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MHC class IIB additive and non-additive effects on fitness measures in the guppy *Poecilia reticulata*

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The genetic architecture of fitness at the class IIB gene of the major histocompatibility complex (MHC) in the guppy *Poecilia reticulata* was analysed. Diversity at the MHC is thought to be maintained by some form of balancing selection; heterozygote advantage, frequency-dependent selection or spatially and temporally fluctuating selection. Here these hypotheses are evaluated by using an algorithm that partitions the effect of specific MHC allele and genotypes on fitness measures. The effect of MHC genotype on surrogate measures of fitness was tested, including growth rate (at high and low bulk food diets), parasite load following a parasite challenge and survival. The number of copies of the *Pore_a132* MHC allele was inversely related to infection by *Gyrodactylus* flukes and it appeared to be positively related to faster growth. Also, genotypes combining the *Pore_a132* or other relatively common alleles paired with rare MHC alleles produced both advantageous and detrimental non-additive effects. Thus, the genetic architecture underlying fitness at the MHC is complex in the *P. reticulata*.

Key words: fitness; genetic quality; growth rate; major histocompatibility complex; parasite; *Poecilia reticulata*.

INTRODUCTION

A full understanding of natural selection requires the identification of the genes underlying fitness (Orr, 2005). A good candidate for a gene important in fitness is the major histocompatibility complex (MHC) because MHC proteins act as the interface between pathogens and the host's adaptive immune system (Klein, 1990; Potts & Wakeland, 1993). Specifically, class I and class II MHC genes code for proteins that bind to pathogen-derived peptides and present them to T cell receptors, which in turn initiates an immune response (Klein, 1990). The mechanisms that maintain diversity in the MHC include balancing selection with either overdominance, in which case MHC heterozygous individuals have increased survivorship because they are able to present a broader array of antigens (Doherty & Zinkernagel, 1975; Hughes & Nei, 1988), or negative frequency-dependent selection, in which case a coevolutionary

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arms race between specific MHC alleles and parasites can lead to the cycling in frequency of alleles (Clarke & Kirby, 1966).

Various studies have used challenge experiments to link specific MHC alleles or genotypes to surrogate measures of fitness. For example, Lohm *et al*. (2002) found a strong survivorship advantage for individuals carrying a high-resistance allele in Atlantic salmon *Salmo salar* L. after being infected with *Aeromonas salmonicida* (also see Langefors *et al.,* 2001; Arkush *et al*., 2002; Grimholt *et al.,* 2003; Miller *et al.,* 2004). Heterozygosity in the MHC has also been linked to higher fitness. For example, Penn *et al*. (2002) showed that heterozygous mice *Mus domesticus* had greater survival, gained more weight and were better able to clear *Salmonella* infections in a laboratory experiment than were homozygous mice (also see McClelland *et al.,* 2003). However, this latter type of analysis does not differentiate among different heterozygous genotypes (see Bernatchez & Landry, 2003). A more direct test of non-additive genetic effects is to examine the effects of specific genotypes and alleles on fitness. This approach was taken by Pitcher & Neff (2006), to examine Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) juvenile survivorship. By developing an algorithm that partitions both additive and non-additive effects of alleles on fitness measures, Pitcher & Neff (2006) found a positive additive effect of one MHC allele, but also a negative non-additive effect of one genotype on survivorship.

Here, the algorithm developed by Pitcher & Neff (2006) was used to assess the MHC class IIB effects on surrogate measures of fitness in the guppy *Poecilia reticulata* Peters. In the northern range of Trinidad, *P. reticulata* inhabit freshwater streams and are subject to a diverse array of parasites (Lyles, 1990). Of these parasites, *Gyrodactylus turnbulli* and *G. bullatarudis* are prevalent throughout the northern range of guppies in Trinidad (Lyles, 1990) and are known to be pathogenic to the guppy (Scott & Anderson, 1984). *Gyrodactylus* can be particularly harmful due to their progenesis (where newborns already contain fully formed embryos) and can reproduce asexually on their hosts with short generation times ($<$24 h at 25° C) (reviewed in Bakke *et al*., 2007). Additionally, Hedrick *et al*. (2001) argued that individuals of the closely related Gila top minnow *Poeciliopsis O. occidentalis* (Baird & Girard) with heterozygotes MHC genotypes could better combat *Gyrodactylus* infection than MHC homozygotes. Recently, van Oosterhout *et al*. (2006) investigated MHC-based selection in *P. reticulata*. While their work did not address the effects of specific alleles or genotypes on infection, they concluded that the parasite community selects for increased MHC diversity. Thus, MHC genes are likely to be important determinants of *P. reticulata* fitness and a detailed analysis of additive and non-additive fitness effects may provide a fuller understanding of natural selection and evolution at the this important locus.

MATERIALS AND METHODS

STUDY ANIMALS

The *P. reticulata* used were descendants from a population collected from a tributary of the Paria River (10° 44′ 42″ N, 61° 51′ 42″ W) in Trinidad. The Paria population was selected in part because individuals are susceptible to *Gyrodactylus* parasitic infection (Houde & Torio, 1992). The stock population was collected 3 years (*c*. six generations) prior to the experiments (Reznick et *al.*, 1997) and was maintained in high numbers, with individuals mating freely.

Females were reared in single-sex tanks from near-birth to ensure their virginity and to ensure that their mate preferences were not influenced by the memory of other males. Males were reared in larger mixed-sex tanks but were transferred to individual tanks 3 days prior to use to ensure full sperm supply (Pilastro *et al.,* 2002). Aquaria were kept at 25° C, on a 12L:12D hour light cycle and the guppies were fed a mix of Tetramin® commercial flake food with carotenoid supplements and brine shrimp *ad libitum* twice daily, unless otherwise noted.

OFFSPRING FITNESS AND MHC

Offspring from known families were examined for MHC-dependent fitness. Broods were generated by mating virgin females to individual males. Before mating, the female was lightly anaesthetized with MS-222 and standard length (L_S) was measured. The following day, the male and female were placed into a 10 l aquarium and observed for 15 min. Following Houde (1997), the frequency of male sigmoid displays and female receptivity was recorded. The pair was then left for 3 days to ensure copulation, after which sires were anaesthetized and photographed to determine their area of orange colouration and L_S (methods in Pitcher & Evans, 2001). The males were then euthanized and kept in 95% ethanol for subsequent genetic analysis. Female L_S and gestation time were also measured to identify and later statistically control for maternal effects. Females were left in a 36 × 21 × 20 cm tank filled to 15 cm until they gave birth. Following birth, dams were euthanized and kept in 95% ethanol.

The fitness of the offspring from each brood was estimated from three surrogate measures of fitness comprising growth rate (at high and low food levels), parasite load and survival. Four offspring were randomly selected for the rest of the experiment. When a brood consisted of less than four offspring, the entire brood was subjected to the rest of the experiment. Each selected offspring was lightly anaesthetized in MS-222 and photographed on their lateral side so that their L_S and area could be measured at day of birth. Each offspring was housed individually in a 1·5 l container and fed either a high or low bulk food levels (two offspring from each brood were assigned to each treatment). Both treatments began with an *ad libitum* diet for the first 7 days. Then the high food group was fed 0·0027 g of flake food and 4 µl of brine shrimp a day (roughly 90% of what one *P. reticulata* would eat *ad libitum*), whereas the low food group was fed 0·0015 g of flake food and 2 µl of brine shrimp a day (roughly 50% of what one *P. reticulata* would eat *ad libitum*) (Hughes *et al*., 2005). Brine shrimp were kept at a density of approximately one tablespoon of eggs per litre of water. Offspring were photographed a second time on day 25 to re-measure length and area. These measurements were used to determine the growth rate of each offspring in each brood (by subtracting the day 1 values and dividing by 24). Temperatures of the tanks were taken each day to ensure uniform conditions.

Following the growth trials, the offspring were infected with *Gyrodactylus* flukes. Each test fish was infected with two individual flukes taken from a stock of *P. reticulata* by contacting an infected caudal fin of the donor fish with the caudal fin of the test fish and waiting for two flukes to transfer. Both the donor and focal fish were lightly anaesthetized and examined under a compound microscope throughout the transfer. The time to transfer the parasites was recorded (mean = 6·0 min, range = 1−18 min), but this time had no effect on the average number of *Gyrodactylus* infecting offspring (spearman's correlation, $n = 18$, $P > 0{\cdot}05$) and was therefore removed from the final statistical models. Offspring were then housed separately for 10 days to avoid cross infection and fed *ad libitum*. Each day during the infection trial, the number of individual flukes on the lateral sides of the host *P. reticulata* was counted by lightly anaesthetizing each fish and examining it under a compound microscope (methods adapted from Houde & Torio, 1992; Hedrick *et al.,* 2001; van Oosterhout *et al.,* 2003). Mortality before the end of the trials was noted.

MHC SEQUENCING

Adults were sequenced at the MHC class IIB exon. Eight offspring from one brood (#11) were genotyped to confirm the independent inheritance of alleles. The IIB exon codes for the pathogen-specific binding domain of the MHC class II gene (Klein, 1990). Two unlinked MHC class II loci appear to exist in guppies (McConnell *et al*., 1998; van Oosterhout *et al.,* 2006). Thus, an individual guppy could have up to four different MHC class II alleles. A combination of single-strand conformation polymorphism (SSCP; Amersham Biosciences www.gelifesciences.com), cloning and sequencing were used to identify alleles. First, DNA was extracted using a kit (Sigma-Aldrich; www.sigmaaldrich.com). The 230 bp exon was then amplified with primers published in van Oosterhout *et al*. (2006) using the following polymerase chain reaction (PCR) mixture: 1 × PCR buffer, 2·5 mM $MgCl_2$, 0·25 mM each dNTP, 1 μM of both the sense and antisense primers, and 0·5 μM *Taq* DNA polymerase in a total volume of 50 μl. The PCR conditions were: 94° C for 1 min, followed by 35 cycles for 30 s at 92° C, 30 s at 57° C, 30 s at 72° C and a final extension for 7 min at 72° C. To clone the gene, the PCR fragment was inserted into either a TOPO TA (Invitrogen; www.invitrogen.com) or a pJET/blunt (Fermentas; www.fermentas.com) vector, following the manufacturer's instructions and the vector was transformed into DH5α competent bacteria and grown on lysogeny broth (LB) agar plates with ampicillin. Up to 10 clones were sequenced in individual with a unique SSCP banding pattern and a total of 411 sequences were analysed. A subset of individuals ($n = 24$) were evaluated a second time with the internal primers and genotypes were compared. Chromatograms were read in BioEdit Sequence Alignment Editor 7.0.5.3 (Ibis Biosciences, Carlsbad, CA) and sequences were aligned using MEGA 3.1 (Kumar *et al.,* 2004). Alleles were identified, following Lukas and Vigilant (2005), as those that occurred more than twice in independent PCR reactions; this approach was used to avoid overestimation of diversity due to *Taq* polymerase error, sequencing errors and heteroduplex mismatching.

STATISTICAL ANALYSIS

First, surrogate measures of fitness MHC homozygote and heterozygote families were compared using a Student's t-test. The relationship between allele number and surrogate measures of fitness were evaluated with a Pearson's correlation.

An algorithm adapted from Pitcher & Neff (2006) was used to examine the effects of specific alleles and genotypes. This algorithm uses inferred offspring genotypes from parental genotypes (Table I) and relates offspring fitness measures to both additive and non-additive genetic effects by using the general linear model: offspring fitness measure $= \mu + \Sigma(a_i \times A_i) + \Sigma(n_{ij} \times N_{ij})$, where μ is the mean of the offspring fitness measure, a_i is the additive coefficient for allele i, A_i is the expected number of copies of allele i in the offspring (ranging from 0·5 to 4), n_{ij} is the non-additive coefficient associated with alleles i and j, and N_{ij} was coded as 1, if the offspring was expected to have a number greater than zero of both alleles i and j, or as 0 otherwise. The first summation is over all alleles and the second is over all combinations of alleles where $i \neq j$. Three-way non-additive effects were not considered. Significance of each coefficient was assessed using a randomization routine. The routine randomizes the offspring fitness measures based on families and then re-calculates values of a_i and n_{ij}. The routine is repeated for 1000 iterations and the resulting distributions are used to determine significance (see Pitcher & Neff, 2006).

Three measures of offspring fitness, comprising growth rate, parasite load and survivorship, were used. Growth rate was measured as the change in area over a 25 day period standardized for food treatment. Area was standardized within a food treatment by dividing each value by the mean value for that treatment. L_S was also initially considered but it was highly correlated with the area measure (Spearman's correlation, $n = 18$, $P < 0{\cdot}001$) and so only area measurements were included in the analysis. The average number of flukes present on a individual across the 10 day trial was used to determine the effects of MHC on parasite load. Three offspring from the analysis were omitted because the flukes did not establish on the *P. reticulata* and thus assumed that the initial transfer was unsuccessful. To determine the effects of MHC on survivorship, the number of days an individual survived throughout the growth and parasite trials was used. Data from all three measures were $\log_{10}(n + 1) + 1$

TABLE I. MHC genotypes for adult *Poecilia reticulata* used in the offspring fitness trials. Data comprise brood ID, brood size (number), dam MHC genotype, sire MHC genotype, average growth rate (mean ± S.E.; mm^2 day^{-1} lateral area) provided for each diet [the high food intake (first line) and low food intake (second line)], average parasite load (mean ± S.E.; number of *Gyrodactylus* flukes over 10 day trial) and average survivorship (mean ± S.E.; number of days survival throughout growth and parasite trials)

Brood	Brood size	Dam genotype	Sire genotype	Growth rate	Parasite load	Survivorship
1	17	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	0·33 0·26	6·7 ± 2·5	22·8 ± 8·1
2	12	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	0·49 ± 0·06 0·41 ± 0·04	3·7 ± 1·3	35·3 ± 0·5
3	14	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	0·51 ± 0·05 0·38 ± 0·02	4·0 ± 1·6	32·0 ± 1·4
4	3	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	0·42 0·27	2·00	19·0 ± 8·0
5	11	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	0·62 ± 0·06 0·43 ± 0·08	3·3 ± 2·1	36·0 ± 0·0
6	1	*Pore_a132, Pore_a132, Pore_iii128, Pore_iii128*	*Pore_a132,Pore_a132, Pore_a132· Pore_fmc*	0·62	9·2	32·0
7	15	*Pore_gmc, Pore_gmc, Pore_gmc, Pore_gmc*	*Pore_gmc, Pore_gmc, Pore_gmc, Pore_gmc*	0·67 ± 0·03 0·58 ± 0·16	13·6 ± 5·2	34·3 ± 0·6
8	1	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	*Pore_a132, Pore_a132, Pore_g190, Pore_p*	0·52	10·4	35·0
9	9	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	0·47	9·0	11·5 ± 5·9

TABLE I. Continued

Brood	Brood size	Dam genotype	Sire genotype	Growth rate	Parasite load	Survivorship
10	1	*Pore_a132, Pore_a132, Pore_iii128, Pore_iii128*	*Pore_a132, Pore_a132, Pore_iii128, Pore_iii128*	0·70	1·71	33·0
11	11	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_emc*	*Pore_dmc, Pore_dmc, Pore_dmc, Pore_dmc*	0·49 ± 0·09 0·41 ± 0·01	7·3 ± 3·4	35·5 ± 0·5
12	16	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	0·49 ± 0·09 0·39 ± 0·02	7·9 ± 5·3	34·3 ± 1·2
13	15	*Pore_b76, Pore_b76, Pore_iii128, Pore_iii128*	*Pore_b76, Pore_b76, Pore_iii128, Pore_iii128*	0·49 ± 0·02 0·34 ± 0·05	4·0 ± 0·7	31·5 ± 1·7
14	5	*Pore_b76, Pore_b76, Pore_iii128, Pore_iii128*	*Pore_b76, Pore_b76, Pore_iii128, Pore_imc*	0·56 0·54 ± 0·05	2·7 ± 1·4	25·3 ± 7·4
15	6	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	0·52 ± 0·03 0·39 ± 0·08	1·6 ± 0·5	33·8 ± 2·3
16	15	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	0·48 ± 0·07 0·43 ± 0·05	3·5 ± 1·7	33·3 ± 1·7
17	9	*Pore_dmc, Pore_dmc, Pore_dmc, Pore_dmc,*	*Pore_iii128, Pore_iii128, Pore_iii128, Pore_iii128*	0·57 ± 0·11 0·52 ± 0·06	1·8 ± 0·4	35·0 ± 0·7
18	4	*Pore_a132, Pore_a132, Pore_a132, Pore_a132*	*Pore_b76, Pore_b76, Pore_b76, Pore_b76*	0·53	2·4	13·7 ± 11·2

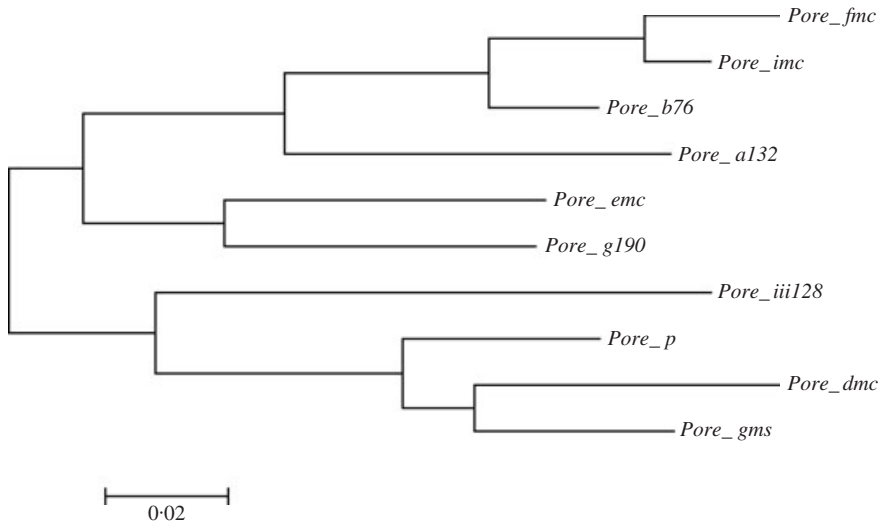

FIG. 1. Neighbour joining tree of the 10 MHC class IIB alleles found in *Poecilia reticulata*. The tree is based on nucleotide distances using the Jukes-Cantor substitution model.

transformed before analysis. Means, plus or minus one standard error, are reported for all juvenile *P. reticulata*.

RESULTS

A total of 10 MHC class IIB alleles were found (Fig. 1; supporting information Table SI). All 10 alleles were variable in the amino acid sequences and contained no insertions, deletions or stop codons, which suggests that the fragments were not part of a pseudogene. Individual *P. reticulata* had a range of one to three different alleles. Thus, these results confirm previous reports of a single duplication event (van Oosterhout *et al.,* 2006). Three alleles were found to dominate, *Pore_a132* with a frequency of 14%, *Pore_b76* with a frequency of 15% and *Pore_iii128* with a frequency of 52%; other alleles had a frequency $<7\%$. Therefore, these three alleles were analysed and the other alleles were binned into a 'rare' category. The binning of rare alleles maximizes statistical power by reducing the number of combinations of alleles (many of which were not observed in the dataset) that must be considered by the fitness algorithm (also see Pitcher & Neff, 2006). The 24 individuals that were analysed with the internal primers showed the same genotypes as the original analysis with the published primers. The genotypes of the offspring in brood 11 contained all three alleles present in their parents. Although the presence or absence of linkage among the alleles could not be assessed statistically due to the small brood sizes in this study population, these results support the previous finding of two unlinked MHC class II loci (McConnell *et al*., 1998; van Oosterhout *et al.,* 2006).

OFFSPRING FITNESS AND MHC

For the offspring measurements, 18 broods (mean brood size $\pm$ S.E. $= 9{\cdot}2 \pm 1{\cdot}3$) were analysed (Table I). Across all the broods, the growth rate average was $0{\cdot}47 \pm$

0·02 mm^2 day^{-1}(lateral area), the mean parasite load was 5·3 ± 0·8 and survivorship was 30 ± 2 days. Based on a correlation analysis, no effect of dam L_S, gestation time, female receptivity, brood size, offspring size at day 1, sire sigmoid frequency, sire relative area of orange pigmentation or sire L_S was found on any of the three measures of offspring fitness averaged across families ($P > 0·05$ for all tests).

The first analysis compared MHC heterozygote and homozygote families. Growth rate was significantly faster in heterozygote families (1·29 ± 0·1 mm^2 day^{-1}) than in homozygote families (0·89 ± 0·04 mm^2 day^{-1}) ($P < 0·05$). Parasite load did not differ significantly between heterozygote families (5·9 ± 1·4) and homozygote families ($n = 4·6 \pm 0·9$) ($P > 0·05$). Also, survivorship did not differ significantly between heterozygote families (30·6 ± 2·4 days) and homozygote families (28·6 ± 2·9 days) ($P > 0·05$). Allele number was not related to growth rate, parasite load or survivorship ($P > 0·05$ for all).

The effects of specific alleles or genotypes were evaluated. Over the 24 day growth trial, individuals in the high food treatment grew faster than those in the low food treatment (high food: 0·52 ± 0·09 mm^2 day^{-1}, low food: 0·42 ± 0·11 mm^2 day^{-1}; t-test, d.f. = 12, $P < 0·001$). In the combined standardized growth dataset, the model revealed a positive additive effect of *Pore_a132* on growth rate that was marginally non-significant (Table II). Fish with at least one copy of the *Pore_a132* grew faster (0·59 ± 0·042 mm^2 day^{-1}) than those without a copy of the *Pore_a132* (0·47 ± 0·02 mm^2 day^{-1}). A significant positive effect of rare alleles was also found on growth rate. Fish with the rare allele grew faster (0·54 ± 0·03 mm^2 day^{-1}) than those without a rare allele (0·44 ± 0·02 mm^2 day^{-1}). Also, a significant negative additive effect of *Pore_b76* was found on growth rate; fish with the *Pore_b76* grew slower (0·43 ± 0·04 mm^2 day^{-1}) than those without the *Pore_b76* allele (0·51 ± 0·02 mm^2 day^{-1}).

Fish with genotypes combining the *Pore_b76* allele and a rare allele also showed a significant positive non-additive effect on growth (Table II). These fish grew at more rapidly on average 0·54 ± 0·03 mm^2 day^{-1} than did fish with a *Pore_b76* allele, but no rare allele (0·39 ± 0·04 mm^2 day^{-1}) and fish with neither a rare nor a *Pore_b76* allele (0·47 ± 0·03 mm^2 day^{-1}). Some caution is warranted when interpreting these comparisons as the genotype consisting of the rare and *Pore_b76* alleles was found in only one brood. No other non-additive effects of MHC were found on growth rate (Table II). Qualitatively, similar results were found when data from the high and low food intake treatments were analysed separately (Table SII).

The MHC genotype also had an effect on parasite load. Across the 10 day parasite challenge trial, a negative additive effect of the *Pore_a132* on parasite number was found (*i.e.* the allele was associated with a decrease in parasite number and was thus beneficial) but also a strong positive non-additive effect of this allele when coupled with a rare allele was found. A significant positive additive effect of the rare allele on parasite number was also found (Table II). Consequently, in the dataset, fish with at least one copy of *Pore_a132* had more parasites ($n = 5·9 \pm 2·3$) than those without a copy of the *Pore_a132* (5·3 ± 1·0) because the *Pore_a132* allele was often paired with a rare allele. Individuals with the *Pore_a132* and rare genotype had an average of 9·8 ± 0·6 parasites, which was much higher than those with a copy of *Pore_a132* and no rare allele (2·1 ± 0·3). The number of parasites was also higher than in individuals with a rare allele and no *Pore_a132* (6·8 ± 1·8) and those with neither a *Pore_a132* nor a rare allele (4·8 ± 1·3). The rare allele had a significant positive

TABLE II. Additive and non-additive genetic effects of MHC class IIB alleles on growth rate, parasite load and days survived in juvenile *Poecilia reticulata*. The results include the r^2 and constant for an algorithm that partitions the variation in performance to additive and non-additive genetic effects. For each additive and non-additive effect of three alleles and a rare allele category, a coefficient value is given. Significant *P* values are in bold

	Growth rate		Parasite load		Survivorship	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
r^2	0·51		0·27		0·22	
Constant	0·30		0·64		1·41	
Additive effects						
Pore_a132	0·014	>0·05	−0·120	**<0·05**	−0·0057	0·48
Pore_b76	−0·006	**<0·01**	0·004	>0·05	0·0002	0·47
Pore_iii128	−0·003	>0·05	−0·012	>0·05	−0·0060	0·33
Rare	0·014	**<0·05**	0·110	**<0·01**	0·0320	0·18
Non-additive effects						
Pore_a132 and *Pore_b76*	0·027	>0·05	0·120	>0·05	−0·520	0·11
Pore_a132 and *Pore_iii128*	−0·046	>0·05	0·065	>0·05	0·140	0·41
Pore_a132 and rare	−0·041	>0·05	0·710	**<0·05**	−0·100	0·38
Pore_b76 and *Pore_iii128*	−0·021	>0·05	0·020	>0·05	0·110	0·38
Pore_b76 and rare	0·088	**<0·05**	0·044	>0·05	−0·310	0·13
Pore_iii128 and rare	−0·016	>0·05	−0·290	**<0·05**	0·073	0·33

N.B. Growth rate is based on standardized values and coefficients and constants for fitness measures are from $\log_{10}(n + 1)$-transformed data (see text).

additive effect on parasite load. Fish with a rare allele had more parasites (6·6 ± 1·6) than those without a rare allele (4·5 ± 1·2). A significant negative non-additive effect of *Pore_iii128* and rare allele was found on parasite number. Fish with a *Pore_iii128* and a rare allele had fewer parasites (4·93 ± 1·3) than those with a rare allele without a *Pore_iii128* (13·6 ± 5·2) but more parasites than those with *Pore_iii128* and no rare allele (4·4 ± 1·3) or neither a *Pore_iii128* or a rare allele (3·5 ± 2·0).

The overall survivorship in broods was also assessed. Across the entire 35 days of trials, no significant additive or non-additive effect of any MHC allele on survivorship was found (Table II). Similar results were found when survivorship during the growth and parasite trials was analysed separately (Table SIII).

DISCUSSION

No effect of MHC class IIB on parasite load or survivorship was found in comparisons of heterozygous and homozygous fish. A significant effect of MHC heterozygosity on growth rate was found, indicating a possible overdominance effect of MHC on general health. However, more complex relationships of MHC on surrogate measures of fitness were found by examining the effects of specific alleles and genotypes. The number of copies of the *Pore_a132* MHC allele was related to infection by fewer gyrodactylus flukes and possibly to faster growth within the first month of life. A negative effect of the *Pore_b76* during the growth trials was also found, but

no additive effect of this allele was found during the parasite trials. Rare alleles had a positive effect on growth rate but a detrimental, negative effect on parasite resistance. The difference in additive effects of rare alleles on growth *v*. parasite resistance might indicate antagonistic effects of a specific allele (*e.g.* Loiseau *et al.,* 2008) or a trade-off in fitness investment (Lochmiller & Deerenberg, 2000). Rare alleles were grouped to increase statistical power in the model and therefore a more detailed analysis of the fitness effect of each rare allele could not be provided. Rare alleles also showed a non-additive effect when present with a *Pore_a132* allele, as individuals with those genotypes performed worse in the parasite trials. However, individuals did better in the parasite trials, when a rare allele was present with a *Pore_iii128* allele. Growth rate and resistance to *Gyrodactylus* are key determinates of survivorship and reproductive success, and fitness early in life is an important indicator of lifetime fitness in *P. reticulata* (Reznick & Endler, 1982; Scott & Anderson, 1984; Houde & Torio, 1992; Reynolds & Gross, 1992). Thus the additive and non-additive effects of the MHC detected here should have a significant effect on individual fitness, and these results suggest a complex relationship between MHC genotype and fitness in *P. reticulata*.

Maternal effects are unlikely to explain these MHC and performance results. Maternal effects can inflate effects of genetic benefits through preferential allocation to fitter offspring (the so-called differential allocation hypothesis; Burley, 1988; also see Sheldon, 2000). Empirical data from the scarab beetle *Onthophagus taurus* show that females can indeed allocate more resources to genetically superior offspring (Kotiaho *et al*., 2003). However, *P. reticulata* is ovoviviparious, provisioning its eggs prior to fertilization, although there is some evidence that there is a small transfer of nutrients between mother and offspring during gestation (DeMarais & Oldis, 2005; Martyn *et al.,* 2006). Attempts were made to quantify maternal effects in *P. reticulata* by measuring gestation time, brood size, offspring size at day 1 and female L_S, but no correlation was found between these and any of the surrogate measures of offspring fitness. This result suggests that females did not affect offspring fitness during gestation by allotting more energy to favourable broods.

The MHC confers resistance to specific pathogens through recognition of pathogen-derived peptides at the variable peptide-binding region. Consistent with this mechanism, several studies have found relationships between parasite resistance and specific MHC alleles. For example, resistance to *Aeromonas salmonicida* in *S. salar* is associated with a specific MHC allele (Lohm *et al*., 2002). Similarly, in the present study, the *Pore_a132* allele was related to reduced parasite load and possibly faster growth rates in the guppy. One other study has investigated MHC genotypes and *Gyrodactylus* infection in *P. reticulata*, but the study did not compare specific alleles and genotypes with infection intensity (van Oosterhout *et al*., 2006). Instead, the authors argued that parasite community may be contributing to the maintenance of MHC diversity, particularly in small populations (van Oosterhout *et al*., 2006). The results of challenge experiments in other studies of *P. reticulata* show that resistance to *Gyrodactylus* has a genetic basis (*e.g.* Madhavi & Anderson, 1985; van Oosterhout *et al.,* 2003). Taken together, these results suggest that the MHC class IIB gene contributes to parasite resistance in *P. reticulata*.

Diversity at the MHC is thought to be maintained through either overdominance or frequency-dependent selection (Bernatchez & Landry, 2003). The results of the present study indicated additive and non-additive effects of specific alleles

and combinations of alleles on surrogate measures of fitness. These results support both the frequency-dependent selection and the overdominance hypotheses. Similarly, Pitcher & Neff (2006) found additive and non-additive effects at the MHC on survivorship in *O. tshawytscha*. Specific non-additive effects were detected in both studies because the model used assessed the effect of individual genotypes and thereby accounted for the inequality of different heterozygous genotypes. For example, heterozygous genotypes consisting of dissimilar alleles may confer greater resistance to pathogens because the genotype is better able to recognize a diverse array of pathogens than a homozygous genotype (Bernatchez & Landry, 2003). Therefore, alleles in heterozygotes may be well matched, as appears to be the case in our study for the *Pore_iii128* and rare alleles, or mismatched, as for the *Pore_a132* and rare alleles, in how well they combat a broad range of pathogens. Consistent with this idea, mean amino acid divergence between *Pore_a132* and the rare alleles was lower than the average divergence across all (14·8 *v.* 18·4 amino acids), whereas the divergences between *Pore_iii128* and rare alleles were greater on average (19·8 amino acids). As in the present study, researchers will be able to gain a better understanding of how selection acts on MHC diversity in natural populations by examining the effect of specific alleles and genotypes.

In addition to direct resistance conferred by MHC genotypes, MHC genotypes may also have an indirect effect on resistance. An effect of MHC genotype not only on offspring resistance to *Gyrodactylus* during a challenge trial but also on growth rate prior to the challenge experiment was found. Both detrimental and advantageous non-additive effects of MHC genotype on both growth and parasite resistance were also found. It seems unlikely that MHC peptides interfere with the recognition of peptides derived from a single pathogen (*i.e.* the *Gyrodactylus*), but instead alleles may be mismatched and unable to recognize a broad suite of pathogens, and therefore decrease condition overall. A second model of MHC-associated fitness may be relevant, in which different MHC genotypes improve body condition by conferring resistance to a suite of pathogens. The improved body condition then confers augments resistance to specific pathogens. Thus, the MHC may also play an indirect role in disease resistance through body condition. Support for this model comes from studies that show MHC-dependent effects on condition (Ottová *et al.,* 2007) and condition-dependent traits (von Schantz *et al*., 1996; Ditchkoff *et al.,* 2001). Conceivably, individuals with disadvantageous combinations of MHC alleles may resort to costly, innate immune defences to combat pathogens and results in increased oxidative stress and poorer condition (Kurtz *et al.,* 2006). Individuals in poorer condition are then more susceptible to pathogens (*e.g.* Møller *et al.,* 1998; Coltman *et al.,* 2001). Although body condition was not directly assessed in the present study, MHC genotype was related to growth rates, suggesting that MHC may be related to condition. This result, coupled with the additive effect of the *Pore_a132* allele, indicates that the MHC may have both direct and indirect effects on fitness in *P. reticulata*.

In summary, MHC genotypes influenced fitness in *P. reticulata* by affecting both parasite resistance and growth rate in *P. reticulata*. The genetic architecture is complex with both additive and non-additive effects, and these results highlight the importance of a careful evaluation of specific alleles and genotypes, in addition to considering MHC heterozygosity *v.* homozygosity.

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Table SI. Summary of amino acid sequences of 10 MHC class IIB alleles in the guppy (*Poecilia reticulata*)

Table SII. Additive and non-additive genetic effects of MHC class IIB alleles on growth with a high and low food intake in juvenile guppies (*Poecilia reticulata*). The results include the r^2 and constant for an algorithm that partitions the variation in performance to additive and non-additive genetic effects. For each additive and non-additive effect of three alleles and a rare allele category, a coefficient value and P value are given

Table SIII. Additive and non-additive genetic effects of MHC class IIB alleles on survivorship during a growth trial and parasite infection trial in juvenile guppies (*Poecilia reticulata*). The results include the r^2 and constant for an algorithm that partitions the variation in performance to additive and non-additive genetic effects. For each additive and non-additive effect of three alleles and a rare allele category, a coefficient value and P value are given

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