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TEMPORAL VARIATION AT THE MHC CLASS IIB IN WILD POPULATIONS OF THE GUPPY (*POECILIA RETICULATA*)

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Understanding genetic diversity in natural populations is a fundamental objective of evolutionary biology. The immune genes of the major histocompatibility complex (MHC) are excellent candidates to study such diversity because they are highly polymorphic in populations. Although balancing selection may be responsible for maintaining diversity at these functionally important loci, temporal variation in selection pressure has rarely been examined. We examine temporal variation in MHC class IIB diversity in nine guppy (*Poecilia reticulata*) populations over two years. We found that five of the populations changed significantly more at the MHC than at neutral (microsatellite) loci as measured by F_{ST}, which suggests that the change at the MHC was due to selection and not neutral processes. Additionally, pairwise population differentiation measures at the MHC were higher in 2007 than in 2006, with the signature of selection changing from homogenizing to diversifying selection or neutral evolution. Interestingly, within the populations the magnitude of the change at the MHC between years was related to the change in the proportion of individuals infected by a common parasite, indicating a link between genetic structure and the parasite. Our data thereby implicate temporal variation in selective pressure as an important mechanism maintaining diversity at the MHC in wild populations.

KEY WORDS: ***Gyrodactylus bullatarudis*, *Gyrodactylus turnbulli*, natural selection, parasite-mediated selection, population differentiation.**

The mechanisms that maintain adaptive genetic diversity in natural populations have long interested biologists. In vertebrates, the genes of the major histocompatibility complex (MHC) are highly diverse and functionally important, and these genes thereby provide an ideal opportunity to examine selection for diversity. The MHC codes for proteins that bind foreign peptides and then present them to T helper cells, which initiates an immune response (Klein 1990). Thus, the MHC acts as the interface between pathogens and the adaptive immune system. The MHC is known to be highly polymorphic in many taxa, specifically at the region of the protein that binds foreign peptides (referred to as the peptide binding region or PBR; reviewed in Bernatchez and Landry 2003). Three major nonexclusive hypotheses have been proposed to explain selection for diversity at the MHC: (1) overdominance, where heterozygotes are able to recognize a broader array of pathogens and therefore have increased fitness (Doherty and Zinkernagel 1975; Hughes and Nei 1988); (2) negative frequency-dependent selection, where pathogens and MHC alleles cycle through a coevolutionary arms-race (Clarke and Kirby 1966); and (3) spatial and temporal variation in selection, where pathogens vary spatially and temporally (Hedrick 2002). Other hypotheses include mate choice for MHC dissimilar alleles mediated by inbreeding avoidance (Penn 2002) and a nonadaptive mechanism proposed by van Oosterhout (2009) that

selection occurs against mutations that accumulate near MHC loci.

Of the hypotheses that exist to explain the maintenance of diversity at the MHC, the most convincing evidence comes from overdominance where heterozygotes have a fitness advantage over homozygotes. For example, Oliver et al. (2009a) found that water voles (*Arvicola terrestris*) that were heterozygous at the MHC were infected by fewer parasites than homozygotes. Similarly, Kekäläinen et al. (2009) found that Arctic charr (*Salvelinus alpinus*) that were heterozygous at the MHC had a lower parasite load, higher condition, and higher survival rate than individuals that were homozygous at the MHC. Evidence for frequency-dependent selection is less common. Support for this hypothesis comes from studies that show a fitness disadvantage for the most common allele or a fitness advantage for rare alleles. For example, in humans the HLA-A11 allele confers resistance to infection with Epstein-Barr virus only in populations in which the allele is rare (de Campos-Lima et al. 1993); in populations with a high frequency of the allele, virus strains are monomorphic for a mutation that prevents presentation of immunodominant epitopes by HLA-A11 molecules. In several other systems, there is little support for either the overdominance or frequency-dependent selection hypotheses or the mechanism responsible for maintaining diversity remains to be determined.

Temporal variation in selection for MHC diversity is arguably the least well-studied mechanism of maintaining diversity, with only a handful of studies having examined the mechanism. Westerdahl et al. (2004) studied MHC allele frequency changes over time in great reed warblers (*Acrocephalus arundinaceus*) and found that MHC alleles tended to vary more among cohorts than microsatellite alleles. They concluded that selection differences among cohorts, and not neutral processes, were responsible for the differences in the MHC allele frequencies. Similarly, Charbonnel and Pemberton (2005) conducted a long-term survey of MHC variation in soay sheep (*Ovis aries*) and found that temporal variation was higher at MHC than neutral loci in one subdivision of sheep and that the strength of selection changed among years. Specifically, they showed that homogenizing selection was present in some years, where MHC population differentiation was more similar than expected by neutral diversity, but not present in other years (also see Jensen et al. 2008; Oliver et al. 2009b). If temporal variation in MHC diversity is common in wild populations, then selection studies on point estimates may miss some of the complexity of selection for MHC diversity. Clearly more studies of temporal variation at the MHC are needed.

Here, we study temporal variation at the MHC class IIB gene in nine wild populations of the guppy (*Poecilia reticulata*). The guppy is a tropical freshwater fish native to northeast South America and the neighboring islands of Trinidad and Tobago (Magurran 2005). In the mountainous regions of Northern Trinidad, populations are typically highly differentiated at neutral loci (Carvalho et al. 1991; Alexander et al. 2006; Crispo et al. 2006; Barson et al. 2009; Suk and Neff 2009). Despite this high population differentiation at neutral loci, in a previous study we uncovered homogenizing selection at the MHC across populations. Based on samples from 10 populations collected in 2006, we found that the MHC was less differentiated than estimates based on microsatellites for 73% of the population pairwise comparisons (Fraser et al. 2010). Similar homogenizing selection was found by van Oosterhout et al. (2006) for two populations within a single river in Northern Trinidad. We also showed that two common external parasites, *Gyrodactylus turnbulli* and *G. bullatarudis* (hereafter referred to as gyrodactylus), were the likely agents of the homogenizing selection. Gyrodactylus is a pervasive parasite that can be lethal to guppies (Scott and Anderson 1984). Specifically, we found that the *Pore_a132* allele was common to all 10 populations and was associated with reduced gyrodactylus infection (Fraser and Neff 2010). The additive genetic effect of the *Pore_a132* on parasite resistance was further supported by an experimental study in which guppies were challenged with gyrodactylus (Fraser and Neff 2009).

Here, we compare MHC class IIB genetic diversity in nine guppy populations that were surveyed in both 2006 and 2007 to determine whether there is temporal variation in selection on the MHC. Our sampling period corresponds to a minimum of two generations in guppies (Reznick et al. 1997). We compare the MHC class IIB genetic diversity within and between years to neutral diversity estimated from six microsatellite loci. We also examine the change in the putative selection pressure from gyrodactylus and relate it to the change in MHC variation across the years. In guppies, the MHC class IIB has undergone a single duplication event as up to four alleles have been found in individuals. Therefore, there are two MHC class II loci in the guppy that appear to be unlinked (McConnell et al. 1998; van Oosterhout et al. 2006; Fraser et al. 2010).

Methods

FIELD METHODS

During May of 2006 and May of 2007, nine populations of guppies from across northern Trinidad were sampled (Fig. 1, Table S1). The populations comprised upstream and downstream locales from the Aripo river and a lower locale from the Guanapo river in the Caroni drainage, upper and lower locales in the Quare and Turure rivers in the Oropouche drainage, and upper and lower locales in the Yarra river on the northern coast. The upper Guanapo population sampled in 2006 was not sampled in 2007 because of difficulty accessing the site. Of note is that about 50 years ago, guppies near the lower Guanapo were transferred to the upper

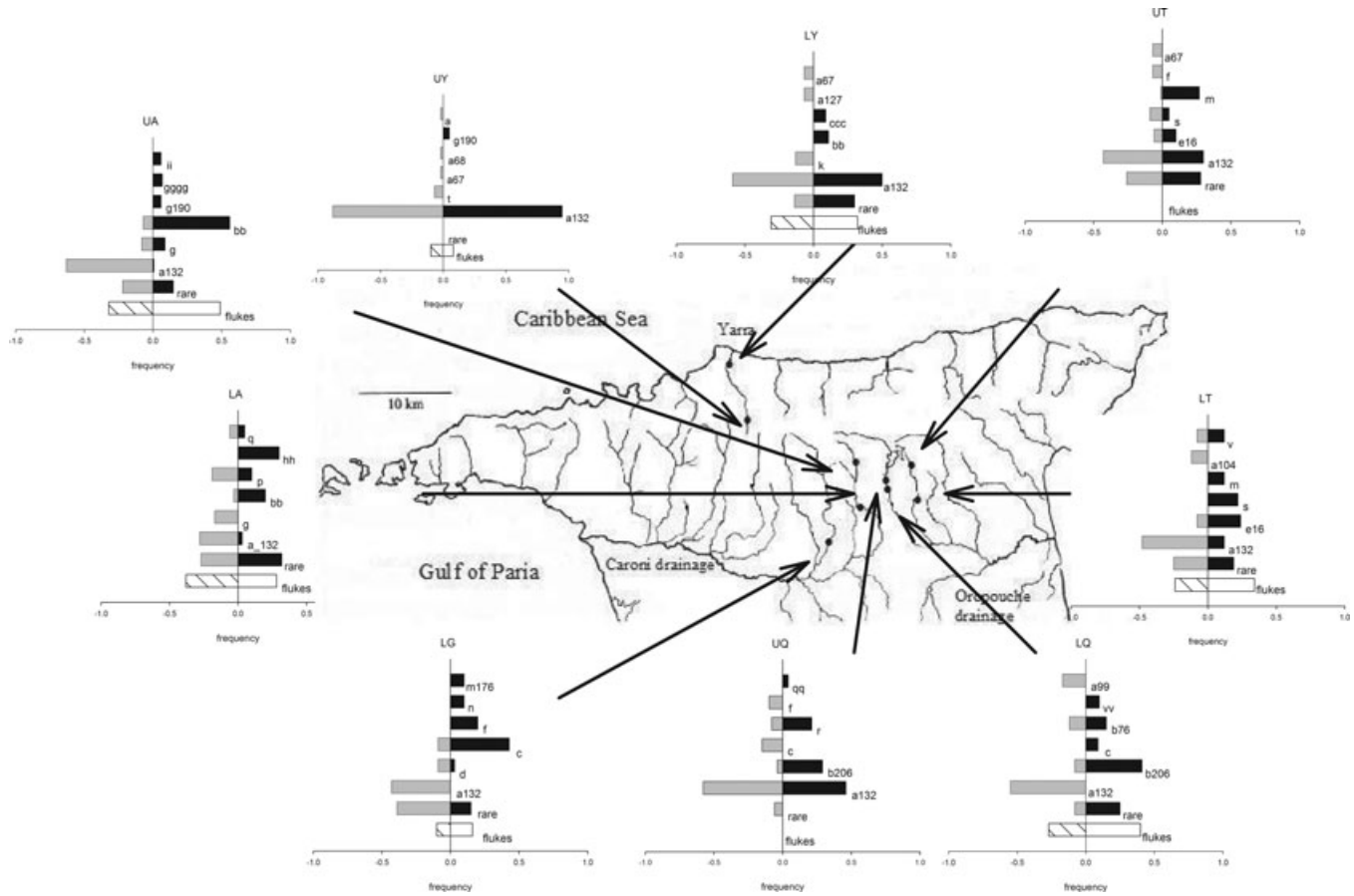

Figure 1. **Location and MHC class IIB allele frequencies of nine guppy (*Poecilia reticulata*) populations in Northern Trinidad. Populations comprise: from the north slope, Lower Yarra (LY), Upper Yarra (UY); from the Oropouche drainage, Lower Quare (LQ), Upper Quare (UQ), Lower Turure (LT), and Upper Turure (UT); and from the Caroni drainage, Lower Guanapo (LG), Lower Aripo (LA) and Upper Aripo (UA). Frequencies are shown for the six most common alleles in each population and the remaining alleles were collapsed into a rare category. Gray bars show frequencies in 2006 and black bars show the frequencies in 2007. The frequency of infected individuals with *Gyrodactylus* flukes are shown in hatched bars in 2006 and white bars in 2007.**

Turure population as part of an experiment (Shaw et al. 1991; Magurran 2005). These two populations remain genetically similar as measured by microsatellite loci (Shaw et al. 1991; Magurran 2005; Suk and Neff 2009).

Fish were collected from each population using seine and dip nets. Fish were individually packaged to avoid cross-contamination of parasites and transported back to our laboratory at the University of the West Indies, St. Augustine where they were housed individually before examination. Examination occurred within 24 h after collection. Fish were lightly anesthetized with MS222 prior to conducting the external examination. Using a compound microscope, the number of *G. turnbulli* and *G. bullatarudis* flukes, without identifying species, on each individual was recorded. The two species have similar pathologies in guppies and infection with either species results in similar parasite load and parasite-related mortality (Cable and Oostehout 2007). The fish were then euthanized with an overdose of MS222 and two gill arches were excised at random and examined (gyrodactylus sometimes migrate to the gills of fish). Standard length and sex were also recorded during the examination. Whole body tissue samples were then preserved in 95% ethanol for genetic analysis.

MOLECULAR METHODS

An average of 15 adults from each population were sequenced at the MHC class IIB (exon 2) for a total 261 individuals. The subset of individuals genotyped were selected randomly from the collected individuals and to ensure that we had accurate representation of infection rates for each population. In 2007, for one population (the upper Quare) only seven individuals could be genotyped because of poor DNA quality. We conducted our analysis with and without this population included. We found that the results were similar and so we present the full dataset. The analysis based on the restricted data is available from the authors. Genotyping followed Fraser et al. (2010). Briefly, samples were first analyzed using single-strand conformation polymorphism (SSCP; Amersham Biosciences, Piscataway, NJ). At

least one individual that corresponded to each unique banding pattern was genotyped by reamplifying their DNA and the PCR product was inserted into a plasmid vector (Promega, Madison, WI) following the manufacturer's instructions. The vector was then transformed into DH5α competent bacteria (Invitrogen Life Technologies, San Diego, CA) and grown on LB agar plates with ampicillin (Sigma-Aldrich, St. Louis, MO). Up to 10 clones were sequenced per sample and a total of 1142 clones were analyzed. Individuals had clones sequenced sequentially such that individuals that had less than four alleles present in the first four or five clones were sequenced at additional clones. This approach reduced the probability that we missed an allele. A subset of SSCP banding patterns were also sequenced from more than one individual to confirm that the genotypes matched. Furthermore, we also designed an alternate forward primer (5′ CAGAGAATAT GAAGTGGATCGTTGTG 3′) to test for potential null alleles. The alternative primer was used in both years and a subset of individuals ($N = 15$) were analyzed with both sets of primers; no difference was detected in the amplified genotypes (see Fraser et al. 2010).

An average of 19 adults from each population were also genotyped at six microsatellite loci for a total of 358 individuals. Microsatellites were genotyped following Suk and Neff (2009). We used three dinucleotide microsatellite loci (*Pr*39, *Pr*92, and *Pr*171; Becher et al. 2002) and three tetranucleotide microsatellite loci (*Pre*8, *Pre*9, and *Pre*15; Paterson et al. 2005).

INFECTION ANALYSIS

We examined three levels of infection across years, proportion of individuals infected, average number of flukes across all individuals, and intensity of infection (average number of flukes on only infected individuals). Differences in proportion of infected individuals between years were detected with paired *t*-tests (samples paired by population). Differences between years in average number of flukes and intensity were evaluated with a two-factor analysis of variance (ANOVA) with year and population as fixed factors. Populations that had no incidence of infection were removed from these analyses.

SPATIAL AND TEMPORAL ANALYSIS

MHC genotypes were determined from the clone data by calculating the most likely genotype using multinomial probabilities. For example, if we had sequence data from six clones and found four clones with allele "a," one with allele "b," and one with allele "c," then the most likely genotype for the individual is "aabc" with a normalized likelihood of 0.76. The genotypes of "abbc" and "abcc" each have a normalized likelihood of 0.095 and the genotype "abcd" (where "d" represents a fourth allele not appearing in the six clones) has a likelihood of 0.05. Population diversity measures comprising expected heterozygosity, nucleotide diversity using the Jukes-Cantor correction, and number of alleles per population were calculated using SPAGeDi version 1.2 (Hardy and Vekemans 2002) or DnaSP version 4.0 (Rozas et al. 2003). Allelic diversity adjusted for variation in sample size was estimated using the rarefaction method using FSTAT 2.9.3 (El Mousadik and Petit 1996; Goudet 2001). F_{IS} and associated Fishers exact test *P*-values for microsatellite loci were calculated using Genepop (Raymond and Rousset 1995). The population differentiation measure F_{ST} was calculated using a polysomic polyploids model (Ronfort et al. 1998), which reflects a situation similar to unlinked duplicated genes as in the guppy MHC class II (McConnell et al. 1998); that is, when alleles cannot be assigned to loci and all possible pairwise combinations are assumed to be equally likely to be inherited (Hardy and Vekemans 2002). F_{ST} measures for microsatellites were estimated using Weir and Cockerham (1984). We also compared our results with the more general population differentiation measure, G_{ST} (Pons and Petit 1995). F_{ST} and G_{ST} measures were highly correlated ($r^2 = 0.99$, $n = 36$, $P < 0.001$) and therefore we focused on F_{ST} for our analysis. The G_{ST} data and analyses can be obtained from the authors. Mantel correlations were estimated in FSTAT 2.9.3 (Goudet 2001). Differences in number of alleles, adjusted allelic diversity, expected heterozygosity, nucleotide diversity, and F_{ST} between years were detected with paired *t*-tests.

We compared MHC F_{ST} estimates to the F_{ST} estimates from the six microsatellite loci between years within populations, and among populations within each year. Ninety-five percent confidence intervals (CI) were generated for the F_{ST} estimates based on the MHC and six microsatellite loci through resampling individuals in populations with replacement for a total of 1000 runs. Significance was determined by comparing the pairwise values for microsatellites and MHC in each randomization run and determining the overall proportion where the MHC estimate was lower or higher than the microsatellite estimate. This method of comparing between F_{ST} estimates using bootstrapped confidence intervals is ideal for small sample sizes because it minimizes the effect of perceived rare alleles (due to sampling error) on estimates of differentiation between populations (Neff and Fraser 2010). An analysis of molecular variance (AMOVA) was used to partition molecular variation to within populations, among populations within rivers, and among rivers using Arlequin 2.0 (Schneider et al. 2000).

Results

MHC SEQUENCE RESULTS

A total of 66 alleles from 261 individuals were found in 2006 and 2007 (Fig. S1). Alleles were extremely variable; 139 of the 218 nucleotide positions (64%) were polymorphic. The 66 nucleotide sequences translated into 58 different amino acids sequences and

63 of the 72 codons (88%) were polymorphic. Twenty-three new alleles were found in 2007, whereas 21 alleles found in 2006 were not seen again in 2007. The alleles that were unique to year were rare; all but two of these alleles were less than 1% frequent in either year. Across both years, 36 alleles were unique to population, three alleles were unique to rivers, and five alleles were unique to drainage. The remaining 22 alleles were found in multiple drainages.

INFECTION ANALYSIS

The proportion of fish infected in each population, average number of gyrodactylus, and intensity of infection did not vary significantly across years (Table 1). No difference was found in the proportion of infected individuals between 2006 (0.19 ± 0.05) and 2007 (0.23 ± 0.06) ($t_8 = 1.39$, $P = 0.20$). There also was no difference in the average number of gyrodactylus flukes between 2006 (0.38 ± 0.12) and 2007 (0.27 ± 0.09) ($F_{1,630} = 0.89$, $P = 0.35$). We found a significant difference among populations in the average number of flukes ($F_{6,630} = 4.95$, $P < 0.001$) and this difference was apparent in both years as there was no interaction between population and year on average number of flukes ($F_{6,630} = 0.50$, $P = 0.81$). We also found no difference in the intensity of infection between 2006 (1.46 ± 0.32) and 2007 (1.78 ± 0.53) ($F_{1,162} = 0.25$, $P = 0.25$). There again was a significant difference in the intensity among populations ($F_{6,162} = 2.4$, $P = 0.029$), but also a significant interaction between population and year ($F_{6,162} = 2.9$, $P = 0.01$). There was no difference in number of flukes between female (0.56 ± 0.064) and male fish (0.47 ± 0.061) ($t_{642} = 1.49$, $P = 0.14$) and there was no correlation between standard length and the average number of flukes on individuals (rho $= 0.059$, $n = 644$, $P = 0.13$). A similar lack of effect of sex on intensity of infection was found (male $= 1.87 \pm 0.16$, female $= 1.92 \pm 0.14$; $t_{174} = 0.25$, $P = 0.81$) and there was no relationship between standard length and the intensity of infection (rho $= -0.061$, $n = 176$, $P = 0.42$).

SPATIAL AND TEMPORAL ANALYSIS

The *Pore_a132* allele was the most frequent allele in all populations in 2006 (range: 0.28–0.88; Fig. 1, Table S1). In contrast, in 2007 the *Pore_a132* was the most frequent allele in only the upper and lower Yarra, the upper Quare, and the upper Tururе populations (range: 0.30–0.95 in these four populations). In the other five populations in 2007, the most frequent allele was different in each population.

Populations were significantly differentiated at the MHC in both years (overall F_{ST} in 2006 was 0.038, $P = 0.003$; overall F_{ST} in 2007 was 0.23, $P < 0.0001$). F_{ST} estimates were consistently higher in 2007 than 2006 ($t_{35} = 8.77$, $P < 0.0001$; Table 2). There was no consistent difference in the number of alleles in 2006 (10.1 ± 1.2) and 2007 (8.4 ± 1.1) ($t_8 = 1.47$, $P = 0.18$) or adjusted allelic diversity in 2006 (4.51 ± 0.52) and 2007 (4.37 ± 0.59) ($t_8 = 0.37$, $P = 0.72$). There also was no consistent difference in the expected heterozygosity between 2006 (0.66 ± 0.06) and 2007 (0.70 ± 0.08) ($t_8 = 1.35$, $P = 0.21$). The nucleotide diversity, however, was consistently lower in 2006 (0.10 ± 0.01) than in 2007 (0.13 ± 0.02) ($t_8 = 2.36$, $P = 0.046$).

MHC F_{ST} estimates of samples between years within populations indicated that five sites changed significantly between years (Table 2). Conversely, the upper Quare and upper Tururе populations and both Yarra populations did not change at the MHC. Based on allele number, the upper Tururе was the most diverse population in 2006, whereas in 2007 the lower Aripo was the most diverse. In contrast, the upper Yarra was the least diverse in both 2006 and 2007.

Table 1. Summary of gyrodactylus infection in nine populations of the guppy (*Poecilia reticulata*). Data comprise the number of fish surveyed (*N*), the proportion of individuals infected, the average parasite load, and the intensity of the infection (average parasite load of those that are infected) for samples collected in 2006 and 2007.

	2006				2007			
	N	Proportion infected	Average	Intensity	*N*	Proportion infected	Average	Intensity
Lower Aripo	42	0.38	0.83	2.19	50	0.28	0.58	2.07
Upper Aripo	31	0.32	0.97	3.00	51	0.49	0.75	1.52
Lower Guanapo	39	0.10	0.13	1.25	50	0.16	0.34	2.13
Lower Quare	56	0.27	0.52	1.93	53	0.40	0.66	1.67
Upper Quare	47	0	0	0	54	0	0	0
Lower Tururе	34	0.24	0.35	1.5	47	0.34	0.45	1.31
Upper Tururе	37	0	0	0	51	0	0	0
Lower Yarra	52	0.31	0.48	1.56	48	0.32	0.58	1.87
Upper Yarra	41	0.10	0.17	1.75	50	0.08	0.44	5.5

Table 2. Summary of the MHC class IIB loci sampled in nine populations of the guppy (*Poecilia reticulata*). Data comprise number of individuals (*N*), number of MHC class IIB of alleles (K), allelic variation adjusted for differences in sample size (A_R), expected heterozygosity (H_E), nucleotide diversity following a Jukes-Cantor correction (π_{JC}), and F_{ST} estimates between years within populations for samples collected in 2006 and 2007.

Population	2006					2007					F_{ST}
	N	K	A_R	H_E	π_{JC}	*N*	K	A_R	H_E	π_{JC}	
Lower Aripo	16	14	6.2	0.85	0.14	15	12	6.1	0.85	0.19	0.091[1]
Upper Aripo	15	12	4.2	0.59	0.08	17	11	4.7	0.67	0.12	0.32[1]
Lower Guanapo	11	13	6.2	0.78	0.12	10	8	4.5	0.77	0.13	0.14[2]
Lower Quare	15	6	3.5	0.66	0.07	17	11	5.4	0.79	0.10	0.21[1]
Upper Quare	13	8	3.7	0.64	0.12	7	4	2.5	0.68	0.11	0.013
Lower Turure	13	9	5.0	0.74	0.12	17	10	5.6	0.86	0.17	0.087[2]
Upper Turure	17	15	6.2	0.80	0.13	15	9	5.2	0.82	0.18	0.022
Lower Yarra	14	9	4.1	0.63	0.07	14	9	4.8	0.73	0.12	0.0029
Upper Yarra	14	5	1.5	0.23	0.05	21	2	0.5	0.09	0.01	−0.001

[1]$P<0.05$, [2]$P<0.001$.

Populations were significantly differentiated at the six microsatellite loci in both 2006 (overall $F_{ST} = 0.22$, $P < 0.0001$) and 2007 (overall $F_{ST} = 0.22$, $P < 0.0001$). There was no consistent difference in pairwise population F_{ST} estimates between 2006 and 2007 at the microsatellite loci ($t_{35} = 0.49$, $P = 0.63$, Table 3). There also was no difference in the mean number of alleles between 2006 (7.96 ± 0.88) and 2007 (7.07 ± 0.86) ($t_8 = 1.94$, $P = 0.088$) or difference in adjusted allelic diversity between 2006 (5.22 ± 0.49) and 2007 (5.24 ± 0.51) ($t_8 = 0.12$, $P = 0.91$). As well, there was no difference in the mean expected heterozygosity between 2006 (0.69 ± 0.057) and 2007 (0.68 ± 0.057) ($t_8 = 0.98$, $P = 0.36$). There was no difference in F_{IS} measures between years ($t_8 = -0.45$, $P = 0.66$). Based on allele number, the lower Quare and lower Turure were the most diverse in both 2006 and 2007, whereas the upper Yarra was the least diverse in both years. By calculating F_{ST} values between years within populations, we found that only the lower Yarra population changed significantly at the microsatellite loci (Table 3). In both 2006 and 2007, there was a significant relationship between MHC F_{ST} and microsatellite F_{ST} (2006: $r^2 = 0.30$, $n = 36$, $P < 0.001$; 2007: $r^2 = 0.74$, $n = 36$, $P < 0.001$). As well, there was a significant relationship between MHC and microsatellite expected heterozygosities (2006: rho = 0.86, $n = 9$, $P = 0.003$; 2007: rho = 0.88, $n = 9$, $P = 0.002$).

When comparing F_{ST} estimates of MHC and microsatellites, we found that five of the nine between-year within-population comparisons showed a greater difference at the MHC than at the microsatellite loci (Fig. 2A). This result indicates that these five

Table 3. Summary of the six microsatellite loci sampled in nine populations of the guppy (*Poecilia reticulata*). Data comprise number of individuals (*N*), average number of microsatellite alleles (K), average allelic variation adjusted for differences in sample size (A_R), average expected heterozygosity (H_E), population inbreeding measure (F_{IS}), and F_{ST} estimates between years within populations for samples collected in 2006 and 2007.

Population	2006					2007					F_{ST}
	N	K	A_R	H_E	F_{IS}	*N*	K	A_R	H_E	F_{IS}	
Lower Aripo	21	9.5	6.1	0.77	0.23[2]	17	8.5	5.9	0.75	0.11	−0.0049
Upper Aripo	19	5.0	3.7	0.46	−0.46	15	5.0	3.9	0.48	−0.052	0.0077
Lower Guanapo	19	9.6	6.9	0.85	0.17[2]	14	8.2	6.3	0.82	0.15[2]	0.016
Lower Quare	38	11.3	6.2	0.79	0.015[1]	21	11.0	7.0	0.81	0.10	0.0031
Upper Quare	39	7.8	4.6	0.70	0.14[2]	7	3.8	3.8	0.68	0.14	0.0023
Lower Turure	21	9.8	6.4	0.78	0.23[2]	18	9.7	6.8	0.84	0.18[2]	0.0016
Upper Turure	19	7.5	5.5	0.78	0.042	15	6.2	5.0	0.72	0.055[1]	0.018
Lower Yarra	17	8.3	5.3	0.76	0.15[2]	20	7.5	5.9	0.69	−0.0052	0.024[2]
Upper Yarra	19	2.8	2.3	0.35	0.09[1]	19	3.7	2.6	0.33	0.15[1]	0.037

[1]$P<0.05$, [2]$P<0.001$.

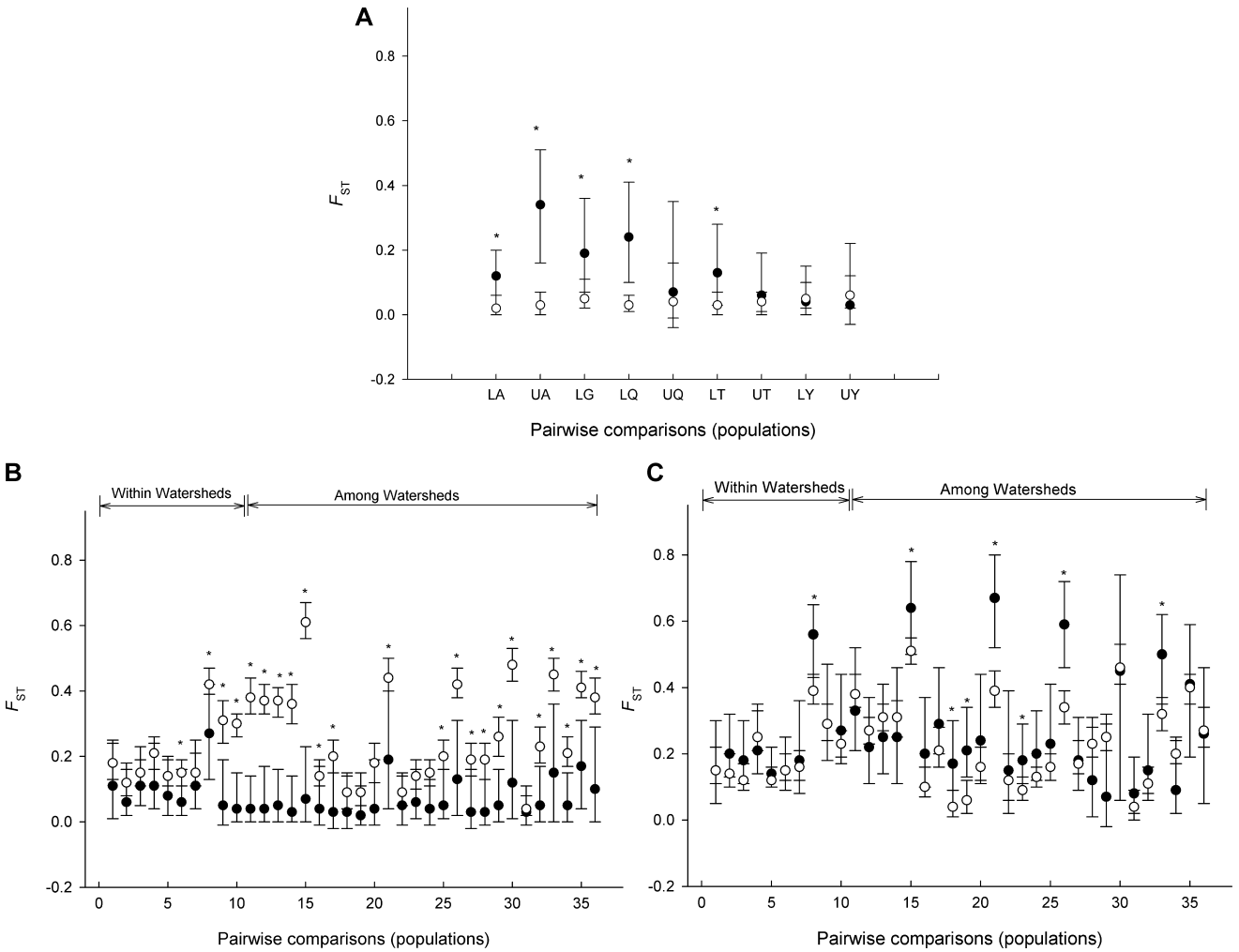

Figure 2. Comparison of F_{ST} values of neutral loci (six microsatellite loci) to MHC class IIB in nine populations of the guppy (*Poecilia reticulata*). (A) F_{ST} values are from comparisons between years 2006 and 2007 for each population. F_{ST} values are from all pairwise population comparisons within (B) 2006 and (C) 2007. Median F_{ST} estimates of microsatellite loci are indicated with open circles and median F_{ST} estimates of MHC class IIB are indicated with closed circles. Error bars indicate 95% confidence intervals and were estimated by resampling individuals within a population with replacement 1000 times ($P < 0.05$ indicated with an asterisk). Pairwise population estimates are grouped by within drainage and among drainages comparisons.

populations changed more at the MHC than expected by neutral evolution. In 2006, 23 of the 36 (64%) pairwise population comparisons were more similar at MHC than microsatellite loci, 13 (36%) showed no difference between the MHC and microsatellite loci, and none had higher F_{ST} values at the MHC than at the microsatellite loci (Fig. 2B). In contrast, in 2007 no pairwise population comparisons were more similar at the MHC than microsatellite loci, 28 (78%) showed no difference between the MHC and microsatellite loci, and eight (22%) had higher F_{ST} values at the MHC than at the microsatellite loci (Fig. 2C).

The absolute difference in the proportion of infected individuals was positively correlated to the MHC F_{ST} estimates between years for each population (rho $= 0.91$, $n = 9$, $P = 0.006$; Fig. 3). This relationship remained significant after removing the upper Quare and upper Turure populations (rho $= 0.89$, $n = 7$, $P = 0.007$); these two populations had no gyrodactylus in either year and remained unchanged at the MHC. The biggest change in MHC frequencies between years was the decrease in the *Pore_a132* allele (Fig. 1, Table S1), and this change was significantly negatively correlated with the change in the proportion of individuals infected (rho $= -0.77$, $n = 9$, $P = 0.015$; Fig. 4). This relationship was marginally nonsignificant after removing the upper Quare and upper Turure populations (rho $= -0.75$, $n = 7$, $P = 0.052$). A similar relationship between the change in the frequency of the *Pore_a132* and intensity of infection was found (rho $= -0.69$, $n = 9$, $P = 0.039$). This relationship remained significant after removing the upper Quare and upper Turure populations (rho $= -0.81$, $n = 7$, $P = 0.030$). There also was a significant negative correlation between the number of gyrodactylus flukes and the number of copies of *Pore_a132* within individuals

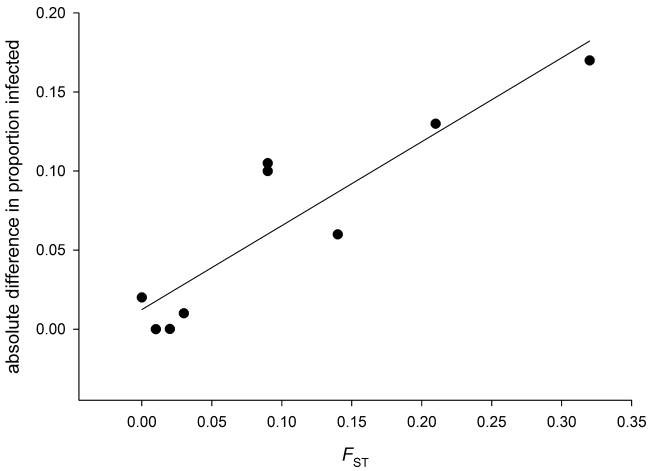

Figure 3. Relationship between the change in genetic composition and parasite infection in nine populations of the guppy (*Poecilia reticulata*). There was a relationship between the difference in MHC class IIB F_{ST} estimates between years (2006 and 2007) and the absolute difference in proportion of infected individuals within populations.

in 2006 (rho $= -0.23$, $n = 98$, $P = 0.021$), but the relationship was not significant in 2007 (rho $= -0.094$, $n = 108$, $P = 0.33$).

Geographic effects on MHC variation appeared to be more important in 2007 than 2006. The AMOVA analysis in 2006 indicated that most of the molecular variation was found within populations, and to a lesser extent among populations within rivers but not among rivers (Table 4). In contrast, the analysis in 2007 also revealed a significant percentage of the variation among rivers (Table 4).

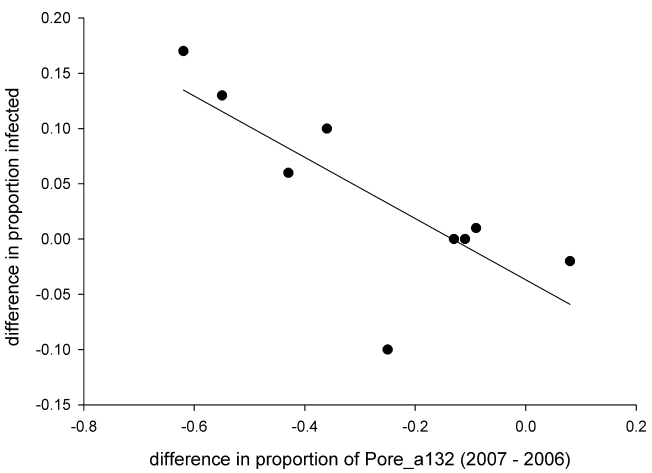

Figure 4. Relationship between the change in genetic composition and parasite infection in nine populations of the guppy (*Poecilia reticulata*). There was a relationship between the difference (2007–2006) in the frequency of the *Pore_a132* MHC class IIB allele and the difference in proportion of infected individuals between years 2006 and 2007.

Discussion

Here we provide evidence for temporal variation at the MHC class IIB in five of nine populations of guppies over two years. The upper and lower Aripo, lower Guanapo, lower Quare and lower Turure populations showed significant temporal variation in the frequency of MHC alleles. We found that these populations had MHC F_{ST} estimates between years that were significantly different than zero. These F_{ST} estimates were also higher than those calculated based on six microsatellite loci, which indicates that the differences at the MHC between years cannot be accounted for by neutral processes such as genetic drift. It is unlikely that sampling error can account for these observations because the microsatellite and MHC data were based on the same individuals for the majority of samples, and we employed a novel bootstrapping approach that is ideal for investigating small sample sizes (Neff and Fraser 2010). Thus, the change at the MHC cannot be accounted for by sampling artifact of rare alleles. Indeed, we found that the change was driven by differences at common alleles and specifically the decrease in the *Pore_a132* allele from 2006 to 2007. Furthermore, the change in MHC diversity was related to the change in infection of the gyrodactylus parasite.

Temporal variation may be the mechanism responsible for maintaining diversity at the MHC class IIB in the guppy. In 2006, selection at the MHC was predominately homogenizing, where F_{ST} values at the MHC were lower than those at neutral loci (Fraser et al. 2010). Infection by gyrodactylus, a pervasive parasite of guppies, and resistance afforded by the *Pore_a132* allele is likely the mechanism underlying the homogenizing selection (Fraser and Neff 2010). However, the MHC in guppies is quite polymorphic and thus the remainder of diversity cannot be explained by this simple guppy–gyrodactylus interaction. Here, based on samples collected the following year, we found little evidence of homogenizing selection at the MHC but instead a signature of either neutral evolution or diversifying selection among populations. This shift in selection led to an increase in the amount of diversity observed across populations and is likely to contribute to the maintenance of diversity at the MHC in guppies.

Ecological parameters may play an important role in the type of selection that occurs at the MHC. It is possible that the variation in selection between years is mediated by rainfall and hence population connectivity and gene flow. In 2007, Trinidad experienced an earlier and more severe dry season than in 2006. In 2007, the dry season started earlier and was more severe than in 2006 with less than half as much rainfall in 2007 as compared to 2006 (2006: 493.3 mm and 2007: 203.4 mm). The wet seasons prior to collection in 2006 and 2007 had similar rainfall (Trinidad and Tobago Meteorological Services at Piarco). The dry season had reduced river flow among populations and therefore dispersal would be reduced, at least in the predominant downstream

Table 4. Summary of the AMOVA results that partition genetic variation of the MHC class IIB locus among nine populations of the guppy (*Poecilia reticulata*). The analysis partitions variation across three levels and is shown for samples collected in 2006 and 2007. Data comprise the numerator degrees of freedom (df), percentage of variation, fixation index, and the *P*-value. The significance values were derived from permutating haplotypes 1023 times. $P < 0.05$ in bold.

	Source of variation	df	Percentage of variation	Fixation index	*P*
2006	Among rivers	4	−2.4	−0.024	0.75
	Among populations within rivers	5	13.3	0.110	**<0.001**
	Within populations	531	89.0	0.132	**<0.001**
2007	Among rivers	4	17.5	0.175	**0.002**
	Among populations within rivers	4	11.2	0.287	**<0.001**
	Within populations	523	71.3	0.136	**<0.001**

direction within rivers (e.g., Barson et al. 2009). Consistent with this reduced connectivity, our AMOVA analysis revealed that variation at the MHC partitioned among rivers in 2007 but not in 2006. In contrast, the microsatellite data indicated a similar high degree of differentiation in both years, which suggests that migration of guppies themselves is not the agent of genetic change at the MHC. Instead, the effect of decreased river connectivity may be on the migration of the parasites. If migration among these communities is reduced during drier times, then the parasites may exert local specific selection pressures, which in turn could increase the effect of subdivision at the MHC locus. The interdependency of host and parasite migration patterns has been documented. For example, Bruyndonckx et al. (2009) found that parasitic wing mite (*Spinturnix bechsteini*) populations showed temporal variation in population differentiation that corresponded with temporal variation of migration of their hosts, Bechstein's bats (*Myotis bechsteinii*). Thus, it is plausible that in our case, variation in population connectivity and migration of guppy parasites accounts for the variation in selection at the MHC in guppy populations. More research is certainly warranted in connectivity in parasite communities and its effect on MHC selection.

Gyrodactylus may nevertheless play a prominent role in mediating both spatial and temporal variation in selection on the MHC in guppies. We found a strong correlation between the change observed at the MHC diversity between years and the change in gyrodactylus infection. For example, the Yarra populations showed no change at the MHC and had the smallest change in proportion of gyrodactylus-infected individuals. The lack of change at the MHC in the gyrodactylus-free populations of the upper Quare and upper Turure is also consistent with the idea that gyrodactylus are driving the variation in selection at the MHC in the other populations we studied. This is the first study that we are aware of that has shown a correlation between change in parasites and MHC. It is clear that MHC and gyrodactylus are tightly linked in both space and time.

Temporal variation in selection at the MHC in guppies may also be maintained by trade-offs between alleles. We found that in 2006 the *Pore_a132* allele was the most frequent allele in all populations, whereas in 2007 it decreased in frequency in eight of nine of the populations studied and was then the most frequent in only four of the populations. This consistent drop in frequency suggests a cost of the *Pore_a132* allele or benefit for rarer alleles. For example, negative frequency-dependent selection could operate through a disadvantage for common alleles. Evidence for negative frequency-dependent selection at the MHC has been shown in other systems. In humans the HLA-A11 allele has a fitness advantage only in populations in which the allele is rare (de Campos-Lima et al. 1993). Also, in soay sheep the most common MHC alleles were associated with decreased survival and high incidence of parasitism from a nematode whereas a rare allele was associated with increased survival (Paterson et al. 1998). Negative frequency-dependent selection could also operate through rare-mate advantage. Individuals may base mate choice decisions for rare or unfamiliar MHC alleles to avoid inbreeding (Penn and Potts 1999), and in the guppy, females have been shown to prefer unfamiliar males (Hughes et al. 1999; Kelley et al. 1999). Of course, any benefit of rare alleles or cost for the *Pore_a132* allele would have to outweigh the benefit of gyrodactylus resistance provided by the *Pore_a132* allele (Fraser and Neff 2010). The benefit of the *Pore_a132* allele is evident in 2007 as the decrease in *Pore_a132* was associated with an increase in gyrodactylus infection. Nevertheless, either natural or sexual selection could account for the decrease in the *Pore_a132* allele between years and contribute to the maintenance of diversity at the MHC across guppy populations.

In conclusion, we have provided evidence of temporal variation in MHC class IIB loci in wild guppy populations. In 2006, there was evidence for homogenizing selection at the MHC, whereas in the following year there was evidence of neutral evolution in some populations and diversifying selection in others.

Our data highlight the value of studies of temporal variation of MHC diversity and the potential importance of temporal variation in selection as a mechanism maintaining genetic diversity in wild populations. In guppies, future studies are needed to fully elucidate the evolution of gyrodactylus virulence as well as selection pressure from the broader parasite community and the role of population connectivity in driving MHC evolution across the landscape. Additional years of sampling will also help to better detail any potential pattern in the temporal variation in evolution at the MHC and the role of various ecological parameters such as rainfall.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Neighbor-joining tree of MHC class IIB alleles found in the guppy (*Poecilia reticulata*).
Table S1. List of MHC class IIB alleles found in the guppy (*Poecilia reticulata*).

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

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Supplementary Material

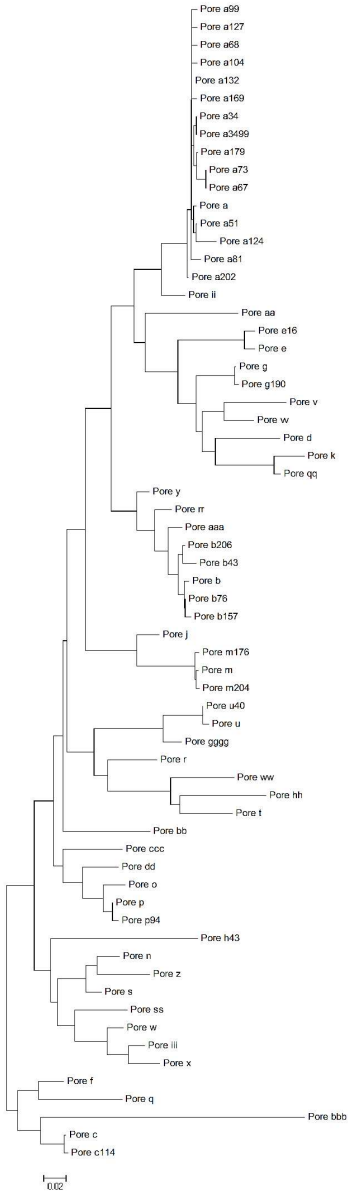
Figure S1: Neighbor joining tree of MHC class IIB alleles found in the guppy (*Poecilia reticulata*).

The scale refers to difference among alleles and uses the Jukes-Cantor correction.

Table S1: List of MHC class IIB alleles found in the guppy (*Poecilia reticulata*). Proportion of alleles in each population in 2006 and 2007 as well as the total proportion in 2006 and 2007 of each allele are shown.

	LA_06	LA_07	UA_06	UA_07	LG_06	LG_07	LQ_06	LQ_06	UQ_06	UQ_07	LT_06	LT_07	UT_06	UT_07	LY_06	LY_07	UY_06	UY_07
a132	0.28	0.03	0.63	0.01	0.43	-	0.55	-	0.58	0.46	0.48	0.12	0.43	0.30	0.59	0.50	0.88	0.95
bb	0.03	0.20	0.07	0.56	-	-	-	-	-	-	-	-	-	-	-	0.11	-	-
c	-	-	-	-	0.09	0.43	-	0.09	0.15	-	0.08	0.06	0.01	-	-	0.07	-	-
b206	-	-	-	-	-	-	0.08	0.41	0.04	0.29	-	-	0.03	-	-	-	-	-
r	-	0.10	-	-	-	-	0.07	0.06	0.08	0.21	0.04	0.03	0.04	0.07	-	0.07	-	-
m	0.03	-	-	0.04	0.02	0.03	-	-	0.02	-	-	0.12	0.01	0.27	-	-	-	-
e16	-	-	-	0.01	-	-	-	-	-	-	0.08	0.24	0.06	0.10	-	-	-	-
s	-	-	-	0.04	-	-	-	-	-	-	-	0.22	0.09	0.05	-	-	-	-
g	0.17	-	0.08	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hh	-	0.30	-	-	-	-	-	-	-	-	-	0.01	-	0.03	-	-	-	-
b76	-	-	*0.02*	-	*0.02*	-	0.12	0.15	-	-	-	-	-	-	-	0.02	-	-
p	*0.19*	*0.10*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
f	-	-	-	-	-	*0.20*	-	-	0.10	-	-	-	0.07	-	-	-	-	-
a73	*0.05*	-	*0.03*	-	*0.05*	-	0.02	-	0.02	-	0.04	-	0.07	-	0.02	-	0.02	-
g190	-	*0.07*	-	*0.06*	-	-	-	-	-	-	-	0.07	-	-	-	-	-	0.05
a99	-	-	-	-	*0.05*	-	0.17	-	-	-	-	-	0.04	-	-	-	-	-
t	-	-	-	-	-	-	-	-	-	-	0.08	-	-	0.10	-	-	0.07	-
v	-	-	-	-	-	-	-	-	-	-	0.08	0.12	-	-	-	-	-	-
q	*0.06*	*0.05*	*0.02*	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-
d	-	0.05	-	-	0.09	0.03	-	-	-	-	-	-	-	-	-	-	-	-
k	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	-	-
a104	0.02	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-	-	-
vv	-	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-
p94	0.03	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-
ii	-	-	-	0.07	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-
b	0.03	-	-	-	-	-	-	0.04	-	-	-	-	-	-	0.02	-	-	-
m204	0.02	-	0.03	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-
a67	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	0.07	-	-	-
n	0.02	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-
ccc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	-
u40	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.07	-	-	-	-
gggg	-	-	-	*0.06*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a68	-	-	*0.02*	-	*0.02*	-	-	-	-	-	0.02	-	-	-	-	-	0.02	-
a172	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-
ss	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a34	-	-	-	-	-	*0.10*	-	-	-	-	-	-	-	-	-	0.07	-	-

o	*0.06*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
m176	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
aaa	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-
j	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-
a51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-
b43	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-
a81	-	-	0.02	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-
z	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
aa	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	0.02	-
a202	-	-	0.02	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-
a169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-
a3499	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-
rr	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-
h43	-	*0.02*	-	-	-	*0.03*	-	-	-	-	-	-	-	-	-	-	-	-
u	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-
iii	-	-	-	*0.03*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ww	-	-	-	*0.01*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
e	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-
a124	-	-	-	-	*0.02*	-	-	-	-	-	-	-	-	-	-	-	-	-
a179	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-
qq	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-
w	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-
b157	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-
bbb	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-
x	*0.02*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
dd	-	*0.02*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c114	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-
y	-	-	*0.02*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Neighbor joining tree of MHC class IIB alleles found in the guppy (Poecilia reticulata). The scale refers to difference among alleles and uses the Jukes-Cantor correction.
120x409mm (400 x 400 DPI)