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Conservation of Synteny between Guppy and *Xiphophorus* Genomes

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ABSTRACT

The guppy and fish in the genus *Xiphophorus* have both been important model systems for the study of natural and sexual selection for over 50 years. Whereas the guppy is unique in the degree to which the environmental variables shaping phenotypic variation are known, *Xiphophorus* has the advantage that genomic resources have been developed due to the utility of this taxon for the study of melanoma. If linkage maps for the guppy and *Xiphophorus* are similar, genomic resources developed in *Xiphophorus* will be useful in the guppy. The authors used an F_2 mapping cross of divergent populations of the guppy to construct partial female and male genetic linkage maps incorporating microsatellite markers derived from *Xiphophorus* mapping efforts. Flanking regions for a sample of microsatellites occurring in maps for both taxa were sequenced in the guppy and compared to published sequences from *Xiphophorus*. This confirmed that these loci were homologous and estimated the divergence in neutral nuclear DNA to be 0.21 substitutions per site. The female map comprises 16 linked markers on six linkage groups, and the male map comprises 24 markers on nine linkage groups. Linkage relationships among loci homologous in the guppy and *Xiphophorus* primarily show conservation of genetic architecture between species, but several major changes were detected.

INTRODUCTION

Fish of the family Poeciliidae have served as model systems for the study of evolution, ecology, behavior, and genomics. Natural populations of guppies (*Poecilia reticulata*) have been studied for more than 50 years,[1] and the unique aspect of this work is that many of the selective forces driving phenotypic variation between populations are well known.[2] *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.[3–5]

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 a well-characterized 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 guppy as a model study system

The guppy has been used to study sexual selection, natural selection, and other evolution-

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ary forces acting on natural populations.[2] There are large amounts of phenotypic variation between populations and between individuals within a population. Where they co-occur with guppies, predatory fish apply natural selection to guppy populations, in a direction opposite to that applied by sexual selection.[1,6] The balance between natural and sexual selection results in drab males and less choosy females becoming prevalent in high-predation areas.[7,8] In addition, Reznick and colleagues[8] have shown that life-history traits such as time to first reproduction and overall reproductive effort vary in guppy populations as a function of predation risk.

Heritable differences are known to underlie much of the phenotypic variation between guppy populations and individuals,[9–11] though the molecular characteristics of the underlying loci are mainly unknown.[12] A genetic linkage map is an important step in locating and characterizing genetic variation underlying adaptive phenotypic variation. Locating and identifying genes influencing phenotypes enables examination of the evolutionary forces shaping complex genetic systems in real ecological settings. Ultimately, understanding the genetic basis underlying the vast amount of phenotypic variation in guppies will be an important advance in the study of the genetics of adaptive variation.

Xiphophorus *as a model study system*

Like the guppy, *Xiphophorus* fish have been extensively studied as a model system.[4] *Xiphophorus* species and hybrids have been used as models for sexual and natural selection.[13,14] Early work in *Xiphophorus* established them as important genetic research organisms with the discovery of genetically controlled melanoma development.[3,4] The ease with which fish that spontaneously develop malignant tumors can be produced has resulted in a wealth of genetic information now available, including genetic linkage maps.[15–17]

Crossing designs producing offspring with malignant tumors in *Xiphophorus* hybrids were first explored more than 70 years ago.[18,19] The most famous crossing design, termed the "Gordon–Kosswig" cross after its creators, uses a *Xiphophorus maculatus* female mated to an *X. helleri* male, backcrossed to *X. helleri*. The resulting backcross hybrid offspring segregate alleles at two unlinked loci such that one quarter develops melanoma.[3,16,20] One locus involved in this melanoma is normally involved in pigment cell production; the other is a tumour suppressor locus.*[16]

Genetic architecture and synteny

Overall conservation of genetic architecture across Poeciliidae has been found in several studies.[21,22] 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.[23]

A high density microsatellite genetic linkage map of *Xiphophorus* already exists, and the microsatellite markers used were examined in two guppy lineages during the production of the *Xiphophorus* map.[16,17] Our study constructs a partial genetic linkage map for the guppy, using some of the microsatellite markers that were used in the construction of the *Xiphophorus* linkage map. There are many similarities and a few differences between the *Xiphophorus* linkage map and the female and male guppy maps. For example, *Xiphophorus* show similar rates of recombination between the sexes,[16,17] but the guppy maps show differences between the sexes in recombination rates in some parts of the maps. Genetic linkage maps developed in *Xiphophorus* will provide important landmarks for mapping in the guppy.

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; we analyzed 42 F_2 offspring. CCFR is derived from one isolated female from a high-predation stream in Cumaná, Venezuela.[24] QUL89 is an inbred line derived from a high-predation population in the lower Quaré River, Trinidad,[25] 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 euthanized by immersion in ice-water for 60–120 sec, pho-

tographed, and preserved in 95% ethanol saturated with EDTA. Carcasses were stored at −20°C. DNA extraction and purification were carried out using standard methods, either following a standard Proteinase K and Phenol/Chloroform method[26] or using a Genomic DNA Isolation Kit (Gentra Systems, Minneapolis, MN), following the manufacturer's instructions.

Concentrations of extracted DNA were measured using a Beckman DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). 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 were taken of purified DNA and diluted to 10 ng/μL, to improve precision during handling.

Microsatellites

Primers for microsatellite loci linked to each other on the *Xiphophorus* linkage map[17] were synthesized for 61 loci (Invitrogen, Carlsbad, CA). An additional eight primer pairs were earlier developed in the guppy by Taylor.[27] 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-labeled with ^{32}P-γ-ATP (Perkin-Elmer, Foster City, CA, or Amersham Biosciences, Piscataway, NJ) using T4 Polynucleotide Kinase (New England Biolabs, Ipswich, MA, 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 labeled 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).

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

Flanking region sequences

We obtained sequences for flanking regions of four microsatellite loci: ATG036, CA069, CA114, and TAGA042 from guppies. Sequences were short, ranging from 47 to 257 bp. Initial PCR was conducted using a protocol similar to that for radioisotope-labeled 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) 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 min at 95°C, followed by 35 cycles of 45 sec at 95°C, 45 sec at the locus-specific annealing temperature, and 45 sec at 72°C, then a final extension step of 5 min at 72°C, and storage indefinitely at 4°C. Temperature changes were limited to 2°C per sec.

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). Sequences were visualized using FINCHTV version 1.3 software (Geospiza, Seattle, WA), edited, and aligned with *Xiphophorus* sequences from GenBank using CLUSTAL W version 1.83.[28]

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

Gels 0.3 mm-thick consisting of 8% polyacrylamide, 32% formamide, and TBE buffer solution were cast using Bio-Rad Sequigen sequencing rigs (Bio-Rad, Hercules, CA). 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,[29] and samples were denatured by heating to 90°C for 5 min, 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 60–130 min, 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) for 90 min, then placed in light-proof metal cassettes with blue-sensitive film (Cole-Parmer, Vernon Hills, Il or Kodak, Rochester, NY). Exposure times for the film varied with the locus and the amount and age of ^{32}P in use, between 24 and 200 h. 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.[30]

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, 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 (http://www.biometris.nl/uk/Software/MapChart/).[31]

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 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 usable when comparing two 2–allele loci, severely restricting sample size for analyses of these loci. LINKMFEX requires heterozygous F_2 individuals at such loci to be coded as missing data.

Segregation distortion

Segregation of alleles at all polymorphic loci (excluding loci in which both parents were heterozygous for the same two alleles)[32] was tested using a log likelihood adjusted χ^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.[33] Critical χ^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 11 were not informative for linkage in the mapping cross. Sixteen variable loci segregated to six linkage

groups in the female map. These 16, plus an additional 8, 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 the guppy, all of the microsatellites segregating to linkage groups in the female map also appear on linkage groups in the male map (Fig. 1). The eight additional loci in the male map occur on three linkage groups unique to the male map, plus two loci occurring on the sec-

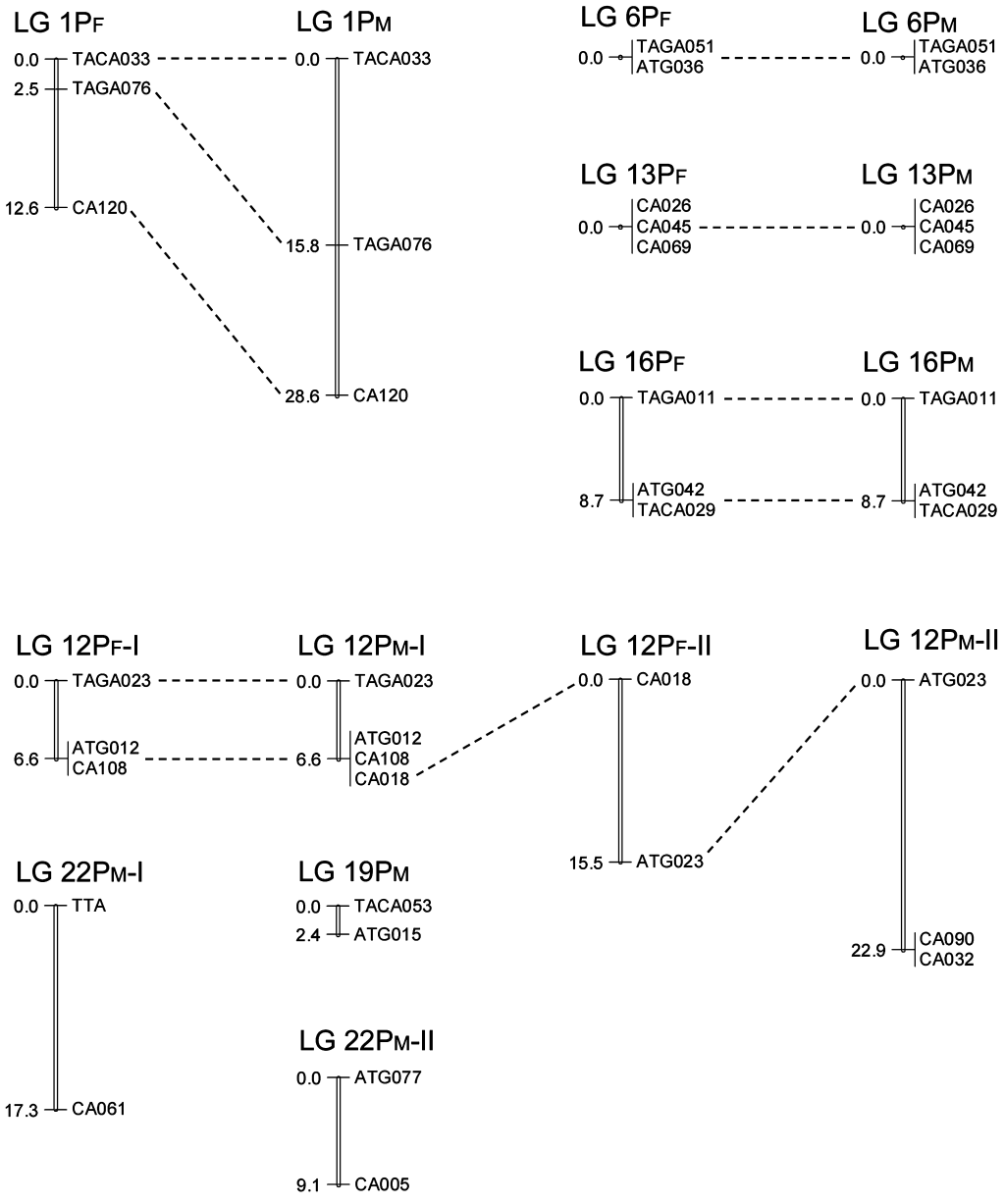

FIG. 1. Female (F) and male (M) linkage groups for *Poecilia reticulata*. Names of microsatellite markers are on the *right*, distances between loci in cM after Kosambi correction are on the *left*, and *dashed lines* indicate identical loci between sexes. The minimum threshold for detection of linkage 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 7M, 8M, and 9M.

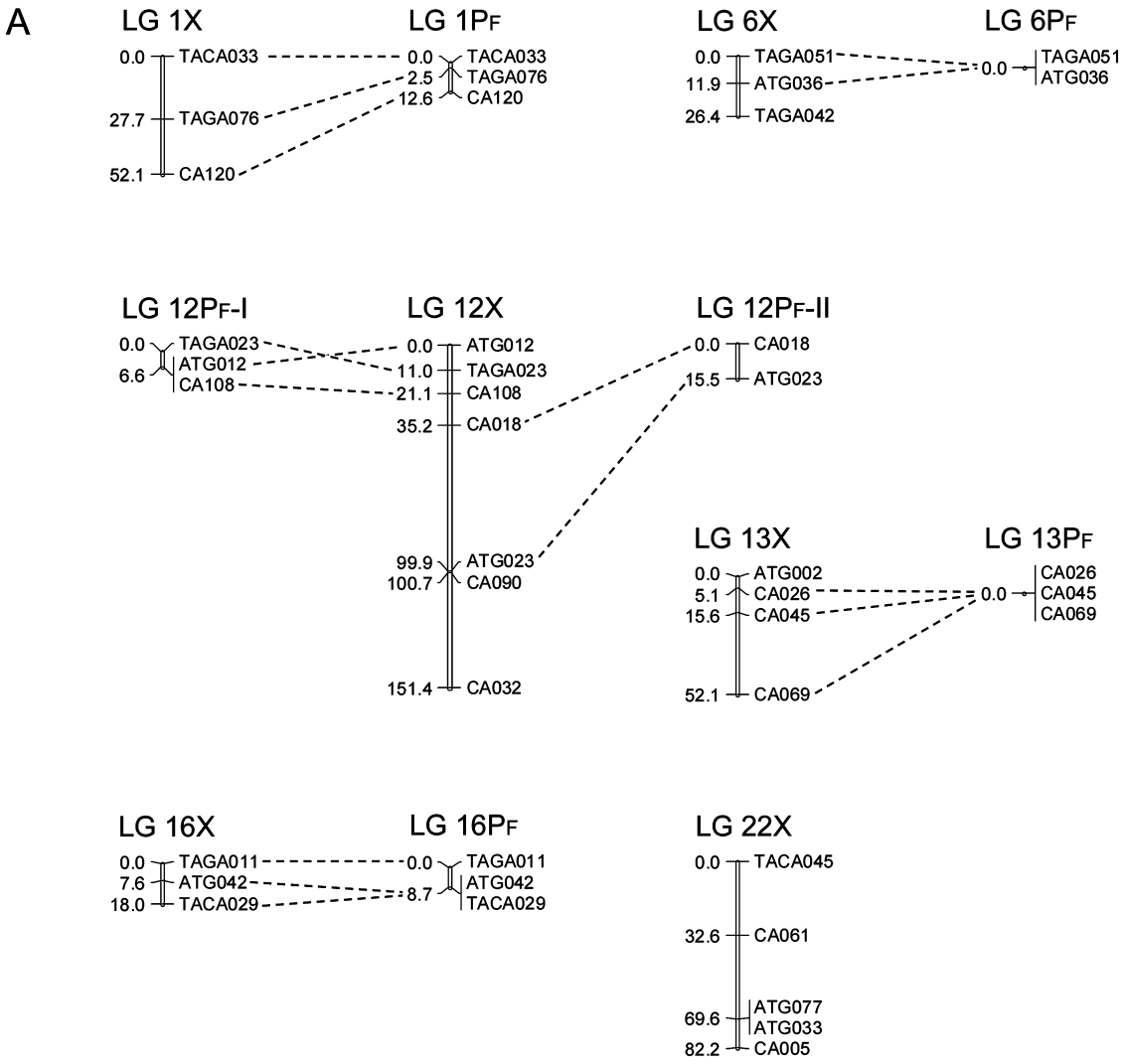

FIG. 2. Comparisons of linkage groups between guppies (*Poecilia reticulata*) and *Xiphophorus maculatus*. (**A**) Female guppy linkage groups (F) show many similarities to *Xiphophorus* linkage groups (X). Most linkage groups show conserved synteny, though no loci from Xiphophorus LG 22 were linked to any other loci in female guppies. Microsatellites TAGA023 and ATG012 appear in inversed order on LG 5F and 12X. (**B**) Male guppy linkage groups (M) also show many similarities to *Xiphophorus* linkage groups (X). Some loci from 22X did show linkage to other loci in male guppies, though the recombination distances between those loci are significantly different between species. The same microsatellites as in females show an inversion between species. Linkage groups for *Xiphophorus* adapted from Walter et al.[17] with permission.

ond 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,[15,34]

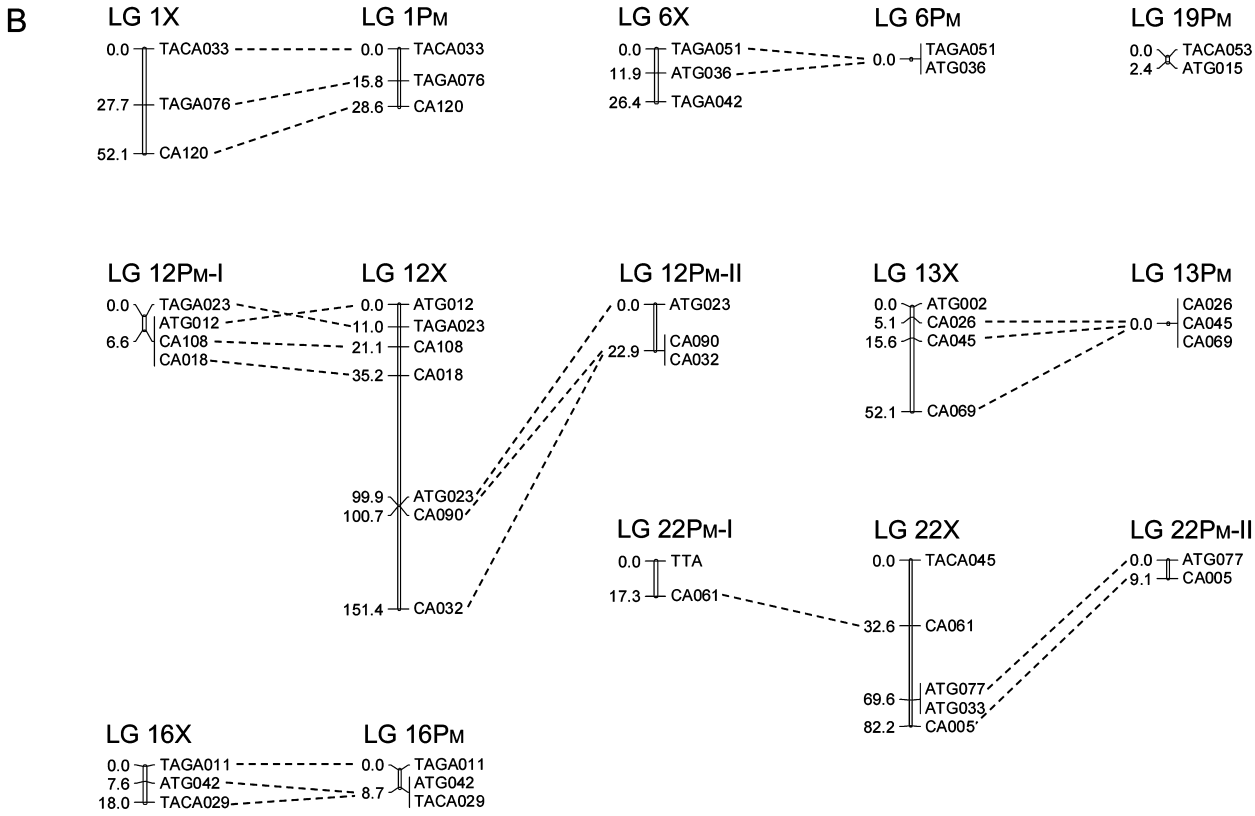

FIG. 2. *Continued.*

appears on LG 22P_M-I. Sat4 was discovered in *Poecilia catemaconis* in Manfred Schartl's lab (Universität Würzburg) 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* (Fig. 2A). 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 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* (Fig. 2B). 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*.[16,17]

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 we found no evidence 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. We found no evidence to suggest these two loci are linked in this guppy mapping cross.

Sequence similarity between guppy and Xiphophorus

We sequenced four microsatellite loci in guppies (ATG036, CA069, CA114, and TAGA042), with at least 47 bp of flanking sequence in *Xiphophorus* for each locus. All four microsatellites were originally developed from *Xiphophorus* from a subgenomic library.[17] We were able to align the guppy sequences with published sequences for *Xiphophorus* (GenBank nos. AY258864, AY258682, AY258791, AY258752). 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.

DISCUSSION

We have constructed partial linkage maps for female and male guppies, using microsatellite loci mainly derived from *Xiphophorus* fish. 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.[35]

The female and male guppy maps show some differences in recombination rates (Fig. 1). 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. Many vertebrates show differences in recombination rates between the sexes (human,[36] mouse,[37] zebrafish,[38] and rainbow trout[39]), but *Xiphophorus* does not.[16,17] Guppies do not show a consistent difference in recombination rates in this study or in other linkage analysis studies of the species,[40,41] although whole-genome sex-specific maps are still lacking for the guppy.

A difference in recombination rates between sexes is termed heterochiasmy, and occurs in many taxa.[42,43] In most species where it has been observed, males show reduced recombination in their gametes relative to females, even in cases where the male is not the heterogametic sex,[42,44] or where individuals are hermaphrodites producing both highly-recombinant female gametes and less- or nonrecombinant male gametes.[45] Numerous hypotheses to explain the phenomenon have been proposed, but clear evidence eliminating or favoring one hypothesis in animals is still lacking.[43,45] Thus it is unclear why one genus, such as *Xiphophorus*, shows no heterochiasmy, while other taxa, such as *Salmonidae*, show a pattern of severely reduced recombination in males.[46]

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 the guppy, 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,[2] 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 the guppy. Kazianis et al.[16] 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.,[40] 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.

The genomes of the guppy and *Xiphophorus* are similar in physical size,[16,40] but are markedly different in map size, with two mapping crosses of guppies[40] having a map size al-

most twice as large as *Xiphophorus*. In this 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. If *Xiphophorus* consistently shows larger map distances between homologous loci than the guppy, this would more likely indicate higher genome-wide recombination rates in *Xiphophorus*, rather than a larger genome. Other linkage analysis studies in the guppy using microsatellites have not used any loci overlapping with the set of markers used in this study.

The level of sequence divergence between the guppy and *Xiphophorus* estimated from four microsatellite flanking regions, 0.21 substitutions per site, is at least 10 times higher than divergence between species within a single genus of rockfish also estimated from microsatellite flanking regions.[47] *Sebastes* rockfish species diverged from each other within the last 2 million years, more recently than did the guppy and *Xiphophorus*.[35,48,49] Our estimate of neutral sequence divergence between the guppy and *Xiphophorus* is the first such estimate using neutral nuclear markers in these taxa of which we are 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.[35]

Microsatellite loci that could be reliably amplified by PCR but did not show any variation in our 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.[16,17] 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 the guppy and the differences between the guppy and *Xiphophorus* in sex determination.

Future mapping efforts in the guppy could compare genetic architecture within the species, using mapping cross designs employing individuals from combinations of low and high predation risk. This would allow examination of the role of environmental factors in shaping the structure of the genome. Haskins et al.[1] discovered linkage relationships among some male color pattern genes varied as a function of predation risk, and it is possible that other adaptive phenotypes also show a pattern of variation in genetic architecture across populations. Further comparisons with *Xiphophorus* and other related taxa may further elaborate the role of the environment in structuring the genome of the guppy.

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 the guppy, 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.

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