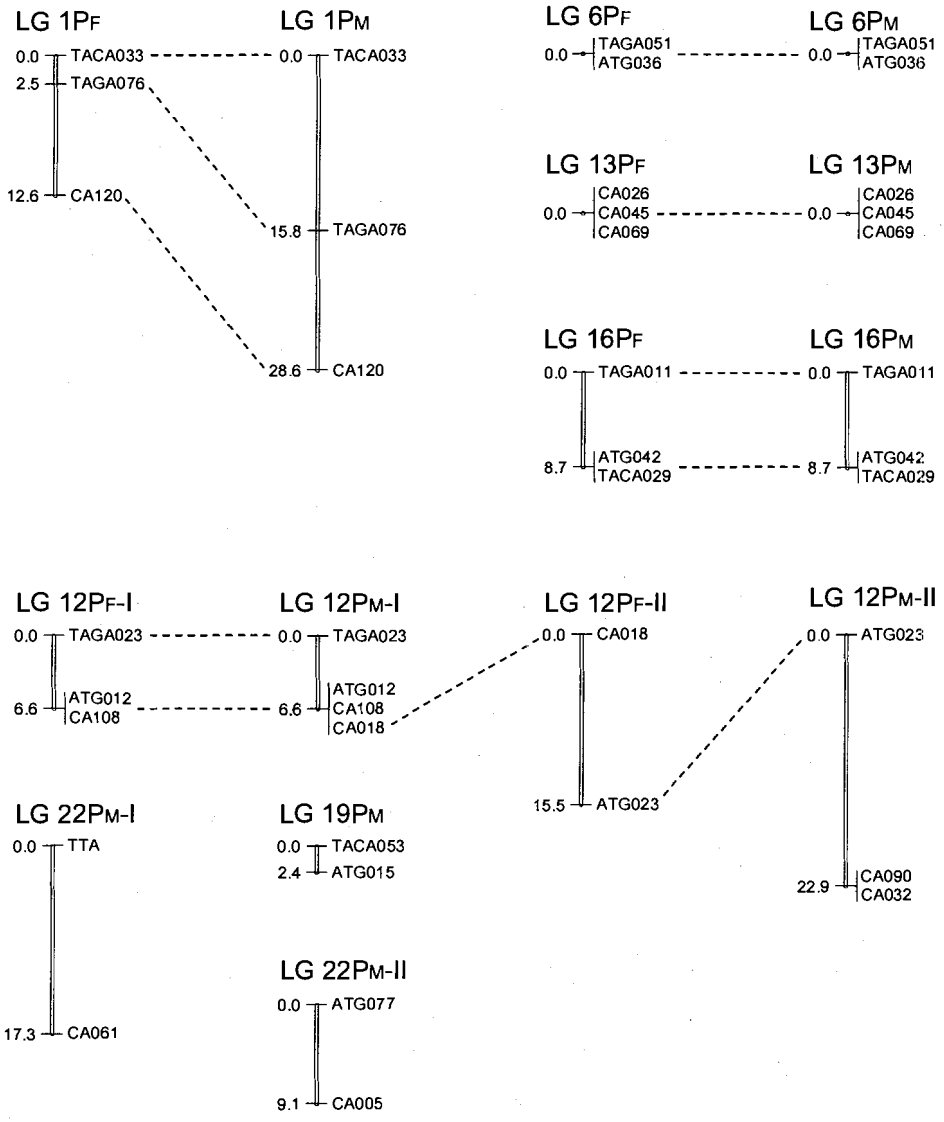


Figure 2. Female (F) and male (M) linkage groups for *Poecilia reticulata*. Names of microsatellite markers are on the right, distances between loci in cM after Kosambi correction are on the left, and dashed lines indicate identical loci between sexes. The minimum threshold for detection of linkage in constructing these linkage groups was a LOD score of 3.0. Loci appearing at the same location on a linkage group showed no recombination between them. The two sexes show colinearity for all loci mapped in both sexes. Six loci did not show linkage in females, but did in males, and have been assigned to linkage groups $19P_M$, $22P_M$-I and $22P_M$-II.

map, plus two loci occurring on the second part of the *Poecilia* male LG 12, named LG $12P_M$-II, which also carries a locus (ATG023) occurring on LG $12P_F$-II. All loci occurring on LG $1P_F$, $6P_F$, $13P_F$ and $16P_F$ appear on the first four linkage groups in males, in colinear positions where that can be determined. Loci appearing on both female and male maps allow construction of a hypothetical sex-average linkage group including seven loci from LG $12P_F$-I, $12P_F$-II, $12P_M$-I and $12P_M$-II and spanning a minimum of 45 Kosambi-corrected centiMorgans (cM_K), and corresponding to a portion of LG 12X in *Xiphophorus*. LG $1P_F$ is shorter than LG $1P_M$, suggesting reduced recombination rates in the female map on this linkage group. Other loci showed linkage in males but not in females, suggesting the opposite pattern for other regions of the genome.

Two microsatellite markers not included in the *Xiphophorus* map (TTA and Sat4) were informative for linkage in this mapping cross. TTA, a locus widely used in guppy studies (Taylor et al. 1999; Kelly et al. 1999), appears on LG $22P_M$-I. Sat4 was discovered in *Poecilia catemaconis* in Manfred Schartl's lab (Universität Würzburg) (Schartl unpublished) and does not show linkage to any other loci in either sex in the guppy maps.

All six linkage groups constructed in the female map carry only loci homologous to loci mapped to five linkage groups in *Xiphophorus* (Figure 3A). Colinearity could be established for all of LG $1P_F$ compared to LG 1X. An inversion was detected among homologous loci occurring on LG $12P_F$-I and LG 12X. Other colinear relationships could not be established because there was no

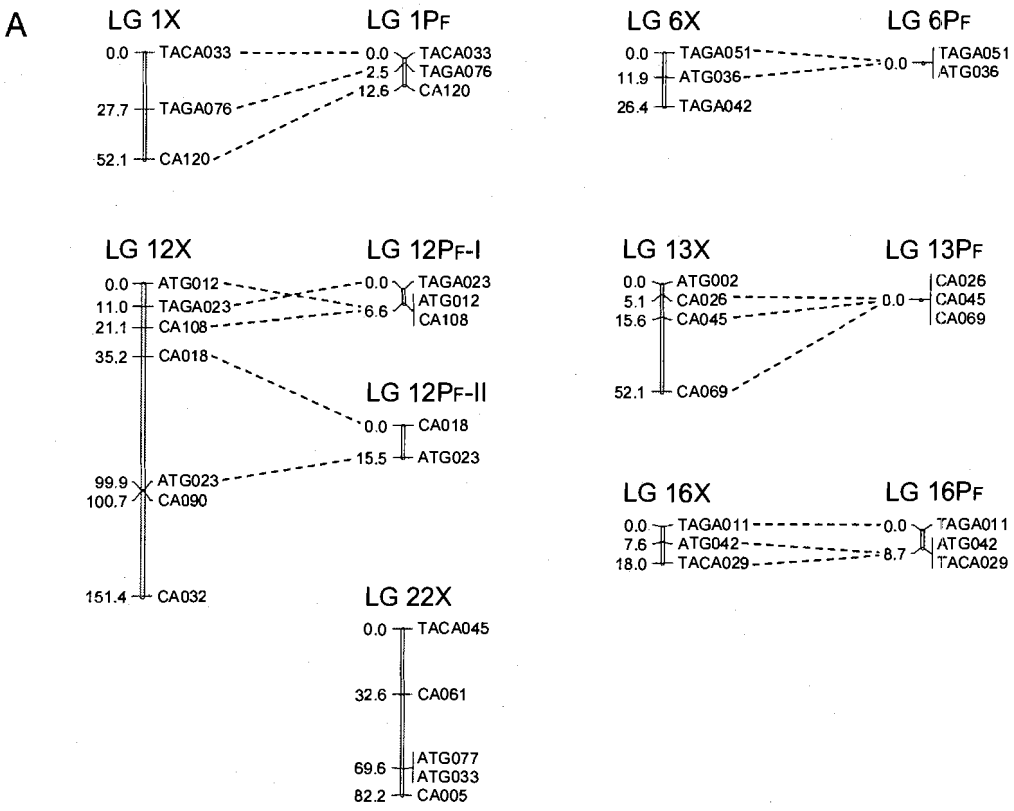
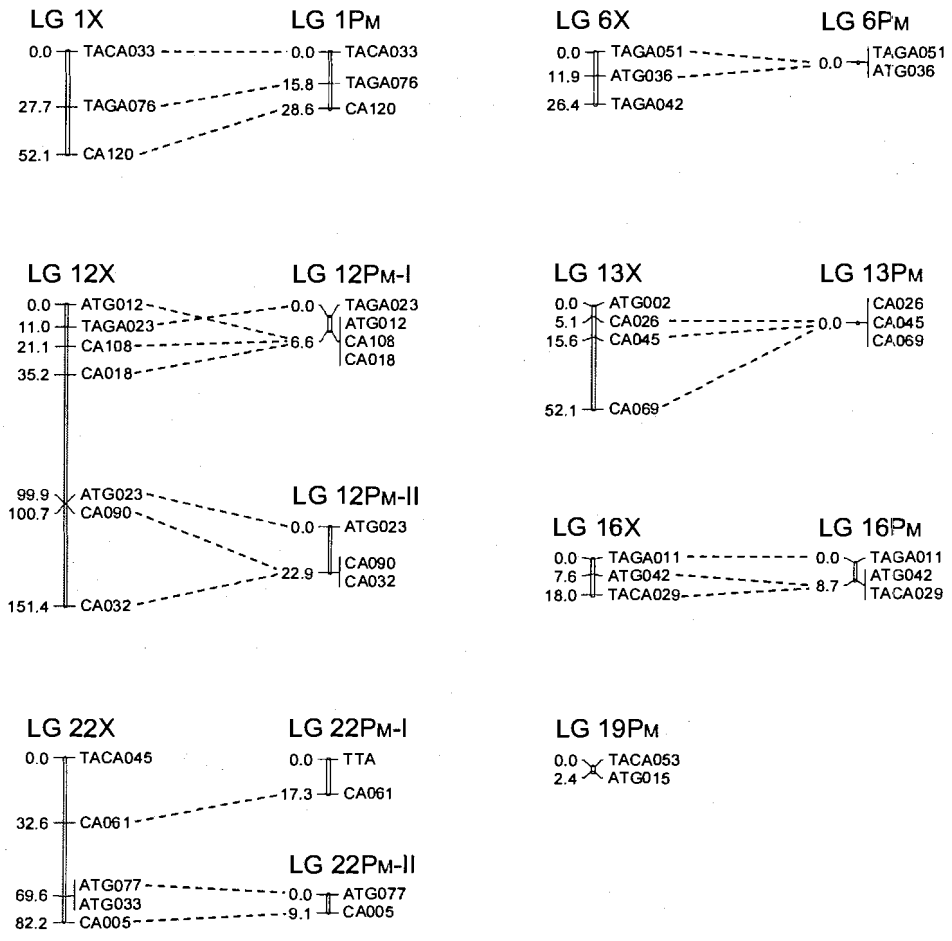


Figure 3. Comparisons of linkage groups between the guppy (*Poecilia reticulata*) and *Xiphophorus*. **A**. Female guppy linkage groups (P_F) show many similarities to *Xiphophorus* linkage groups (X). Most linkage groups show conserved synteny, though no loci from *Xiphophorus* LG 22 were linked to any other loci in female guppies. Microsatellites TAGA023 and ATG012 appear in inversed order on LG 12P_F-I and 12X. Linkage groups for *Xiphophorus* adapted from Walter et al. (2004) with permission.

B



B. Male guppy linkage groups (P_M) also show many similarities to *Xiphophorus* linkage groups (X). Some loci from 22X did show linkage to other loci in the male guppy, though the recombination distances between those loci are significantly different between species. The same microsatellites as in the female map show an inversion between species. Linkage groups for *Xiphophorus* adapted from Walter et al. (2004) with permission.

recombination in the female map for several pairs of loci, particularly on LG $13P_F$ and LG $16P_F$.

Twenty-three loci on nine linkage groups in the male map appear homologous to loci mapped to seven linkage groups in *Xiphophorus* (Figure 3B). Colinearity between the male guppy map and the *Xiphophorus* map could be established for LG $1P_M$ and LG 1X, as for the female map. The same inversion was detected comparing the male map to *Xiphophorus* as was found in the female map, on LG $12P_M$-I and LG 12X. One microsatellite locus on LG $22P_M$-I, TTA, does not have a known equivalent position on the *Xiphophorus* map, but the other locus on LG $22P_M$-I, CA061, does have an equivalent position on LG 22X, suggesting a TTA homologue may map to this linkage group in *Xiphophorus* as well. LG 19X, carrying the microsatellite marker ATG015 is not shown as no other loci from that linkage group screened were informative in the guppy mapping cross. ATG015 occurs on LG $19P_M$, along with TACA053, a locus that was not mapped to any linkage group in *Xiphophorus* (Walter et al. 2004; Kazianis et al. 2004).

The linkage maps of both sexes of guppies differ from the *Xiphophorus* map in the recombination frequency between homologues to microsatellite markers ATG023 and CA090, on LG 12X, where these two loci are tightly linked. In the male guppy map, they are separated by 22.9 cM_K, and in the female map, no evidence was found to suggest they are linked at all. A similar pattern can be seen in the linkage of ATG077 and ATG033, occurring on LG 22X with no

recombination between them. I found no evidence to suggest these two loci are linked in this guppy mapping cross.

Sequence similarity between guppy and *Xiphophorus*

I sequenced four microsatellite loci in guppies (ATG036, CA069, CA114 and TAGA042), with at least 47 bp of flanking sequence for each locus. All four microsatellites were originally developed from *Xiphophorus* from a sub-genomic library (Walter et al. 2004). I was able to align the guppy sequences with published sequences for *Xiphophorus* (GenBank nos. AY258864, AY258682, AY258791 and AY258752, respectively). Sequence similarity of the flanking regions ranged from 61.4% (35 of 57 nucleotides in common) at CA114 to 81.5% (22 of 27) at ATG036. Mean sequence conservation was 75.3% across 361 bp of homologous sequence between these species, or 0.21 substitutions per nucleotide. For aligned sequences, please see Appendix C.

Discussion

I have constructed partial linkage maps for female and male guppies, using microsatellite loci mainly derived from *Xiphophorus* fishes. This has allowed a comparison of portions of the guppy genome with portions of the *Xiphophorus* genome, across a boundary of at least five million years of independent evolution (Breden et al. 1999). I was able to obtain sequence data for flanking regions of four of the microsatellites used in the construction of the maps. All four loci were homologous to their counterparts in the *Xiphophorus* linkage map (Kazianis et al. 2004, Walter et al. 2004) based on sequence data

for *Xiphophorus* in GenBank. I am confident that the other microsatellites also represent homologous loci, and differences in linkage relationships represent real differences between these taxa.

The female and male maps of guppies show some differences in recombination rates (Figure 2). The maps are more similar if constructed using a threshold LOD score of 2.0 instead of 3.0 (Figure 4). A lower threshold LOD score generally increases linkage, particularly on the female map. In the female maps, LG 22P is not produced at LOD 3.0, but appears as two short linkage groups at LOD 2.0, similar to the male map at both LOD thresholds. Additionally, the female map gains a complete version of LG 12P at LOD 2.0, while the male map retains two separate linkage groups among those loci. Maps for both sexes gain linkage of TACA048 to LG 13P at the lower LOD threshold.

At both LOD thresholds, the female map shows reduced recombination relative to the male map among loci on LG 1P, but the male map shows reduced recombination relative to the female map in other surveyed regions of the genome (Table 1). Many vertebrates show differences in recombination rates between the sexes (human, Gyapay et al. 1994; mouse, Dietrich et al. 1996; zebrafish, Knapik et al. 1998; rainbow trout, Sakamoto et al. 2000), but *Xiphophorus* does not (Walter et al. 2004; Kazianis et al. 2004). The female guppy map shows slightly higher recombination rates than the male across the surveyed portion of the genome, but does not show a consistent difference in other linkage analysis studies of the species (Khoo et al. 2003; Watanabe et al. 2004), although whole-genome sex-specific maps are still lacking for the guppy.

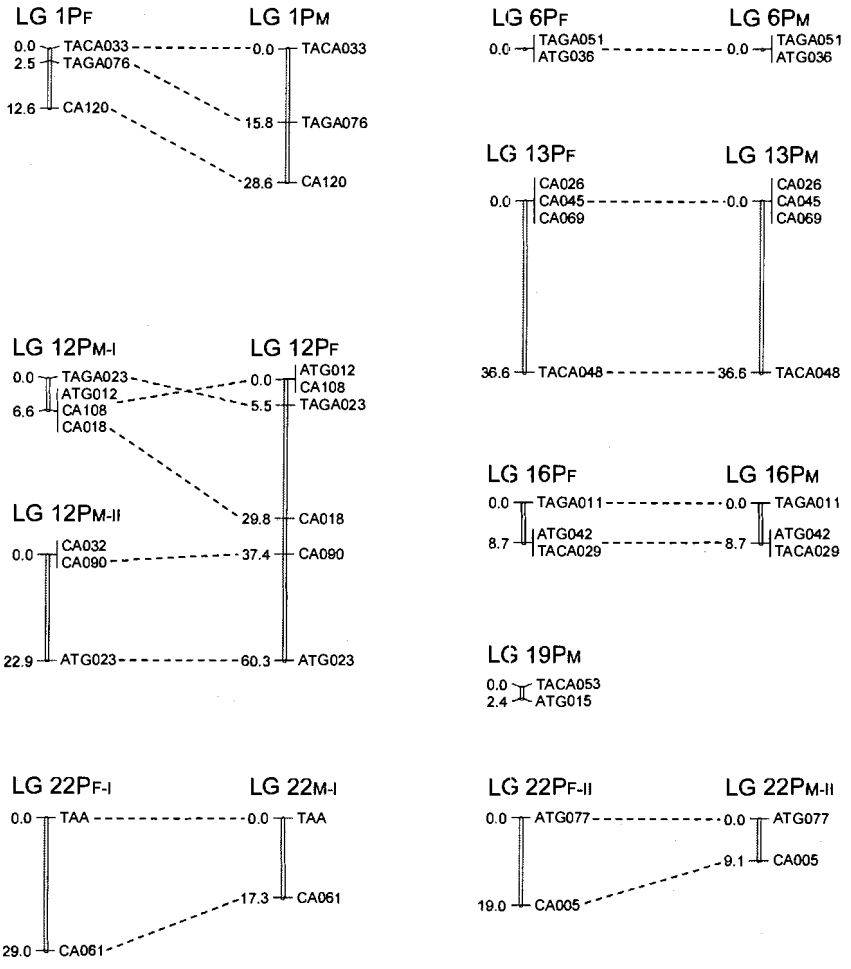


Figure 4. Female (F) and Male (M) guppy linkage groups using a minimum LOD of 2.0. Compared to maps constructed using LOD 3.0, these maps show more linkage in both sexes. Additionally, the maps for the two sexes are more similar, with most linkage groups represented in both sexes.

Table 1. Pairwise comparison of linkage in the female and the male guppy using a minimum threshold LOD 2.0. Averages of these values were used to construct the LOD 2.0 sex-averaged guppy linkage map (Figure 5). Linkage distances greater than the standard X- maximum of 28.6 for 42 offspring can be accounted for by Kosambi correction of distances, which tends to increase linkage distances, and strong skew among categories of recombinant offspring for some genotypes.

Loci pair	*Linkage Groups*	*Female Recombination*	*Male Recombination*
TACA033 – TAGA076	1F, 1M	2.5	15.8
TAGA076 – CA120	1F, 1M	10.13	12.77
TAGA051 – ATG036	6F, 6M	0	0
ATG012 – CA108	12F, 12M-I	0	0
CA108 – TAGA023	12F, 12M-I	5.52	6.63
TAGA023 – CA018	12F, 12M-I	24.23	0
CA018 – CA090	12F	7.65	Unlinked
CA090 – ATG023	12F, 12M-II	22.87	22.87
CA090 – CA032	12M-II	Unlinked	0
CA026 – CA045	13F, 13M	0	0
CA045 – CA069	13F, 13M	0	0
CA069 – TACA048	13F, 13M	36.57	36.57
TAGA011 – ATG042	16F, 16M	8.68	8.68
ATG042 – TACA029	16F, 16M	0	0
TAA – CA061	22F-I, 22M-I	28.95	17.25
ATG077 – CA005	22F-II, 22M-II	18.95	9.09
TACA053 – ATG015	19M	Unlinked	2.4

Other linkage analysis studies in the guppy using microsatellites have not used any loci overlapping with the set of markers used in this study.

Colinearity between species maps could be established only for LG 1, with loss of colinearity (an inversion) detected near one end of LG 12X for both sexes of guppies, on LG $12P_F$-I and $12P_M$-I. Extremely low recombination rates in other surveyed portions of the guppy genome prevent assessment of linear arrangements of microsatellites. A paired backcross design to accommodate loci

with only two alleles within the mapping cross would likely be able to determine whether these arrangements have been conserved between these taxa, particularly regarding LG 13X and 16X, because phase relationships among markers would be known. A successful guppy backcross design would need to use highly inbred strains because the first generation female could not be mated to any of her own offspring due to her ability to store sperm from her first mate (Houde 1997), and the uncertain paternity resulting among her offspring after multiple matings.

The *Xiphophorus* genome consists of 24 chromosomes in the haploid set, one more chromosome than in guppies. Kazianis et al. (2004) estimated the total genome size of a *Xiphophorus* male-derived map to be 2485.8 cM, with a haploid genome size of 8.3×10^8 bp and 334 kb/cM. Khoo et al. (2003), using a similar method, estimated the total size of a pair of sex and strain derived maps in guppies to be 4410 cM and 4060 cM, with a haploid genome size of 7.0×10^8 bp and 159 or 173 kb/cM respectively.

The genomes of the guppy and *Xiphophorus* are similar in physical size (Khoo et al. 2003; Kazianis et al. 2004), but are markedly different in map size, with two mapping crosses of guppies (Khoo et al. 2003) having a map size almost twice as large as *Xiphophorus* (Kazianis et al. 2004). In the present study, *Xiphophorus* shows higher rates of recombination between microsatellite loci than either female or male guppies across most of the surveyed portions of the guppy genome. A linkage map for the guppy including more *Xiphophorus*-derived markers across more linkage groups and chromosomes would help to

explain this apparent contradiction between the current study and previous surveys of the guppy and *Xiphophorus* genomes.

Reducing the threshold LOD score is one way to add markers to the guppy maps. At the lower LOD threshold of 2.0, a sex-averaged guppy map shows more similarities and more differences to the *Xiphophorus map* (Figure 5). This sex-averaged map was constructed using the mean recombination distance between each pair of markers, assuming loci unlinked in one sex are actually $50cM_K$ from any locus linked in the other sex, except in one case where a pair of loci (CA032 and CA090) were unlinked in the female, but linked at $0cM_K$ in the male, where it was assumed the true linkage was $0cM_K$. The lower statistical confidence and the many assumptions made in constructing this sex-average map reduces confidence in the veracity of the linkage relationships presented. Thus, this sex-average map should be considered to be for illustrative purposes only, and should not be construed as a presentation of statistically well-supported information.

The level of sequence divergence between the guppy and *Xiphophorus* estimated from four microsatellite flanking regions, 0.21 substitutions per site, is at least ten times higher than divergence between species within a single genus of rockfish also estimated from microsatellite flanking regions (Asahida et al. 2004). *Sebastes* rockfish species diverged from each other within the last two million years, more recently than did guppies and *Xiphophorus* (Rocha-Olivares et al 1999, Breden et al. 1999, Hulsey et al. 2004). My estimate of neutral

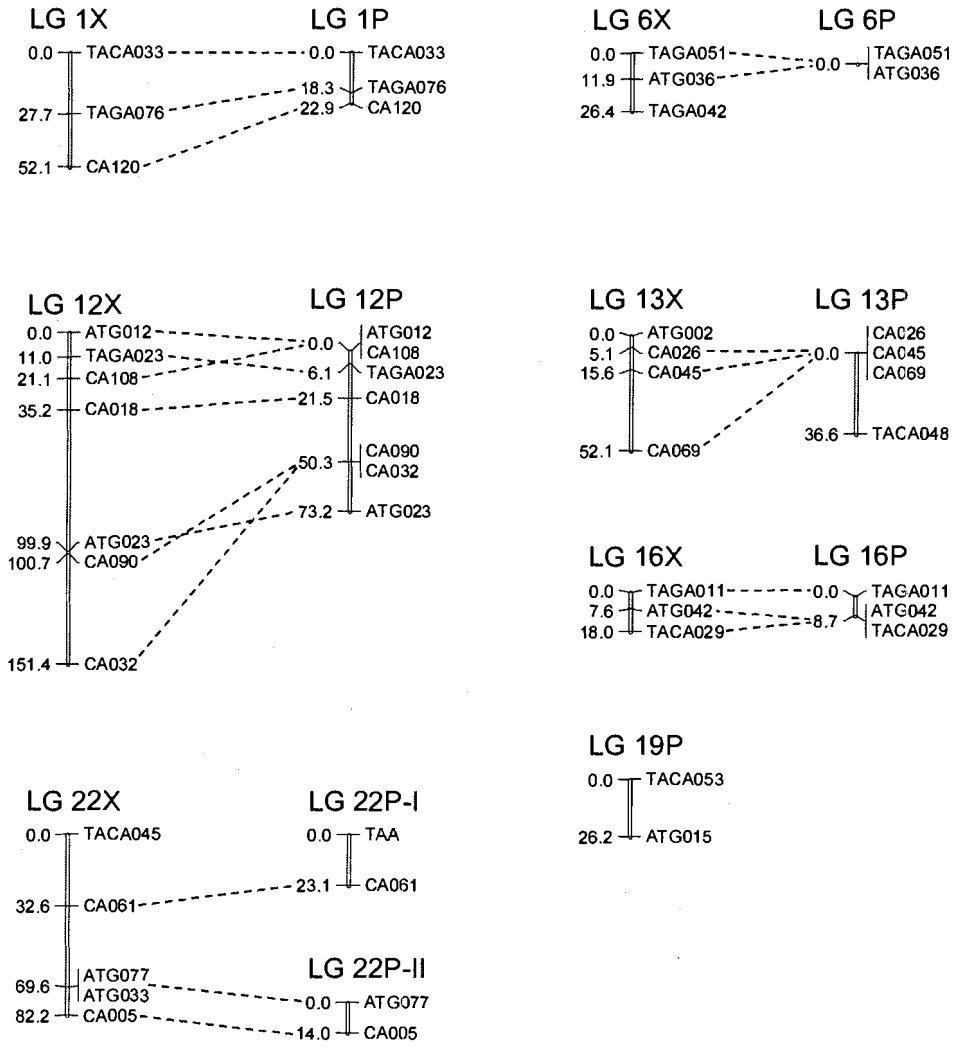


Figure 5. Comparison of sex-averaged guppy linkage groups (P) to *Xiphophorus* linkage groups (X). Guppy linkage groups were constructed using intersex mean recombination rates between loci and a minimum LOD threshold of 2.0. This comparison reveals many of the same similarities and differences as the pair of LOD 3.0 sex-specific comparisons, including the colinearity of LG 1 in both taxa and the inversion near one end of LG 12. A possible second inversion on LG 12, involving loci ATG023, CA090 and CA032, is not well supported in the guppy maps due to low statistical confidence and differences between the sexes.

sequence divergence between guppies and *Xiphophorus* is the first such estimate using neutral nuclear markers in these taxa of which I am aware. This agrees with other estimates of divergence between the guppy and *Xiphophorus* based on mitochondrial gene ND2, which were 0.211 and 0.220 substitutions per site, respectively, using two populations of guppies and *Xiphophorus nigrensis* (Breden et al. 1999).

Microsatellite loci that could be reliably amplified by PCR but did not show any variation in this mapping cross may be useful for other studies of guppies involving microsatellites. In particular, microsatellite ATG037 provided a reliable amplification product without variation in this guppy mapping cross, and is located in *Xiphophorus* on LG 24X, the same linkage group as the sex determining locus (Walter et al. 2004; Kazianis et al. 2004). No informative marker in this mapping cross showed linkage to the phenotypic marker sex, a result that was expected because no other markers on LG 24X were informative in the guppy mapping cross. Further investigations including ATG037 in guppies may be useful for elaborating the sex-determining system in guppies and the differences between guppies and *Xiphophorus* in sex determination.

It is only possible to compare linkage maps between species when homologous markers are available on both maps. Constructing a linkage map in one species using homologous markers originally developed for another species allows comparative genomics, and brings valuable genetic tools into the study of a particular species or system. These partial linkage maps for guppies, and the comparison possible with *Xiphophorus*, provide preliminary information on the

differences between the genomes of these important model systems and provide a collection of characterized microsatellite markers for future studies of guppies.

APPENDICES

Appendix A: Microsatellite loci examined in *Poecilia reticulata*

Poecilia linkage groups are named as in Results, above. For *Xiphophorus* linkage groups, NL: Not linked, NP: not present

1A. Microsatellites mapped to Linkage Groups in *Poecilia reticulata*

Locus Name	*LG Xiphophorus*	*LG Poecilia*	*Number of alleles in mapping cross*	*Forward Primer*	*Reverse Primer*
ATG012	12	$12P_F$-I, $12P_M$-I	2	CAAAAGCATCTCATTGCAGGG	ACTGGATTGGACGAGGCATC
ATG015	19	$19P_M$	4	GAATGAGACGTTCTAGATGCACATG	GGATGGTCAGATTGAAGCGC
ATG023	12	$12P_F$-II, $12P_M$-II	3	CTGCTGTCTCCATTCCTGAAGG	GCGAGCTGAGGCAGAAGATC
ATG036	6	$6P_F$, $6P_M$	2	TGTGTATGCCGGAGGACGT	CGGAGCAGCGTTTTAGTTCG
ATG042	16	$16P_F$, $16P_M$	2	TTTGGTGACCTAGTGACTCCGA	CAAGGTGGCGTCCACAAGA
ATG077	22	$22P_M$-III	3	CTTTGGACTGGGTTTGGTTCC	CCAGCAGCATCTTCATCCTGT
CA005	22	$22P_M$-II	2	TCATTGGCCTTCAGTGTATGTGT	ACGGCTTCCACAGCGTGT
CA018	12	$12P_M$-I, $13P_F$-II	3	CCACATTTAATGCTGTCAAGCC	GACAAGGTTTCCACTTTCAGAATTC
CA026	13	$13P_F$, $13P_M$	2	CATTTCACAGTAAAAGCAGGTGGA	CCCACAGGCTGTATCCTAATGC
CA032	12	$12P_M$-II	3	CCTCCGCCGACCAGC	CGGATCCCCGTGATCATC
CA045	13	$13P_F$, $13P_M$	2	TTTTGAGGTCGCTATAAGTCCTCA	GGTCGGCTTCAGACCTAGCAG
CA061	22	$22P_M$-I	3	CCTACTCTGGTGAGCTGAGTGTTGTCA	GCCAGCTCTTCCAGGAGACTT
CA069	13	$13P_F$, $13P_M$	2	ATGTCAGAGGTGCAGTGGAACAT	TCTGCACATTTTAAATGGTCTACGTC
CA090	12	$12P_M$-II	2	GGTGACCCACATGAGCTGAA	TGCTCTTCTGCTGCGCACT
CA108	12	$12P_F$-I, $12P_M$-I	2	AAGGTTGCTGTTGGCAATGC	CGTTCCCTGACATGGTGTCTC
CA120	1	$1P_F$, $1P_M$	3	TTGCTGCTCCTGCAAATCAA	CATGAAGCTCCCTGCAAAGG
TTA	NP	$22P_M$-I	4	GTGACCGAACGAAAGGATA	CCCCAAAGGAACACTGTAT

continued

TACA029	16	$16P_F$, $16P_M$	2	TCTGCAAAAGAATGTGCCAAA	TCGATTACTAAATTCGCTGATGTCTATT
TACA033	1	$1P_F$, $1P_M$	4	GCAAAGTGAATACAGATGCAACAA	GCTTAAAATGGTTCTGAAATGCATT
TACA053	NL	$19P_M$	3	ATCAGTGCAATTCCAAATACTCATAATAT	GGAAACAACAAGAATGACACATCAGT
TAGA011	16	$16P_F$, $16P_M$	2	TCTAAGACTGTGCAGAAACTGAAAGTT	TGTTAGGAAGATGATGGTAATAGAGAAGATA
TAGA023	12	$12P_F$-I, $12P_M$-I	3	TCTGGTTGTCTTCTATTTTCTTTGAGTAA	TGCAAACTGGAGGTCCCG
TAGA051	6	$6P_F$, $6P_M$	2	GCATCCCCACAGTATAATTCTGCT	CACGTGGTTTGAAAATGTCGAA
TAGA076	1	$1P_F$, $1P_M$	4	GCAAAATAGACCACAAATATTAAACATCAT	TACACGTTTTGGATTTCTTTGTGAAT

1B. Microsatellites showing no linkage to any other locus in *Poecilia*

Locus Name	*LG Xiphophorus*	*Number of alleles in mapping cross*	*Forward Primer*	*Reverse Primer*
ATG002	13	2	ATCAACTCAGAAGGGTGGAACG	CATCACCATTGTCCTTGCCC
ATG033	22	2	GAAAAGAAGACCTCATCACAACCA	TCCACTCAGAGGGAACCAGG
CA006	1	3	AGGAGCACTGCTTGTGCCA	CATGAGCATCAATCTGATTCAGACT
CA114	4	3	CCGCTTGCACGCCTGT	CAGCAGCCCCAGAACCTTC
TACA045	22	2	TACGTGTCCAGTTAAACCAAAAAAGTAT	TCTGCAAAAGTCATGTTATCAAAACA
TACA048	23	2	TGGTCCATTATTCATTGACTTTAATCA	TCCTCACTTCAACACATGAATGC
TAGA033	23	3	GGGATTAGTGGCTGTTATTAATGGG	TTGGACGAGTAAGAAGAGTAATCAAATT
TAGA042	6	2	CTGCCCTACTGTGGTGAGTTACC	TGAAAACAGTCAACCTTGTGCTAAT
Sal4	NP	3	GAGCTCCACTTGAATGACA	AACCACTCACGAGCCACT

1C. Microsatellites uninformative for linkage in *Poecilia*

Locus Name	*LG Xiphophorus*	*Number of alleles in mapping cross*	*Forward Primer*	*Reverse Primer*
ATG037	24	1	AAGAAGAGATTTTGGTACAGTTTCCGT	ATGTCGGTCATACTGTTTCTACAATGT
ATG120	13	1	GCACATTTTAACTTTCTACTACCTACAATAACA	ATATGATGCTGGAATGCAGGAA
ATG123	13	1	CCATCGTCATTGCTGTCGTT	GTTATTTCATGACAAGTCCAGCTTTAA
CA052	12	1	TCTTTCTGTACTGGCGCGG	GGCAGAAGGCTGGGATCAG
CA384	16	1	TGCCATTGGTCAGCTAGTGAAC	TGGCACAAGCACTCAGAGGT
TACA003	1	1	CATCGTTGTGGACTTTGAGGG	GGGTTGTTTTGCATGGACTTAAC
TACA010	1	1	GACCAAATTAATCCACCCATCC	TGTTTTACACATGTCAGTCATATTGGAC
TACA021	12	1	GAATAAGAGGGACAGGGAGAAGAGA	GCATAATTGTAGCAATGAAACCAAAAT
TACA050	12	1	TTTATCGGCTTTCTGAACAGTTTCT	TCTGCTGACGGTGATCATCC
TAGA035	11	1	CATGTAGGAAAATGCATTGTGCC	GAACATCGAAACTCAGAAACAGAGA
TAGA084	12	1	TCAGAGTGTTTCAGGGCCAG	TGTTTCAGAGAACCAGAGCAATGT

1D. Microsatellites failing to provide reliable PCR product in *Poecilia*

Locus Name	*LG Xiphophorus*	*Number of alleles in mapping cross*	*Forward Primer*	*Reverse Primer*
ATG006	12	0	CTGGGTAGTAAATGGCATCCG	GCGTGTGTAAAAGACATAGTTCTCAAC
ATG008	8	0	CACAATAGCAATGTTTCTGCAATG	TTGTAGATTTAATACTTATGTCTTTGCGG
ATG034	NL	0	TGTTCTGGATCAGTTTGGAGATTG	TTGCCTTCAACACATCAGAGCT
ATG046	20	0	TTGGAAACGCAGCGGGT	GCCTCTAGCTGGCAGTAATGATG
ATG082	1	0	TCGGCTTCTGAGTTTCTCACG	ATGTCACGGGCTCGACTGA
CA010	23	0	TTCTTGTGCTATATTTCTGTTTCATGG	CTTGGAGCTAGTGAAGGCTGATC
CA094	13	0	TCAATCAGCCTAATCGTCTCAGAT	AAAATGTTGCATATTTCAACTGATGTT
CA101	NL	0	ACGACGGCAGCGATGC	GTAATGTGTCACACCTGAATGCAG
CA110	NL	0	CGTCCATGTATGAGAATGAAACG	GCACAAACACTGCATGGAACA
CA186	NL	0	CCACTGCTGAAGCTAAGAAAACCT	GCACAGCGACAGCGGTG
CA243	4	0	GATAACCCTGGAGTCCTGCG	GTCTGACCATCAAGCCTCTGG
CA263	5	0	GTGTAAAGCACATGGTGCACATT	CCTCTGCTTACAAATATCCACCTCTAT
TACA049	13	0	ATTCAGAGACTGCAACTGCTGC	TCACATCCAAAATGATAAGTAATAGAACATT
TAGA002	13	0	GGTGCATGCCATGATGTTTCT	CCCATCGACTGCACCCTC
TAGA009	13	0	CACACCCCCATCGACTGC	AAACGGGTGCATGCCATG
TAGA014	NL	0	GAGACGTGACTCCACCAATCAAG	TCTGTAAAAGTAATATGGATACGAGACCA
TAGA060	16	0	GATCTCAGTTTAACCACAACAGGGT	CCCTGCTGGTTCGTCTGG
TAGA070	3	0	AACATCCACATGTTGTCTATGTATACAAAA	CCCATCCAACAATAGCTGTAGCA
TAGA075	NL	0	CCACCTGCAGAATCATGTTTGTA	GCCTCGAGCAAGCACCAA
Sat1	NP	0	ACAGCAGCTGTTCACGGC	TCGGAGGAAAAACAGATGAC
Sat2	NP	0	GCTGTGATACTTATTGCAGA	ATAAACCCTGGAGCCAAAC
Sat3	NP	0	GCC AGG CTT CGG GCT CGA	TCGTCATCAGTGGCTTCGAA
Sat5	NP	0	CTCAATAATGGCCACTTTGT	TGATGGCACTGAAGAGTCA
Sat6	NP	0	CGCATTAACGGTGACGGA	GTACCATATAAGCAAAGTTC

Appendix B: Sequence Alignment

ATG036

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Poecilia      TCACCTAGCGCCCNTTANCTGACG(CAT)6 CACGA
Xiphophorus   TCAGCTAGCGCCCCTTAGCTAACA(CAT)6 CATCA
                 *                  *  *        **
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CA069

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Poecilia      GTATGTTGGTTTGATAA-TATATCCGGACTGACTCAGTGT
Xiphophorus   GTATGTTGGTTTGATAAATATATCCGGACTGACTCAGTGT

Poecilia      GTGACACTGATGGCTGCTAGTGTGTGTTTGCATATGGA
Xiphophorus   GTGACACTGACGGCTGCTA-TACAGAGACGTAGACCAT
                        *          ******** * * ****
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CA114

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Poecilia      TTAT-TANCTGNGTGTGCGTACCACG-CGTGCGTGCATGC
Xiphophorus   TCACACACTTATTTGTGCGTGCGGGGGTGCACATGCAGGC
               * * *  * * *        * ***  * ** *    *

Poecilia      GAGNGAGANATATAGANACACA
Xiphophorus   GAGAGAGAGAGACAGAGCTATA
                     * *    ** *
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TAGA042

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Poecilia      CCCTGAGCATAATATGAACACGACGTCTGTTTTTATACGC
Xiphophorus   CCCTGAACATAATATGAACACCACGTCTGTTTTTATACGC
                    *               *

Poecilia      GCTGTGAAATGTGAGTTTAGGAGGTTGGCATTTATTTGTT
Xiphophorus   GCTGTCAAATGTGAGTT-AGGAGGTTGGCATTTATTTGTT
                   *

Poecilia      TTGGGAGGAGCGAATTATCTTCTGATTTATCTGATCATTA
Xiphophorus   TTGGGAGGAGCGCATTATCTTATGATTTATTTGATC---A
                        *         *         *

Poecilia      ACCAGGAATGAGTCACCTAGCTTTCTGTACTATGTA--TG
Xiphophorus   ACCAGGAATGAGTCACCTAGTAGTCATTAGCACAAGGTTG
                                  ***  **  ** ****

Poecilia      TATGTATTCATT-CTGTCTTTAATAGAAATAGAATATCC
Xiphophorus   ACTGTTTTCAATATTAGATTTATTAGCTATCCTCTTTAT
              **   *    *  * ***    *   **  **** * **
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