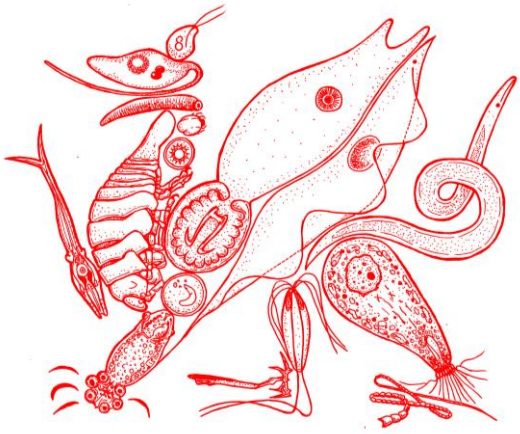

The impact of Major Histocompatibility Complex composition on fitness and life history traits of a vertebrate model, the guppy (*Poecilia reticulata*)

Willow Smallbone

September 2017

A thesis submitted to Cardiff University for the partial fulfillment of the requirements for the degree of Doctor of Philosophy in the group
Cardiff Research into Infection and Parasites in Ecological Systems, School of Biosciences,
Cardiff University

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This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisors Prof. Jo Cable and Prof. Cock van Oosterhout for their support throughout my Ph.D. Particularly 'Grandma Jo', who has put up with me for the last 5 years, helped me with paper submissions, thesis preparation and lots of laughs.

Huge thanks go to Dr 'Auntie' Amy Ellison, without whom I think I would still be stuck in the genetics lab trying to work out how to do a PCR and create the appropriate gel bands, all of your guidance and help really is appreciated. The help that Dr Karl Phillips has given me when it came to MHC supertyping and confirming that what I had done was correct has been great (and the videos you sent to Jo always lightened the mood). Thanks to Hefin for all of the culinary delights and guidance.

All of the Ph.D. students across the 5^{th}, 6^{th} and 7^{th} floors have been a great support, especially Fred, Ifan, Rhidain, Emma and Jade for great discussions and lots of lunches. Huge thanks goes to Mike Reynolds, I think this process would have been a hell of a lot tougher without you there to bounce ideas with and have boost / coffee time. The undergraduate students that have helped me in the laboratory over the last few years have been great and taught me alot. Thanks to Liam Maggs, Dayna Lea, Owen Wright, Karan Gupta and Dean Tulett for your help. Big thanks to Linda for her laboratory knowledge and assistance, we were sad to see you leave.

Finally, my family (especially mum and dad), without whom I would never have go this far and although I'm sure you often wondered why my life revolved around some little fish continued to support me. Massive thanks to Don - for having to deal with the consequences of my hands and arms being in water for vast amounts of time each day. He knows what a support he has been through the ups and downs over the last years and also knows how much I hate PDA.

This research project was funded by the Leverhulme Trust (RPG-2013-305), approved by Cardiff Ethical Committee and conducted under UK Home Office License (PPL 302876).

LIST OF PUBLICATIONS

Reynolds, M*., Smallbone, W.* and Cable, J. *Submitted.* Assessing the effects of natural parasitism on the swimming performance of two coexisting poeciliid hosts. *Journal of Fish Biology.*

Smallbone, W., Cable, J. and Maceda-Veiga, A. 2016a. Chronic nitrate enrichment decreases severity and induces protection against an infectious disease. *Environment International*. **91**, 265-270.

Smallbone, W.A., Chadwick, E.A., Francis, J., Guy, E., Perkins, S.E., Sherrard-Smith, E. and Cable, J., 2017. East-West Divide: temperature and land cover drive spatial variation of *Toxoplasma gondii* infection in Eurasian otters (*Lutra lutra*) from England and Wales. *Parasitology*. **144**, 1433-1440.

Smallbone, W., van Oosterhout, C. and Cable, J. 2016b. The effects of inbreeding on disease susceptibility: *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*. *Experimental Parasitology.* **167**, 32-37.

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THESIS SUMMARY

The Major Histocompatibility Complex (MHC) is a multi-gene family that includes most vertebrate immune genes. Life history traits have been associated with MHC allelic variation, including offspring survival, reproductive success, kin recognition, inbreeding avoidance, body mass gain, mate choice and parasite resistance. The studies reported in this thesis used laboratory and field investigations to identify differences in MHC genetic variation between truly wild, wild type and domesticated conspecifics and the implications of this for fitness, across the entire life history of a vertebrate, the guppy (*Poecilia reticulata*). Specifically, the effects of host inbreeding and domestication on parasite susceptibility are assessed in relation to MHC allelic and supertype composition.

Laboratory studies showed that inbreeding and domestication lead to increased susceptibility to *Gyrodactylus turnbulli*, which was also linked to the presence of particular functional groups of MHC. A multi-site field sampling supported this finding; revealing that natural parasite communities reflected host MHC functional groups, as well as the river of origin. Truly wild fish had greater MHC genetic diversity than wild type (wild population maintained in the laboratory for ~ 3 years), which, in turn, were more genetically diverse than ornamental (domesticated) conspecifics. The accidental and deliberate release, into the wild, of domesticated fish is common. The release of infected and uninfected ornamental guppies into a wild type laboratory population increased parasite prevalence and abundance, due to the integration of a more susceptible individual into the social group.

Mate preference is often linked to MHC similarity, whereby individuals select mates that are dissimilar or optimally similar at the MHC. The effects of sexual selection, MHC similarity and parasitism on mate choice, were assessed, indicating that a combination of factors are important in a female's preference. Female guppies spent more time interacting with males with redder colouration and less MHC alleles in common. An experimental F1 generation revealed that offspring with parents sharing more MHC alleles and supertypes were more susceptible to parasitic infection. This research suggests that MHC functionality is at least as important as allelic and supertype diversity, with regards to individual fitness and life history traits.

CHAPTER 1

General introduction

1.1. MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

The Major Histocompatibility Complex (MHC) is a multi-gene family that includes most vertebrate immune genes. It is a complex of cell surface proteins, with an important role in the immune system of jawed vertebrates (Cooper and Alder 2006), identifying foreign substances and pathogens and initiating the immune system (Hedrick 2002). The MHC forms part of the adaptive immune response; the second line of defence against infection, initiated following the innate immune response. The high allelic variation and genetic diversity of MHC is associated with disease resistance, sexual attraction, mate choice and heritable disorders. Genetic diversity at the MHC plays an important role in disease resistance to pathogens in vertebrates (Hedrick et al. 2000; Milinksi 2014). MHC polymorphism is maintained through sexual selection and the selective pressures exerted by parasitism (Doherty and Zinkernagel 1975; Hedrick 1998; Piertney and Oliver 2006). Amino acid polymorphisms at the peptide-binding region are the basis for the functional classification of MHC alleles into supertypes.

1.1.1. MHC class I and II

MHC molecules present 'self' and 'non-self' peptides to T-cells, which trigger immune reactions in response to foreign peptides (Klein and Figueroa 1986). Leucocytes differentiate and distinguish between healthy and infected cells, as well as identifying the pathogens, using MHC class I. MHC class II proteins, on the surface membrane of certain types of immune cells, aid inter-leucocyte communication. Upon pathogen infection, an antigen is presented on the MHC class II peptide-binding region, leading to a cascade of events, which culminate in the destruction of the pathogenic cells and production of antibodies.

The innate immune system is initiated first when an infection takes place and a pathogenic cell enters the body (see Fig. 1.1 for overview). Mass cells and basophils

release chemicals, such as histamine, to dilate blood vessels and transport phagocytic macrophages, containing digestive lysosomes, to the site of infection. When a macrophage (containing digestive enzymes) finds a cell presenting a pathogenic antigen, it engulfs the pathogen to form a phagosome vacuole (Fig. 1.2a). Lysosomes fuse with the phagosome and initiate the break down the pathogenic cell. As it is broken down, some of the pathogenic proteins (antigens) are placed onto the MHC class II proteins (Fig. 1.2b).

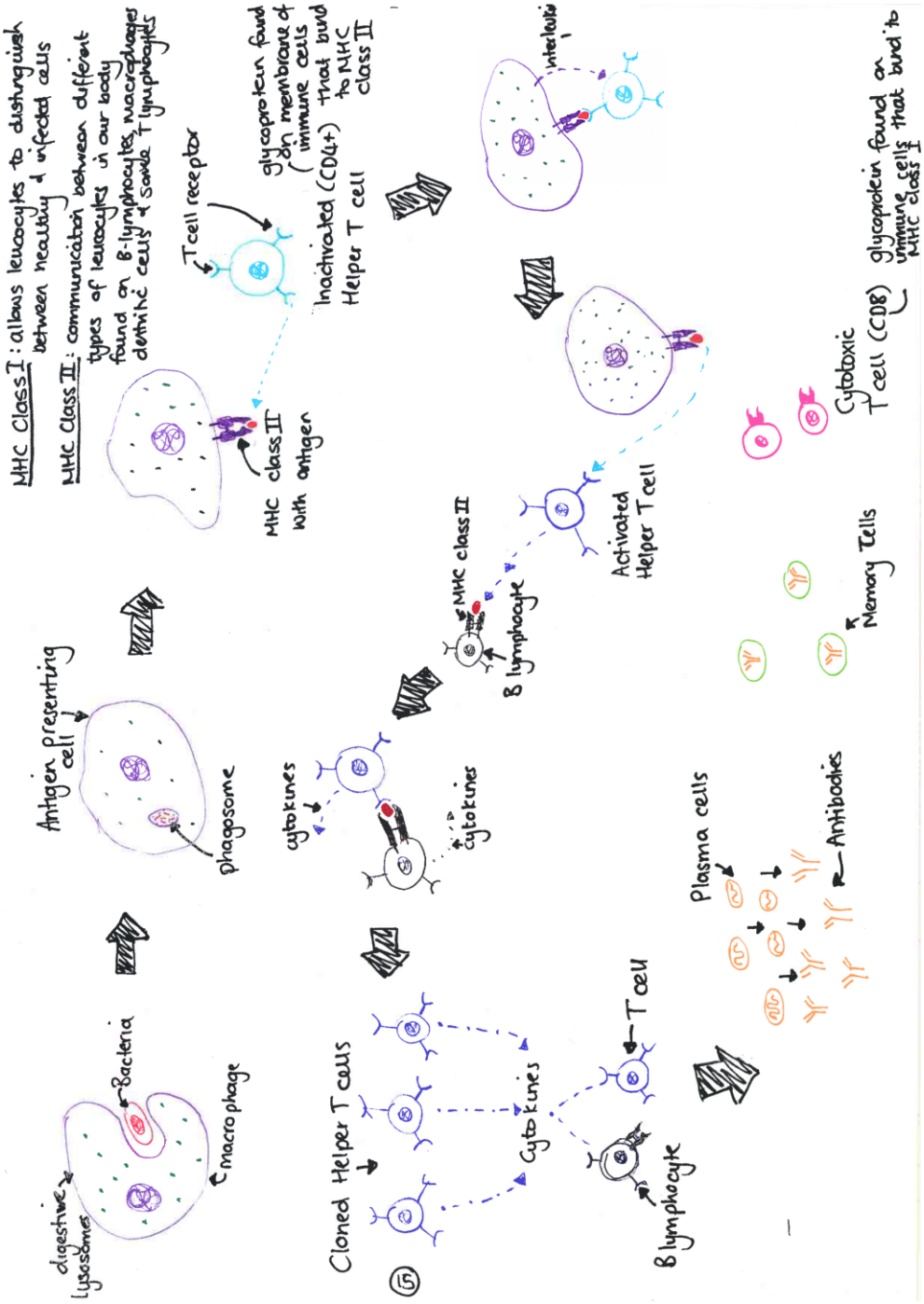

Figure 1.1: Overview of the immune system, illustrating the role of the Major Histocompatibility Complex (MHC).

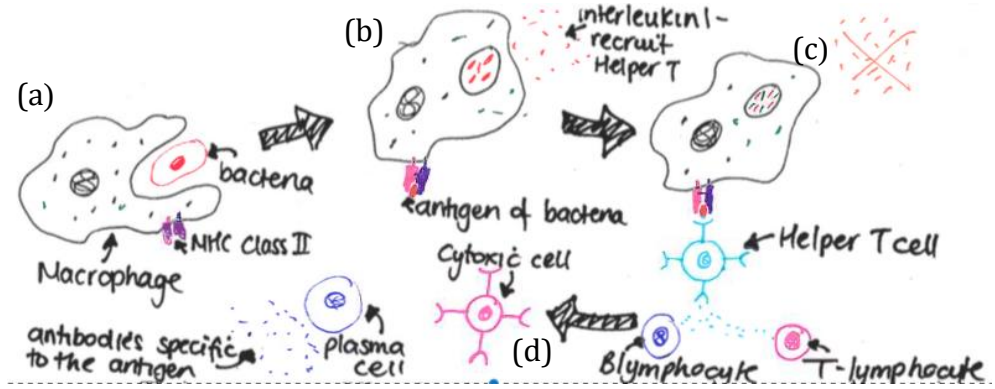

Figure 1.2: Initiation of immune response after an initial infection and a pathogenic cell enters the body.

1.1.1.1. MHC class I

Most nucleated cells have MHC class I proteins, which are comprised of four bound polypeptide units (beta2, alpha1, alpha2 and alpha3; Fig. 1.3), with the Alpha 3 connected to the cell membrane. MHC class I allows leucocytes to distinguish between healthy and infected cells.

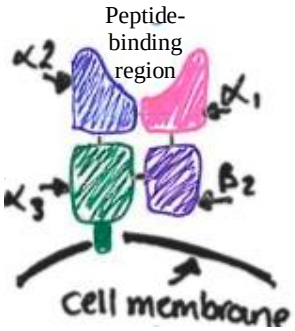

Figure 1.3: Structure of Major Histocompatibility Complex (MHC) class I.

A healthy cell will bind one of its normal 'self' antigens created within the cell onto the clef portion of the MHC class I between alpha 1 and alpha 2 (Fig. 1.4a). An approaching leucocyte will recognise whether the protein that is bound to the MHC class I is a self-antigen (a protein formed by this cell) or not. If the cell is infected with a pathogen, the infected cell will contain pathogen proteins. The cell incorporates one of these pathogen proteins on to the MHC class I protein instead of the self-antigen. The pathogen antigen on the MHC class I will be identified by a cytotoxic T cell, which will bind to it (Fig. 1.4b). This will initiate an immune response that will destroy the infected cell known as cell mediated immunity.

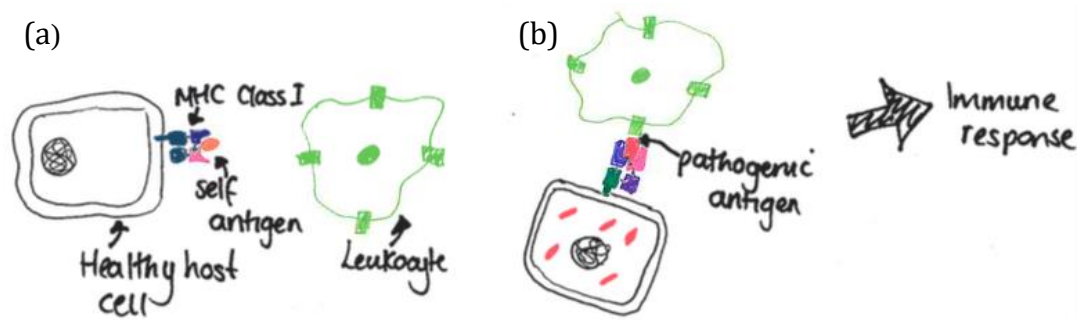

Figure 1.4: The pathway to initiating an immune response from an intracellular infection.

1.1.1.2. MHC class II

MHC class II proteins are found on: B-lymphocytes, macrophages, dendritic cells and some T lymphocytes. They are composed of 4 polypeptide regions, but unlike MHC class I, are made up of homologous pairs (alpha 1 and 2 and Beta 1 and 2; Fig. 1.5), in the case of MHC class II both Alpha 2 and Beta 2 are connected to the cell membrane.

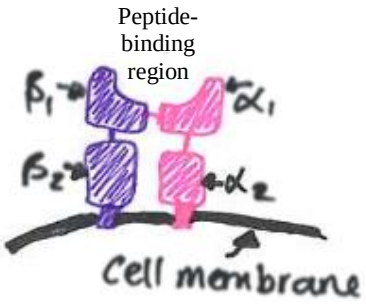

Figure 1.5: Structure of Major Histocompatibility Complex (MHC) class II.

Presentation of a pathogen antigen on the MHC class II protein initiates the release of interleukin 1, which recruits inactivated helper T cells (Fig. 1.2b), enabling inactivated helper T cells (CD4+, a glycoprotein on the membrane of the T cell that means it can bind to MHC class II) to communicate with the macrophage. Upon the arrival of the pathogenic antigen to the infected cell, receptors on the membrane of the inactivated helper T cells recognises the pathogen antigen that is bound to the MHC class II. The inactive helper T cell binds to the infected macrophage eliciting the release of interleukin 2, activating the helper T cell (Fig. 1.2c). Active helper T cells move to find pathogen specific B-lymphocytes and T-lymphocytes (Fig. 1.2c).

The B-lymphocyte and T helper cell bind to make a complex. Subsequently, the two cells release cytokines inducing the Helper T cell to clone itself by mitosis producing multiple cloned helper T cells. These clones release another group of cytokines that induce B-lymphocytes and T cells to mature and differentiate into specialised cells. Differentiation of B-lymphocytes produces memory B cells and plasma cells, which have extensive endoplasmic reticulum to create antibodies (humeral immunity) that are complementary to the pathogen antigens (Fig. 1.2d). T-lymphocytes produce memory T cells, which store the antibodies in the cells enabling an individual host to react to an identical pathogen infection in the future - adaptive immunity (learnt); and cytotoxic (killer) T cells (CD8 glycoprotein), containing specific antibodies that can bind to the specific pathogenic antigen.

1.1.2. MHC supertypes

Many different MHC molecules have similar functional characteristics, which can be grouped together as supertypes (del Guercio et al. 1995). Each MHC molecule within a supertype binds essentially the same peptides (Sette and Sidney 1999; van Oosterhout 2013). Supertypes are based on the molecular binding properties of the positively selected sites (Fig. 1.3) thus; best reflect the functional differences among alleles. They are defined by five methods: 1) structural similarities; 2) primary sequence; 3) tertiary structure; 4) shared peptide binding motifs; and 5) identification of cross-reacting peptides. Most of the MHC polymorphism is located within the peptide-binding region and each variant binds a unique repertoire of peptide ligands. The peptide-binding region is highly adaptive to pathogen recognition and is, therefore, under strong directional selection. Functional differences of alleles are likely to be dependent on variation at the peptide-binding region. Initially, supertypes were defined by manual inspection of the set of peptides each allele binds. The development of bioinformatics allowed supertypes to be statistically characterised, based on inferred shared amino acid functional properties of the MHC genes (Doytchinova et al. 2004; Doytchinova and Flower 2005). Typically, MHC variants are grouped, either by clustering or discriminant principal components analysis, using the properties of amino acids at positions in the peptide binding region that are under strong positive selection and, thus, best reflect the functional differences among alleles (Doytchinova et al. 2004; Doytchinova and Flower 2005).

1.2. MHC AND LIFE HISTORY TRAIT VARIATION

A central question in biology concerns how selection on genetic variation affects fitness traits (Bernatchez and Landry 2003). Several studies of natural populations have shown associations between MHC allele variation and life history traits (Fernandez-de-Mera et al. 2009), including offspring survival (e.g. von Shantz et al. 1997), reproductive success (Kalbe et al., 2009), kin recognition and inbreeding avoidance (e.g. Penn and Potts 1999), body mass gain (Ditchkoff et al. 2001), mate choice (e.g. Yamazaki et al. 1976) and parasite resistance (e.g. Acevedo-Whitehouse et al. 2005). The presence of specific MHC alleles has been linked to mortality (in Soay sheep, Paterson et al. 1998; Chinook salmon, Pitcher and Neff 2007) and life expectancy (Seychelles warbler, Brouwer et al. 2010). Pathogen resistance and susceptibility is a likely link between individual survival and MHC composition. When attempting to link survival to specific functional groups of MHC, some studies have identified such an association (Sepil et al. 2013), whilst others have not (Radwan et al. 2012). Faster individual growth rate is associated with MHC heterozygosity (Fraser and Neff 2009). This supports the concept that increased MHC diversity increases an individual's ability to survive and be successful.

1.2.1. Parasite susceptibility

The higher the number of different MHC alleles an individual has, the broader the spectrum of infectious diseases the individual can fight. Differences in MHC molecules, particularly in the peptide-binding site, lead to variation in the ability of an individual to initiate an immune response against particular infections (Milinski 2014). Certain MHC alleles, or combinations of alleles, result in greater host resistance to parasitism and pathogens (Dunn et al. 2013). High MHC diversity increases host population survival; it is assumed that parasites maintain the high MHC diversity, due to the role of the MHC in the immune response (Parham and Ohta 1996; Dunn et al. 2013). Low genetic diversity in the MHC has been suggested to cause increased susceptibility to infectious disease (e.g. Siddle et al. 2007), which could lead to an "extinction vortex" (Brook et al. 2002), due to increased disease-driven mortality rate.

Traditionally, many evolutionary and parasitological studies have focused on the link

between genetic diversity at the MHC and parasite resistance (Bernatchez and Landry 2003; Piertney and Oliver 2006; Spurgin and Richardson 2010). Changes in MHC allele frequencies have been associated with shifts in parasite fauna, and individuals with certain MHC alleles or genotypes may have a consistently higher (or lower) parasite burden (Westerdahl et al. 2004; Aguilar and Garza 2006; Hess 2007; Eizaguirre et al. 2009; Oliver et al. 2009; Fraser and Neff 2010; Eizaguirre et al. 2012a; Eizaguirre et al. 2012b). Few studies have, however, focused on the function of MHC alleles (supertype) and the association of functionally similar MHC alleles with parasite intensity (exceptions include: Schwensow et al. 2007; Fraser and Neff 2010; Pilosof et al. 2014) and variation between wild and domesticated stocks.

1.2.2. Mate choice

Sexual selection also plays a role in maintaining MHC polymorphism (Wedekind et al. 1995; Millinski 2006; Chaix et al. 2008). In humans, mice and fish, mate choice is partly based on odour cues associated with the MHC (Millinski et al. 2005). Hamilton and Zuk (1982) suggested that females select males based on genetic resistance, to produce fit offspring with greater resistance against parasites and pathogens. The first mate choice experiment involving inbred individuals used mice; the choosing (male) individual selected a mate with differing MHC alleles to itself (Yamazaki et al. 1976). This result has been replicated in many different species, where offspring fitness was increased with mating that increased MHC diversity (including Reusch et al. 2001; Aeschlimann et al. 2003; Milinski et al. 2005; Milinski 2006; Agbali et al. 2010; Ejsmond et al. 2014, exceptions: Pitcher and Neff 2007). Potential mate attractiveness is thought to be related to odour, associated with MHC-related sensory discrimination (Yamazaki et al. 1979). When given the choice, a female chooses mates with a dissimilar odour (and, therefore, MHC alleles) to herself, increasing allelic diversity in their offspring (Milinski 2006; Eizaguirre et al., 2009). This mechanism may aid inbreeding avoidance and kin recognition (Potts and Wakeland 1990; Penn 2002).

It has been suggested that females increase individual heterozygosity and population-wide polymorphism at the MHC loci by choosing males with many different MHC alleles, and selecting for MHC genotype dissimilarity (Reusch et al. 2001; Wegner et al. 2004). In contrast, the optimum MHC diversity theory suggests that an optimal

level of MHC dissimilarity between mates is important in resistance to local parasitism (Eizaguirre and Lenz 2010). An excessively high MHC diversity is predicted to deplete the T-cell repertoire and impair the immune response of individuals (Novak et al. 1994). The optimal level of individual MHC diversity ensures that individual lifetime reproductive success is maximised (Kalbe et al. 2009). Many other factors, including colour, parasitism and size, have been shown to be important in mate selection (including Millinksi and Bakker 1990; Houde and Torio 1992; Zajitscheck et al. 2006). Few studies have, however, considered multiple factors simultaneously when researching mate choice.

1.3. SELECTIVE BREEDING

Fitness-related traits can be affected through inbreeding depression (Saccheri et al. 1998; van Oosterhout et al. 2000b; Keller and Waller 2002). Specifically, survival can be reduced (Coltman et al. 1998), reproductive success hampered (Spielman et al. 2004), sexual ornamentation and courtship behaviour weakened (van Oosterhout et al. 2003b), and parasite susceptibility increased (Coltman et al. 1999; Hedrick et al. 2001; MacDougall-Shackleton et al. 2005; Rijks et al. 2008). Natural selection drives wild populations phenotype, whereby natural mate choice can occur to ensure traits are selected that increase offspring fitness.

The farming of animals for human consumption and companionship has led to a reduction in genetic, morphological, behavioural and physiological trait diversity from wild conspecifics. Selective breeding of captive animal stocks to ensure the presence of commercially valuable traits, such as increased body quality (Gjedrem et al. 2012) and growth rate (Cook et al. 2000) or desirable colouration/patterns, is a fundamental practice of agricultural, aquaculture, pet store and aquarium industries globally. Though beneficial for the human economy, the restricted gene mixing and resultant increase in homozygosity (Charlesworth and Willis 2009) can adversely affect offspring fecundity (Radwan 2003), mating and susceptibility to environmental stressors, including parasitic infection (Smallbone et al. 2016a).

1.3.1. Aquarium trade and aquaculture

The aquarium trade and aquaculture industry use artificially selective breeding to maximise profits. Artificially selective breeding from bottlenecked populations leads to reduced genetic diversity (Charlesworth and Willis 2009), which in turn is likely to increase parasite susceptibility. Differences between captive and wild populations, with respect to immune activation, duration and intensity, can be expected due to regular cleaning of captive facilities, specific pathogen control programmes, reduced energy expenditure and the increased stability characteristic of the captive environment (Friend et al. 1999; Joop and Rolff 2004; Buehler et al. 2008b). There is thought to be depletion in MHC alleles from the genome of domesticated individuals compared to their wild counterparts, due to reduced exposure to parasite fauna in the farmed environment.

Farmed fish are increasingly being accidentally and deliberately released into the wild, along with the rise in intensive aquaculture practices and the ornamental pet trade (Philippart 1995; McGinnity et al. 2003; Naylor et al. 2004; Copp et al. 2006; Lorenzen 2008; Bell et al. 2008; Laikre et al. 2010). These released fish tend to be more inbred than their wild counterparts and are likely to be more susceptible to parasites, but little research has been conducted to identify the effect of their release on wild populations. The release of fish from captivity is not the only method of pathogen exchange; wild fish are attracted towards the food and shelter provided by fish farms (Naylor et al. 2000; Dempster et al. 2009). The open systems commonly used allow water to flow freely between the wild and captive fish, providing a pathogen transmission pathway (Johansen et al. 2011; Jackson et al. 2015; Fig. 1.6). The risk of pathogen transmission is also increased through the movement of aquaculture stocks between fish farms and the supplementation of wild stocks (Naylor et al. 2000; Fig. 1.6). Understanding the risks of deliberate and accidental release of captive bred fish into wild populations on parasite transmission is one of importance for sustainable aquaculture, fisheries and fish conservation (Lorenzen et al. 2012).

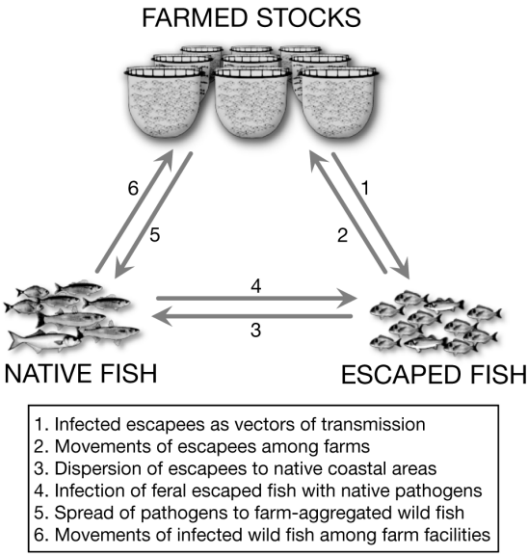

Figure 1.6: Flow chart of potential transmission through the movement of wild and domesticated fish (Archavala-Lopez 2013).

1.4. STUDY SYSTEM

This thesis is based on a well-studied fish host–parasite system, the guppy (*Poecilia reticulata*) and its highly contagious monogenean gyrodactylid, *Gyrodactylus turnbulli*.

1.4.1. The guppy

Guppies are small, live-bearing, sexually dimorphic tropical fish, native to the streams of Trinidad, Tobago and South America (Houde 1997; Fig. 1.7). They are an important ecological and evolutionary model and have been well studied in both natural and controlled environments (Magurran 2005). Guppies have been introduced widely for mosquito control and are also important in the aquarium trade; the ornamental fish trade is estimated to be worth $278 million US dollars, with an estimated one billion ornamental fish exported globally, annually. Their widespread use has meant guppies are found in the wild in a range of habitats and have invaded all but one of the continents (Fig. 1.7). There are over 100 guppy strains that have been bred, selected largely on the basis of male colour patterns (FAO 1996-2005; Dykman 2012). Guppies have high allelic variation at the MHC loci, but selective breeding has reduced the allelic richness of domesticated guppies, compared to natural wild populations (van Oosterhout et al. 2006a).

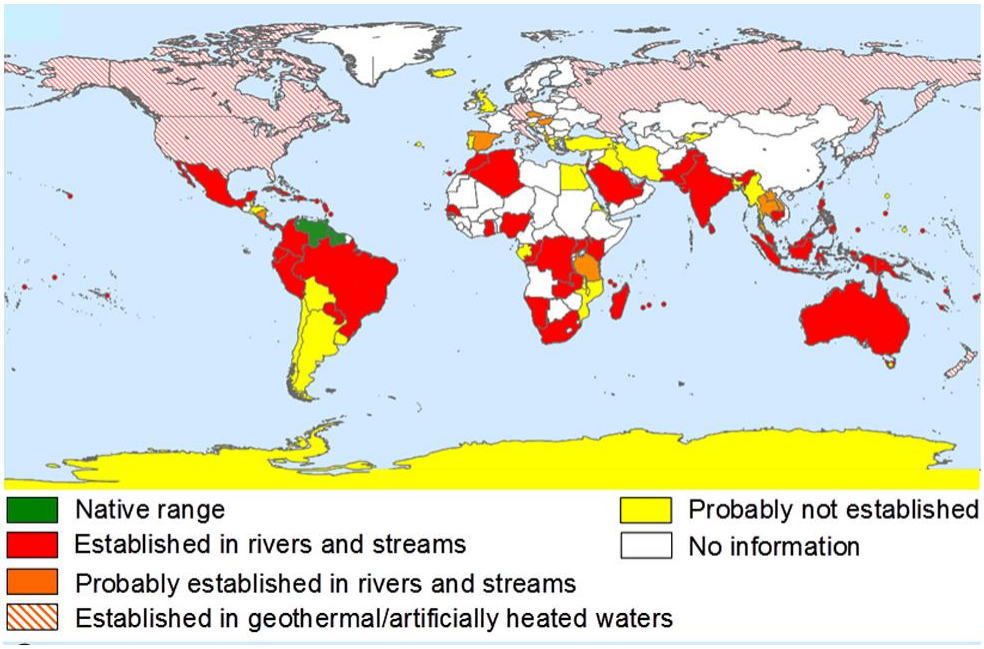

Figure 1.7: Global distribution of guppies (*Poecilia reticulata*). From: Deacon et al. (2011).

1.4.2. Gyrodactylus turnbulli

Gyrodactylids are common ectoparasitic worms that live on the skin, fins and gills of teleosts (Harris et al. 2004). These parasites are important in both the aquarium trade and food industry (Bakke et al. 2007). Infection with gyrodactylids can have devastating consequences for both wild and captive fish populations (Bakke et al. 2007). There is no specific transmission stage for *Gyrodactylus* spp.; they give birth to live young and transmission largely occurs through host contact (Bakke et al. 2007). Exponential population growth is achieved as adult worms reproduce *in situ* on the host, with a generation as short as 24 hours, at 25 ºC (Scott 1982). *Gyrodactylus* spp. adopt a 'Russian doll' reproduction strategy, whereby newly born parasites already contain a fully developed daughter (F1) *in utero*, and within this F1 generation the F2 is already developing (Bakke et al. 2007). Severe changes to host pathology can be caused due to the rapid proliferation of parasites (van Oosterhout et al. 2008). The key advantages of using gyrodactylids in the laboratory include the ease with which they can be manipulated and the entire infection trajectory monitored (Bakke et al. 2007). Laboratory experiments have shown that gyrodactylid infection can lead to marked effects on host behaviour, including courtship and feeding (Kennedy et al. 1987; van Oosterhout et al. 2003b). Without a clearer understanding of host susceptibility and gyrodactylid transmission, these parasites

remain a serious threat to fish stocks.

1.5. THESIS OUTLINE

The overall objective of this thesis is to identify differences in MHC genetic composition between truly wild, wild type and ornamental strains of guppy (*Poecilia reticulata*) and the implications of these for a range of fitness and life history traits of a vertebrate, the guppy (*Poecilia reticulata*). Fitness effects of MHC variation will be analysed through parasite trajectory, mate choice (before conception), and offspring survival (after conception) experiments. This thesis contains two key sections; the first assesses the effect of inbreeding and domestication on MHC composition and parasite susceptibility (**Chapters 2, 3 and 4;** Fig. 1.8), and considers the implications for transmission between domesticated and wild stocks (**Chapter 5;** Fig. 1.8); the second links MHC allelic and supertype composition with individual and offspring fitness and life history traits (**Chapters 6 and 7;** Fig. 1.8**).**

Initially, the effect of inbreeding depression on host susceptibility to parasitism is investigated (**Chapter 2).** This phenomenon is further examined in **Chapter 3**, which focuses on the difference in wild type (naturally selected from a bottlenecked population in captivity) and ornamental (artificially selected) guppy MHC allelic and supertype composition. Truly wild guppies are sampled from the field in **Chapter 4**, which considers the effect of MHC on the entire natural parasite community. MHC genetic composition of truly wild guppies is also compared with that of wild type and ornamental counterparts. **Chapter 5** considers the implications of results from Chapters 3 and 4, and the release of domesticated individuals into the wild, by introducing infected and uninfected ornamental guppies into a wild type population.

The effect of sexual selection, MHC similarity and parasitism on mate selection, was assessed in **Chapter 6**, which is one of very few studies to consider multiple potentially important factors simultaneously. **Chapter 7** uses the F1 generation of specific crosses of known parentage to determine the effect of parental MHC similarity on growth rate, survival, swimming performance and parasite susceptibility. Finally, **Chapter 8** brings together the conclusions from the experimental chapters and considers the broader implications and opportunities for future research.

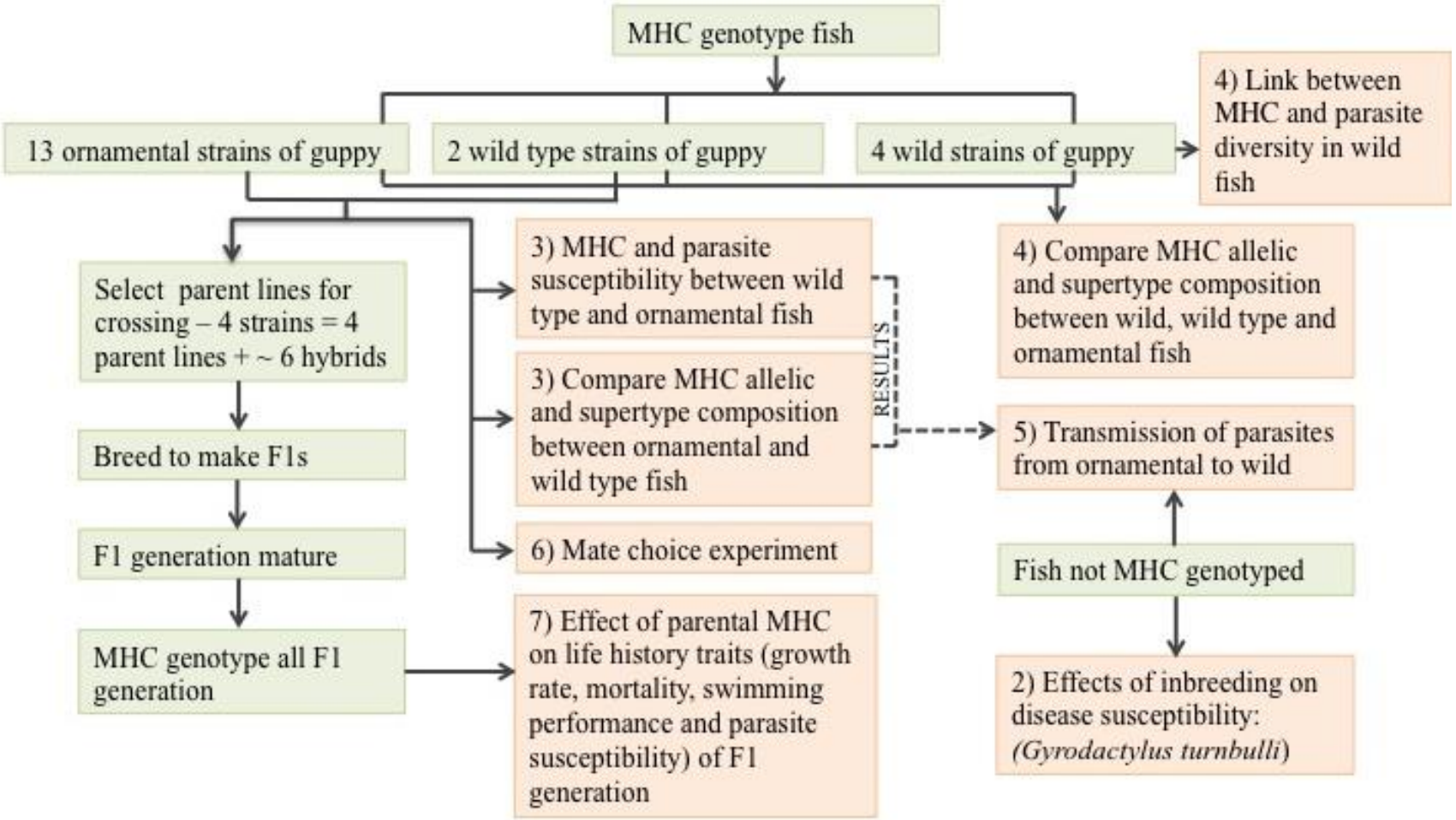

Figure 1.8: Overview of Ph.D. plan with the link between experimental chapters shown. Numbers corresponds to the thesis chapter. Green box represents background methodology and orange box shows the experimental overview.

CHAPTER 2

The effects of inbreeding on disease susceptibility: *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*

A version of this study has been published in Parasitology (Smallbone et al. 2016b). Jo Cable and Cock van Oosterhout collected the data for this chapter prior to the start of this Ph.D. studentship.

ABSTRACT

Inbreeding can threaten population persistence by reducing disease resistance through the accelerated loss of gene diversity (i.e. heterozygosity). Such inbreeding depression can affect many different fitness-related traits, including survival, reproductive success, and parasite susceptibility. Empirically quantifying the effects of inbreeding on parasite resistance is therefore important for *ex-situ* conservation of vertebrates. The present study evaluates the disease susceptibility of individuals bred under three different breeding regimes (inbred, crossed with full siblings; control, randomly crossed mating; and fully outbred). Specifically, the relationship between inbreeding coefficient (F-coefficient) and susceptibility to *Gyrodactylus turnbulli* infection in a live bearing vertebrate, the guppy *Poecilia reticulata*, was examined. Host-breeding regime significantly affected the trajectories of parasite population growth on individual fish. Inbred fish showed significantly higher mean parasite intensity than fish from the control and outbred breeding regimes, and in addition, were slower to clear their gyrodactylid infections. It is argued that the increased disease susceptibility of inbred individuals could contribute to the extinction vortex. This is one of the first studies to quantify the effects of inbreeding and breeding regime on disease susceptibility in a captive bred vertebrate of wild origin, and it highlights the risks faced by small (captive-bred) populations when exposed to their native parasites.

2.1. INTRODUCTION

Small and isolated populations are particularly vulnerable to environmental and demographic stochasticity, which can result in the loss of genetic variation due to random genetic drift (Keller and Waller 2002). Both drift and inbreeding tend to accelerate the loss of gene diversity (i.e. heterozygosity), and this can result in individuals being less resistant to environmental change and increase mortality (e.g. Fox and Reed 2010; Bijlsma and Loeschcke 2011). Inbreeding depression can affect many different fitness-related traits (Saccheri et al. 1998; van Oosterhout et al. 2000b; Keller and Waller 2002), including survival (Coltman et al. 1998), reproductive success (Spielman et al. 2004), sexual ornamentation and courtship behaviour (van Oosterhout et al. 2003b), and parasite susceptibility (MacDougall-Shackleton et al. 2005; Rijks et al. 2008). Inbred individuals tend to have higher pathogen susceptibility compared to outbred counterparts (e.g. Coltman et al. 1999; Hedrick et al. 2001). Genetic diversity has been negatively associated with susceptibility to parasitism in many animals, including insects (e.g. Whitehorn et al. 2011), birds (e.g. MacDougall-Shackleton et al. 2005; Ortego et al. 2007), mammals (e.g. Roelke et al. 1993; Rijks et al. 2008) and fish (e.g. Arkush et al. 2002; Consuegra and de Leaniz 2008; Ellison et al. 2011; Eszterbauer et al. 2015).

Understanding the effects of inbreeding on fitness traits in fish is important because of their economic value and the constraints imposed on aquaculture by limited brood stock, high stocking densities and infectious disease. Reduced sexual activity has been reported in several inbred fish species (Farr and Peters 1984; van Oosterhout et al. 2003b; Mariette et al. 2006; Frommen et al. 2008). Even one generation of full sibling inbreeding can lead to a reduction in male sexual motivation and mating success (Mariette et al. 2006; Frommen et al. 2008), but the effects of inbreeding are significantly more pronounced after multiple generations, possibly due to epistatic interactions (van Oosterhout et al. 2003b). Several inbreeding avoidance mechanisms have evolved in vertebrates in nature, including disassortative mating based on MHC dissimilarity, which is thought to increase offspring resistance to parasitism (Penn et al. 2002; Rauch et al. 2006; Forsberg et al. 2007; Consuegra et al. 2008; Evans and Neff 2009).

It is important to understand the impact of *ex-situ* breeding of wild populations on an individual's ability to fight off pathogenic infection, which may affect their reintroduction success and is important for conservation (van Oosterhout 2007a; IUCN/SSC 2013). Inbreeding is a key consideration in conservation genetics; reducing the effective population size will accelerate the rate of inbreeding and disease susceptibility. Most natural vertebrate populations have had long-term exposure to host–pathogen interactions (May 1988). Resistance traits in the wild are costly and captive bred animals are likely to lose resistance in the absence of pathogen infection and parasitism, due to a lack of acquired immunity from previous pathogen exposure and stress-induced immunosuppression (Altizer et al. 2003; Matthews et al. 2006). Reintroduction programmes may lead to increased pathological effects (Viggers et al. 1993), particularly when susceptible captive bred individuals are released in genetic supplementation programmes (Faria et al. 2010). For example, parasitic infection in reintroduced naïve fish (*Poecilia. reticulata*) reached similarly high levels to those reported on experimentally infected naïve laboratory individuals when released into mesocosms alongside native fish (van Oosterhout et al. 2007b). Reintroduced inbred individuals had a lower survival rate from parasitic infection than their outbred counterparts (van Oosterhout et al. 2007b).

This study examines the relationship between the level of inbreeding and susceptibility to *Gyrodactylus turnbulli* infection in the live bearing guppy under controlled laboratory conditions. Guppies are introduced widely for mosquito control, the success of which depends on their ability to survive under natural conditions, including exposure to parasites (Elias et al. 1995; Cavalcanti et al. 2007). Examining the susceptibility of inbred individuals to parasitism in a controlled environment has been scarcely studied (but see Hedrick et al. 2001; Spielman et al. 2004). Infection with gyrodactylids can have significant fitness consequences in both wild and captive fish populations, including marked effects on host behaviour (e.g. courtship and feeding, Kennedy et al. 1987; van Oosterhout et al. 2003b; Bakke et al. 2007; van Oosterhout et al. 2007a) and survival of fish stocks (Cable et al. 2000). This study aims to determine whether inbreeding reduces the immuno-competence of guppies and increases their susceptibility to gyrodactylid infection. The parasite trajectories on individual fish from three different breeding regimes (i.e. the inbred, the control and the outbred regime) and (i) parasite intensity; (ii) parasite persistence;

and (iii) maximum parasite numbers were analysed. It is hypothesised that inbred fish will have higher parasite susceptibility than their outbred and randomly bred counterparts.

2.2. MATERIAL AND METHODS

2.2.1. Study system

Gyrodactylus turnbulli is a highly contagious, monogenean ectoparasite with a direct transmission pathway (Cable 2011) and "Russian doll" reproduction (Bakke et al. 2007). Guppies are small, live-bearing tropical fish, native to the streams of Trinidad, Tobago and South America (Houde 1997) that are an important ecological and evolutionary model (Magurran 2005) with a short generation time. The study system allows for parasite trajectories to be monitored regularly over a period of time without the need for destructive sampling.

2.2.2. Host population

Guppies (standard length: 13.5-29.0 mm) were collected from the Upper Aripo (UA, grid reference PS 931 817) and Lower Aripo (LA, PS 938 786) River in the northern mountain range of Trinidad and transported to the UK in October 2001.

2.2.3. Breeding regime

The present study analyses guppies that have been bred for four generations in one of three breeding regimes: control, inbred, and outbred. The control group was derived from random mating within a small population (n=16) of breeding individuals. Pairings were assigned randomly using computer simulations, which occasionally resulted in full-sibling crosses. Inbred lines were full-sibling inbred. The outbred regime prevented inbreeding by using pedigree information and equalising the family size by allowing only two offspring per family to reproduce into the next generation. Individual inbreeding coefficients (F-coefficients) were calculated using the co-ancestries in pedigrees using a Minitab 12.1 macro (van Oosterhout et al. 2000a).

2.2.4. Experimental infections

Guppies were kept individually in 1 L containers where they remained isolated for the duration of the infection trial. The population origin of individual guppies remained concealed until completion of the blind experiment. Fish were fed live newly hatched *Artemia* every day and water was changed every second day. Fish from each population were assigned randomly to the experimental *G. turnbulli* infection group or the negative control group, which was kept uninfected. A total of 72 experimental fish (size range: 12.5-29 mm; 18 UA males, 14 UA females, 32 LA males and 8 LA females) and 30 control fish (12 UA males, 6 UA females, 5 LA males and 7 LA females) were parasite screened to account for variation in mortality. Experimental infections utilised the Gt3 strain of *G. turnbulli*, which was isolated from a Nottingham aquarium shop in October 1997 and subsequently maintained in laboratory culture for ca. 4 years on inbred guppies prior to this study.

At the start of the experiment on Day 0, fish were lightly anaesthetised with 0.2% MS222 and each experimental fish was infected with two individual gyrodactylids. Extreme care was taken in transferring parasites using a dissection microscope with fibre optic illumination (following standard methods of King and Cable 2007). Worms from donor fish were transferred to the caudal fin of recipient hosts after they had naturally attached to insect pins. To avoid the possibility that the initial two parasites transferred were too old to reproduce, all fish were re-examined the day after infection and those fish which had lost both parasites were immediately re-infected with a further two specimens of *G. turnbulli*, and for these fish the time was re-set to Day 0. Parasite infections were then monitored every 48 h when fish were anaesthetised (both controls and infected fish) and the total number of gyrodactylids counted. This study reports on the infection dynamics during the first 17 days after inoculation. Control fish were anaesthetised and sham-infected, and were monitored at the same time as experimental fish.

2.2.5. Statistical analysis

All statistical analysis was conducted using R version 2.15.1 (R development Core Team 2008). A survival curve of parasitised fish with different breeding regimes

(inbred, control and outbred) was created using the survival library and the survfit function; this determines the percentage of fish survival over time. A survival curve was also plotted using the survival library and survfit function to determine the rate of extinction of parasites (parasite persistence) depending on the breeding regime of their host. A Chi-Square (χ^2) test was performed to determine any significant difference between fish survival or parasite persistence and breeding regime.

G. turnbulli intensity, defined as the number of worms on an infected host (Bush et al. 1997) were analysed using a Generalised Linear Mixed Model (GLMM) with negative binomial distribution using the *glmmadmb* package and the *glmmadmb* function, with breeding regime, host standard length, sex and time (days from initial infection) as fixed factors. As parasite intensity was recorded for each individual fish at different time points, 'Fish ID' was included as a random effect in the GLMM to avoid pseudo-replication by incorporating repeated-measures. Breeding regime and time were also included as interactive terms to determine the effect on intensity of parasites over time. Fish length was included in the initial model, but was removed because it did not explain significant variation, which makes the model more efficient (Thomas et al. 2013). A negative binomial General Linear Model (GLM) was used to analyse the effect of breeding regime, host standard length and sex on the maximum parasite intensity, i.e. the highest parasite intensity reached during the experimental period, using the function *glm.nb* in the library MASS. Host standard length was not significant and was removed from the model. The models were refined using analysis of variance and stepwise deletions of the least significant terms, until only the significant terms remained (Crawley 2007). An analysis of variance ("Anova" function in R) was employed as a measure of goodness of fit using the maximum likelihood-ratio test. Only significant terms are reported.

2.2.6. Ethical note

Approved by Cardiff Ethical Committee and conducted under UK Home Office License (PPL 30/2876).

2.3. RESULTS

The mean (± SEM) individual inbreeding coefficient (F-coefficient) of fish within the control regime was F = 0.042 (± 0.010), which is representative of many zoo populations of larger vertebrates (Boakes et al. 2007). The full-sibling inbred regime resulted in an inbreeding coefficient of F = 0.5 (± 0.0). The F4 individuals from the outbred regime, using pedigree information to prevent inbreeding, had no consanguineous reproduction for (at least) 4 generations (F = 0.0 (± 0.0)).

There was no significant difference in host survival rates between the three breeding regimes over the 17 day experiment (χ^2 = 0.179; d.f. = 2; p = 0.915). The persistence of the infection did, however, differ significantly between the breeding regimes (Fig. 2.1; χ^2 = 15.970; d.f. = 2; p < 0.001). The infection persisted for longest (on 89% of hosts) on inbred guppies, which differed significantly from that on the control (46%; χ^2 = 14.081; d.f. = 1; p ≤ 0.001) and outbred regime fish (57%; χ^2 = 7.082; d.f. = 1; p = 0.008).

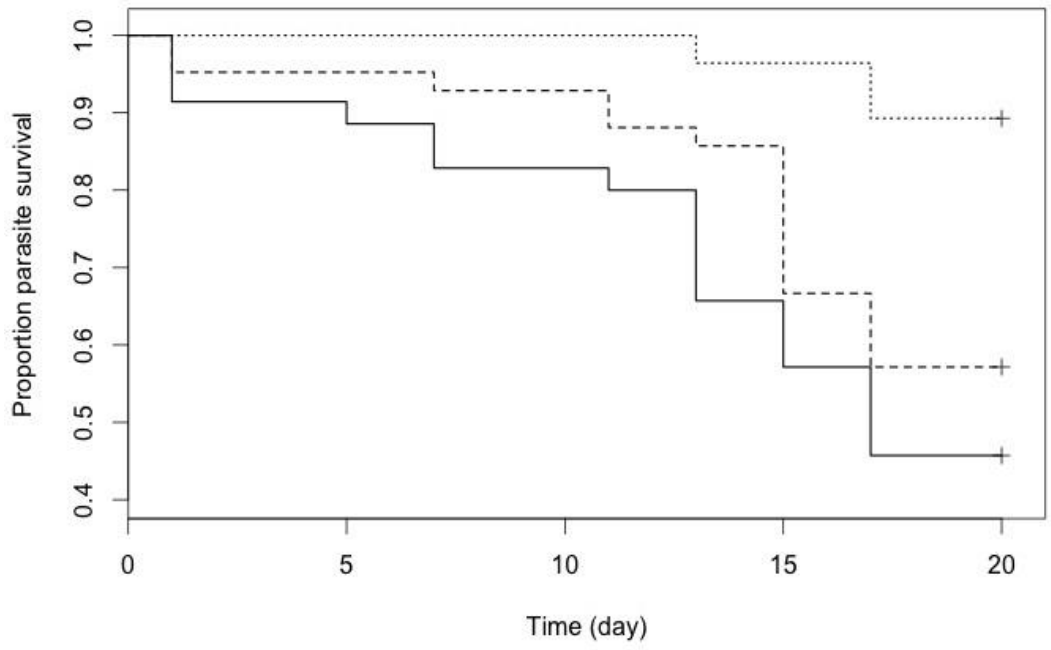

Figure 2.1: Proportion of parasite (*Gyrodactylus turnbulli*) extinct over time (days) from different breeding regimes: inbred (dotted), outbred (dashed) and control (solid).

Parasite number significantly increased through time on all fish, and was significantly affected by breeding regime (χ^2 = 20.552; d.f. = 2; p < 0.001; Fig. 2.2). Parasite mean intensity was higher on inbred fish compared to the control and outbred fish over time (glmm: $z_{2,1438}$ = 4.49, p < 0.001; glmm: $z_{2,1438}$ = -4.03, p < 0.001, respectively; Fig. 2.2). Females had a significantly higher maximum parasite intensity compared to males (glm: $t_{1,149}$ = -3.144, p = 0.002), but the mean parasite intensity did not differ significantly between the sexes (χ^2 = 3.750; d.f. = 1; p = 0.053). Larger fish, however, had a higher overall parasite intensity than smaller fish (Anova: $F_{1,1438}$ = 6.30, p = 0.012).

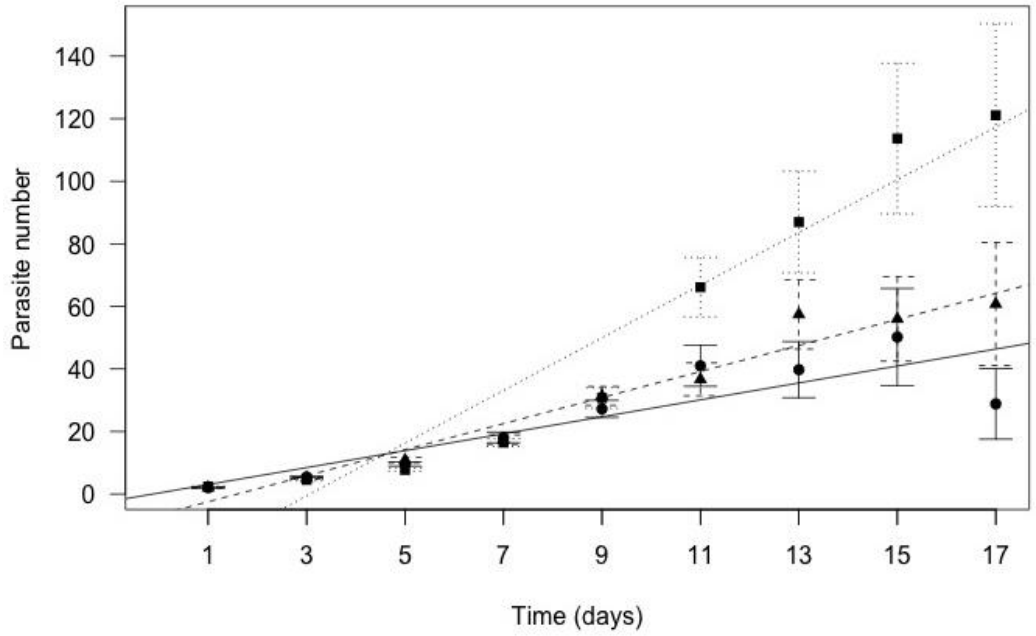

Figure 2.2: The effect of breeding regime, inbred (dotted); outbred (dashed); control (solid), on the number (± SEM) of parasites (*Gyrodactylus turnbulli*) on guppies (*Poecilia reticulata*) over time (days).

Over a 17 day period there was significantly higher maximum parasite intensity on the inbred fish compared to those in the control (glm: $t_{2,149}$ = 2.914, p = 0.004), but there was no significant difference between the maximum parasite intensity of the inbred and outbred regimes (glm: $t_{2,149}$ = -1.622, p = 0.107). Fish from the control breeding regime reached their maximum parasite load earlier (day 9) than those in the inbred regime (day 14) (glm: $t_{2,101}$ = 2.134, p = 0.035).

2.4. DISCUSSION

Overall, the data from the current study suggests that the immune system of the "inbred" guppies (with inbreeding coefficient F = 0.5) was less effective in controlling the *Gyrodactylus turnbulli* parasite infection compared to the randomly mated "control" guppies (with F = 0.042) and outbred fish (F = 0), although the inbred and outbred guppies did not differ significantly in the maximum parasite intensity that was reached. The mean parasite intensity was higher on inbred individuals compared to their control and outbred counterparts, supporting findings from previous studies on wild animals in their natural environment (including the host-specific ectoparasitic lice on Galapagos hawk and kestrels: Whiteman et al. 2006 and Ortego et al. 2007, respectively). The parasite infection also persisted significantly longer and reached higher maximum parasite intensity on inbred hosts than on fish from the control regime (but not compared to the fish of the outbred regime), suggesting that the inbred (F = 0.5) were unable to clear infection. Previous studies have shown individuals with higher inbreeding coefficient have reduced parasite resistance (e.g. bacterial infections in *Drosophila*, Spielman et al. 2004). Individuals from the control regime reached their maximum parasite load earlier and at a lower level than their inbred counterparts. This suggests that either the innate immune response acts more efficiently in the control regime guppies, or that the acquired adaptive immune response was more rapidly mounted in these fish than in the inbred individuals. Maximum parasite intensity is rarely considered as a measure for parasitism; however, it is an important variable to gauge the efficiency of an individual's immune response, and assess the infectivity of an individual's infection.

In the current experiment, the inbred guppies had approximately 50% reduced gene diversity (heterozygosity; F = 0.5) compared to the outbred counterparts and ancestral (wild caught) fish. Low heterozygosity is likely to have impaired the ability of the inbred fish to mount a rapid immune response (Altizer et al. 2003). Genetic diversity at the Major Histocompatibility Complex (MHC) plays an important role in disease resistance to pathogens in vertebrates (Hedrick and Kim 2000; Milinksi 2014). The MHC forms part of the adaptive immune response, and this is the second line of defence that is mounted after the innate immune response. The multigene family codes for cell surface proteins that identify foreign substances and pathogens

and initiate the immune system (Zinkernagel 1979). MHC is not the only immune defence; the vertebrate immune system is multi-layered. Nevertheless, despite the fact that there are numerous genes involved in mounting an immune defence, it is argued that the loss of MHC variation in the inbred regime guppies is a likely candidate that can largely explain the current results. This statement is based on four lines of evidence. Firstly, natural gyrodactylid burden has been linked to MHC variation in wild guppies (Fraser and Neff 2010). Secondly, in the current study there was no significant difference over the first 9 days of the parasite trajectory between the fish of the three breeding regimes. This suggests that the innate immune response was not significantly different between the inbred and the control/outbred regime fish, or that there is a lot of variation in *G. turnbulli* population growth rate between each individual fish before day 9, which potentially disguises any population differences (Ramirez et al. 2012). Thirdly, the parasite population started to decline significantly later on the inbred fish (on day 11, which equates to more than 30 parasites extra on the inbred compared to the control). This suggests that the efficiency of the adaptive (MHC-mediated) immune response was impaired in the inbred fish. Finally, inbreeding is expected to have a particularly profound effect on highly polymorphic genes in multigene families (e.g. MHC) because it will simultaneously reduce the variation at multiple paralogous gene copies. The optimum MHC diversity theory suggests that an optimal level of MHC dissimilarity between mates is important in resistance to local parasitism (Eizaguirre and Lenz 2010), this may explain why the control regime ($F = 0.042$) was less susceptible than the outbred group ($F = 0.0$). Further work is required genotyping the immune genes in inbred and non-inbred fish to examine this hypothesis explicitly.

Increased susceptibility of inbred hosts may have implications for conservation programmes in which wild populations are supplemented with *ex-situ* bred individuals (van Oosterhout et al. 2007b). Previous studies on guppies and gyrodactylids showed that parasites play a crucial role in the reintroduction success of captive-bred animals (van Oosterhout et al. 2007b), and that individuals pre-exposed to parasites before being released had lower parasite load after reintroduction (Faria et al. 2010). Guppies are used widely as a form of mosquito control (Elias et al. 1995; Cavalcanti et al. 2007). If a small founder population of wild guppies was bred in captivity for release it would be wasted effort if the

introduced populations went extinct due to parasitism. This is a model system and the results from this study can be scaled to other animals bred for reintroduction, which often originate from small gene pools without the exposure to natural enemies (both parasites and predators). Due to increased density of susceptible hosts and lack of herd immunity, such a breeding programme could exacerbate the risk of parasite outbreaks and increase mortality, which in turn could contribute to an extinction vortex (Gilpin and Soulé 1986).

CHAPTER 3

Major Histocompatibility Complex supertypes in wild type and domesticated fish drive parasite susceptibility

ABSTRACT

Genetic diversity at the Major Histocompatibility Complex (MHC) plays an important role in disease resistance to pathogens, with MHC heterozygote individuals more resistant than homozygotes. The functional characteristics of MHC alleles determine their role in adaptive immunity. Several studies have associated the presence of particular MHC alleles with the presence or absence of a parasite species. Depletion of MHC alleles from the genome of domesticated individuals has been suggested, due to inbreeding and reduced exposure to diverse parasite fauna in the farmed environment. The present study assesses the implications of MHC class II genotype and supertype variation between wild type and ornamental host strains (Trinidadian guppies, *Poecilia reticulata*) for parasite (*Gyrodactylus turnbulli*) susceptibility and infection trajectories. Ornamental fish had a significantly reduced MHC richness, expressed both at the level of the individual (number of MHC alleles and MHC supertypes per individual), as well as the population level (total number of MHC alleles and supertypes found across all individuals within a strain). In addition, ornamental fish were significantly more susceptible to *G. turnbulli* infections, accumulating a 10 times higher intensity at the peak of the infection, compared to their wild type counterparts. The feeding rate of ornamental fish was significantly lower than that of wild types. Ornamental fish feeding rate was significantly reduced when infected, but this was not the case for wild type fish, possibly as a result of the high infection intensity. It is important for aquaculture and other industries that use captive animals to consider the importance of allelic and functional genetic diversity. A reduction in genetic diversity or artificial selection of traits for breeding could lead to the loss of key functional alleles, with implications for parasite susceptibility and other fitness traits within the population.

3.1. INTRODUCTION

The Major Histocompatibility Complex (MHC) is a multi-gene family that plays an integral part in raising an adaptive immune response that is unique to jawed vertebrates (Cooper and Alder 2006). Genetic diversity at the MHC plays an important role in disease resistance to pathogens (Milinksi 2014). Differences in MHC molecules, particularly at the peptide-binding region, lead to variation in the ability of an individual to initiate an immune response against particular infections (Milinski 2014). Many studies have shown that MHC heterozygote individuals are more resistant than homozygotes to various pathogens (e.g. Hepatitis in humans, Carrington et al. 1999; *Gyrodactylus turnbulli* in Top minnows, Hedrick et al. 2001; infectious hematopoietic necrosis virus in Winter-run Chinook salmon, Arkush et al. 2002; *Renibacterium salmoninarum* in Atlantic salmon, Turner et al. 2007). High MHC diversity of heterozygous individuals increases their relative fitness, by heightened pathogen-driven antigen presentation and detection, compared to individuals with lower MHC diversity (homozygotes; Hughes and Nei 1989; Penn 2002). Increased MHC heterozygosity facilitates broader pathogen recognition (Cassinello et al. 2001; Penn et al. 2002; Harf and Sommer 2005), and this phenomenon is known as over-dominance or heterozygote superiority.

Various theories have been proposed as to whether intermediate or high MHC genetic diversity is optimal. Depletion of the T-cell repertoire by excessively high MHC diversity may impair the immune response of individuals (Novak 1994). Experimental evidence has also suggested an optimal, intermediate, level of individual MHC diversity ensures that individual lifetime fitness is maximised and gives the greatest pathogen resistance (Wegner et al. 2003; Kalbe et al. 2009). More linear relationships have also been evidenced; guppies with more MHC alleles had a lower parasitic (*Gyrodactylus*) intensity than those with fewer (van Oosterhout et al. 2006a).

It is important to consider the functional characteristics of MHC alleles in order to determine their role in adaptive immunity (Sette et al. 2002). MHC class II functionality is based on inferred shared functional properties of the amino acids of the peptide binding region (Doytchinova et al. 2004; Doytchinova and Flower, 2005). Supertype clustering groups alleles based on five physicochemical metric

descriptors of each amino acid; hydrophobicity, trostatic potential, steric bulk, polarity, and electronic effects (Doytchinova and Flower, 2005). For example, the human MHC class I, or human leukocyte antigen (HLA), alleles are clustered into nine different supertypes, and in guppies, a previous study identified 15 supertypes. Several studies have correlated the presence of particular MHC alleles with the presence or absence of a particular parasite species (e.g. Zhang et al. 2015; Lei et al. 2016). Human MHC (HLA) supertypes are predictive of HIV viral load (Trachtenberg et al. 2003). In the fat-tailed dwarf lemur (*Cheirogaleus medius*), one MHC supertype was associated with a higher number of different nematode infections and infection intensity, whereas another rare MHC supertype was mainly found in individuals with a reduced parasite burden (Schwensow et al. 2007). The classification of MHC alleles into supertypes has been valuable in vaccine development by targeting specific groups of antigen epitopes (Sidney et al. 1996; Sette and Sidney 1998; Sette et al. 2002). Few studies have, however, focused on the function of MHC alleles (supertype) and the association of functionally similar MHC alleles with parasite intensity (exceptions include: Schwensow et al. 2007; Fraser and Neff 2010; Pilosof et al. 2014) and variation between wild and domesticated stocks.

Natural selection drives wild population phenotypes, whereby natural mate choice effectively selects traits that increase offspring fitness. In contrast, farming of animals for human consumption and companionship has led to a reduction in morphological, behavioural and physiological trait diversity (e.g. Price 1999; Geiser and Ferguson 2001; O'Regan and Turner 2004). MHC alleles are predicted to be depleted from the genome of domesticated individuals, due to reduced exposure to diverse parasite fauna in the farmed environment, as suggested by the accordion model of multi-gene evolution (Klein et al. 1993). The Accordion Model suggests that spatial and temporal variation in parasite pressures leads to the expansion and contraction of the number of MHC genes to facilitate host survival. Over generations of inbreeding with limited exposure to parasites, captivity may lead to alleles becoming fixed at loci, consequently reducing fitness (van Oosterhout et al. 2006a; Eimes et al. 2011). Hence, both the reduction of copy number variation (due to the Accordion Model), and the loss of gene diversity within loci (due to relaxed parasite-mediated selection and increased level of inbreeding in captivity) are predicted to result in a loss of allelic variation at the MHC.

Few studies have compared the immune function of captive wild type or wild, naturally living, animals with captive-bred conspecifics (exceptions: rodents, Pi et al. 2015; Abolins et al. 2011; Devalapalli et al. 2006; Boysen et al. 2011; Viney et al. 2015; and Red Knots, Buelher et al. 2008). Differences between captive and wild populations, with respect to immune activation, duration and intensity, can be expected due to regular cleaning of captive facilities, pathogen control programmes, reduced energy expenditure and the increased stability characteristic of the captive environment (Friend et al. 1999; Joop and Rolff 2004; Buehler et al. 2008a). In Kenyan spotted hyenas, wild individuals exposed to natural pathogens had a significantly higher serum antibody concentration, providing heightened protection against pathogens, compared to captive raised individuals (Flies et al. 2015). Wild mice are more immunologically responsive than lab-domesticated comparators, probably reflecting greater exposure of wild caught animals to pathogens (Viney et al. 2015). Wild individuals are reported to have greater immune function and higher concentration of antigen-specific Immunoglobulin G (IgG) than laboratory captive conspecifics (e.g. rats, Devalapalli et al. 2006; mice, Abolins et al. 2011). Despite these known differences in the immune responses of wild and captive individuals, very few studies have considered this in terms of susceptibility to parasitism.

The *Gyrodactylus*-guppy system is a well-studied model for monitoring host-parasite dynamics, due to ease of maintenance of the live-bearing tropical fish and monitoring of *Gyrodactylu*s infection on an individual, over time, without destructive sampling. Guppies occur in the wild in a range of different habitats and have invaded all continents with the exception of Antarctica. In addition, ornamental populations have been artificially selected worldwide over many generations (>100) to produce ~100 strains with distinct colour patterns, tail size and body size (FAO 1996-2005; Dykman 2012). Guppy MHC loci, first identified by Sato et al. (1995), have high allelic variation amongst wild fish, and vary between populations (van Oosterhout et al. 2006a). Selective breeding has, however, reduced the allelic richness of domesticated guppies (van Oosterhout et al. 2006a). Ornamental, artificially selected, guppies are reported to have 20% less diverse MHC compared to wild conspecifics (van Oosterhout et al. 2006a). Particular MHC polymorphism in guppies may be associated with defense against common *Gyrodactylus* spp. (viviparous monogenean parasites) (Fraser and Neff 2010). Populations with low genetic diversity have a

reduced feeding rate, especially with high parasite intensity (van Oosterhout et al. 2003a).

The link between reported lower genomic and MHC variation of ornamental guppies (compared with wild; van Oosterhout et al. 2006a), and parasite susceptibility at an individual level, is yet to be studied. The present study assesses *Gyrodactylus* parasite trajectories and host (Trinidadian guppies, *Poecilia reticulata*) susceptibility to infection, as well as MHC genotype and supertype variation, between wild type and ornamental strains of guppy. Specifically, change in parasite intensity over time on fish with varying levels of MHC diversity (wild type and ornamental) and the pathological impact of the parasite to the host are assessed. The current study will determine the differences in MHC class II allelic and supertype genotype between wild type and ornamental populations of fish. It will also identify how individual, functionally important, MHC supertypes impact parasite trajectories. Based on Chapter 2, which showed that inbred individuals are more susceptible to parasitism, it is hypothesised that: (1) ornamental fish will be more susceptible to parasitism compared to their wild type conspecifics; (2) wild type fish will be less affected by parasitism, in terms of feeding ability; and (3) individuals with greater MHC diversity (wild type fish) will fight the infection better than individuals with reduced diversity.

3.2. MATERIALS AND METHODS

3.2.1. Host population

Experimental infections were performed on two wild type strains of guppy, originating from the wild and kept in captivity for 4 years, without selective breeding, but from small founder populations (n = ~ 300). Wild type guppies were collected from the Lower Aripo (10°35'00"N 61°14'00"W) and Tacarigua (10°37'00"N 61°24'00"W) rivers in Trinidad and transported to the UK, in 2012, where they were maintained. Wild type fish were selected at random for this study conducted in 2016 (Tacarigua, n = 43; Lower Aripo, n = 16).

In addition, experimental infections were performed on 8 strains of ornamental guppy. Of these, 7 strains were purchased from a pet shop supplier in November

2014 (n = 114; Strains: Black, n = 11; Blonde Red, n = 20; Cobra Green, n = 14; Flame, n = 9; Neon Blue, n = 8; Sunset Blonde, n = 3; and Yellow German, n = 17). These ornamental fish had undergone intense selective breeding, are phenotypically very similar within strain. The other ornamental strain originated from a Nottingham pet shop in 1997 (n = 8) and has been maintained at Bristol (until 2000) and then Cardiff ever since without selective breeding; these fish have been parasite free from 1998.

Genetic analysis was performed on 11 strains of ornamental guppies (n = 762), including the 8 strains previously described for use in experimental infections (Balcony, Black, Blonde Red, Cobra Green, Flame, Neon Blue, Yellow German and Sunset Blonde). Also included were: a further ornamental strain (Leopard) purchased with those from the pet shop supplier in 2014; and two strains purchased from Tartan Guppy (Black strain) and Frisby Aquatics (Red strain) in 2015 and 2005, respectively, and reared at Hull University before being transferred to Cardiff University in 2015. Genetic analysis was performed on 294 wild type guppies, including those used for experimental infections. In total, genetic analysis was performed on 1056 (762 ornamental and 294 wild type) fish.

All fish used for experimental infection and/or genetic analysis were maintained at Cardiff University under 24 ± 1 ºC and 12 h light: 12 h dark cycle, and fed twice daily with AQUARIAN® tropical fish flakes and weekly with frozen bloodworms.

3.2.2. Experimental infection

Fish for experimental infection (n = 180) were kept across 5 identical 70 L aquaria (mixed strain, individuals identified by Visible Implant Elastomer – described in 3.2.4.1) for 5 days of acclimatisation, prior to the start of the study. During this time fish were fed on *Artemia* daily. The aquaria had been set up for 7 days prior to introduction of the fish and seeded with 5 L of water from tanks that the fish were transferred from.

Guppies were kept individually in 1 L containers (Dispo-Safe), where they remained isolated for the duration of the infection trial. Fish were fed live newly hatched *Artemia* every day and water was changed every second day. Fish that had known

MHC genotype were experimentally infected with *Gyrodactylus turnbulli* (strain Gt3), which has been maintained in a laboratory culture on guppies since October 1997, when it was isolated from a Nottingham aquarium shop. Fish were anaesthetised using 0.2% MS222 and each experimental fish was infected with two individual *G. turnbulli* on day 1. Worms from a donor fish were transferred to the caudal fin of the recipient experimental fish, by placing the donor fish in close proximity to the recipient and allowing the worms to jump. Fish were re-examined 12 h later to ensure the parasites had attached; all fish retained their infection. Parasite infections were then monitored every 48 h, when fish were anaesthetised (both controls and infected fish) and the total number of gyrodactylids counted. This study assessed the infection dynamics for 17 days after inoculation. Control fish were anaesthetised and sham-infected, and were monitored at the same time as experimental fish.

3.2.3. Feeding ability

Feeding behaviour, as a key life history trait, was used to identify the pathological impact of *G. turnbulli* on individual fish. The isolated fish were fed 0.2 ml newly hatched *Artemia* nauplii in water. Feeding response was recorded: (i) time taken for each fish to take its first bite; and (ii) the number of lunges for food during 60 seconds (feeding rate). This was conducted prior to infection (day 0) and on days 3, 7, 11 and 15, to assess feeding response throughout the experimental trajectory (van Oosterhout *et al*. 2003a).

3.2.4. MHC molecular methods

3.2.4.1. Fin clipping

Eight to ten months prior to the experimental infections, fish (n = 1056) were anaesthetised using 0.2% MS222 and a fin clip removed from the caudal fin. Fish were individually marked with a Visible Implant Elastomer (VIE) on either the left or right, above or below the spine. The fin clip was placed into a 2 ml Eppendorf tubes with absolute ethanol (99.9%) and stored at -18 °C before processing. Tools and workstation were sterilised between each individual. In total 762 ornamental

(from 11 strains) and 294 wild type (from 2 rivers) fish were fin clipped for genetic analysis.

3.2.4.2. MHC genotype methods

DNA was extracted from fin clips using the Zymo Research ZR-96 Quick-gDNA kit. The concentration of DNA was quantified using Qubit dsDNA kit. A 217 base pair (bp) fragment of MHC class IIb (encompassing all but three codons), predicted to comprise the peptide-binding region (Brown et al. 1993; Bondinas et al. 2007), were amplified using the degenerate primer pair Po_ii_2_01F 5' GTTGTGTCTTTARCTCSHCTG 3' (Herdegen et al. 2014) and eg2inR 5' ATCGGCTCACCTGATHTA 3' and trimmed to compare to previously published data (Lighten et al. 2014). Primers were validated previously by Herdegen et al. (2014). Primers were modified with a 10-bp barcode at the 5' end (Roche Diagnostics Technical Bulletin TCB No.005-2009). A unique combination of dual barcoded primers (forward and reverse) were used when samples were amplified enabling each individual's amplicons to be identified after de-multiplexing within the pools of 810 individuals. Genomic DNA from individual fish was amplified in 20 μL PCR reactions. The polymerase chain reaction (PCR) mixture contained 4 μL of ~60 ng genomic DNA, 10 μL Qiagen 2x PCR Master mix, 2 μL Qiagen Q and 2 μL 0.5 μM of both forward and reverse primers. The reaction conditions were as follows: 15 min denaturation step at 95 °C; 40 cycles of 30 s at 95 °C; 90 s at 52 °C; 90 s at 72 °C with a final elongation step of 10 min at 72 °C before being held at 10 °C.

PCR amplicons were split into two pools based on approximate concentration, quantified using Qubit dsDNA kit, cleaned using Qiagenquick PCR purification kit and prepared for 150-bp paired-end Illumina MiSeq (Illumina, Inc. San Diego, CA, USA) sequencing using the Illumina PCR-free TruSeq library protocol with 20% PhiX spike in. A total of 20,965,456 reads were generated. Validation of the repeated sequencing runs and the genotyping protocol was performed to ensure repeatability within and between sequencing runs. After de-multiplexing and tag identification, the mean (± SE) number of reads per individual was 4656.69 (± 23.04).

3.2.4.3. Genotyping with AmpliLEGACY (Lighten)

Raw sequences were converted to individual genotypes using the automated clustering and genotyping method AmpliLEGACY Lighten (part of the AmpliSAT package, Biedrzycka et al. 2017). AmpliLEGACY Lighten uses the genotyping method described by Lighten et al. (2014), which clusters variants to decipher real alleles and artefacts before using the degree of change (DOC) method, which looks for a drop in cumulative frequency of the alleles. The DOC method first entails an error correction (clustering) step, where sequences that differ by 1-3 bp from a higher frequency variant (parental) are considered putative errors and their reads are added to the parent sequence. AmpliLEGACY Lighten was run using the clustering and genotyping parameters described in Lighten et al. (2014): mismatches from parental putative alleles = 1-3 bp; relative pre-dispositional effects (RPEs) with respect to parental putative alleles = < 2% depth; maximum number of expected alleles to calculate DOCs = 10 to ensure all are encompassed; sequences not in-frame classified as artifacts = 1; non exact length sequences considered artifacts; minimum amplicon depth = 600 to ensure enough depth for at least 3 loci; maximum amplicon depth = 5000 due to computational power. A total of 164 individuals that had < 600 reads were removed from downstream analyses. A subset of genotypes was selected at random and checked against manual interrogation, where an individual had < 4 alleles and 5000 amplicon read depth there was 100% similarity between manual interrogation and AmpliLEGACY (Lighten). Discrepancies were identified when individuals had been given > 4 alleles or where read depth was < 5000 per individual. All individuals whose amplicons had a read depth of less than 5000 or more than 4 alleles identified by AmpliLEGACY (Lighten), the genotypes were manually checked and where necessary the genotypes were updated. This curation changed the automatic calling of 58 (3.9%) of all genotypes.

3.2.4.4. Lighten et al. (2014) method in brief

Data analysis involved three major steps: (1) sequence pre-processing, (2) error correction and (3) genotype estimation. The third step included two complementary approaches; the copy number variation of genes or alleles model method and the DOC method. The former method provided estimates of total number of MHC alleles per individual (A_i), number of loci per individual (L_i) and copy number

variation of genes or alleles pattern. The latter independently estimated A_i and provided a quality filter that allowed for amplicons to be assessed identifying those of high enough quality to yield reliable genotypes; this relies on the assumption that all artefacts will be significantly less common than true alleles within an amplicon.

3.2.4.5. Tests of selection and PBR identification

Many different MHC molecules have similar specificities and functions based on their amino acid substitutions at the peptide-binding region; these can be grouped together as MHC 'supertypes'. Supertypes are based on the molecular binding properties of the positively selected sites and are expected to bind similar antigenic 'supermotifs', potentially characterising a host's immune defence functionally rather than by looking at individual MHC alleles (Sidney et al. 1996; Doytchinova and Flowers 2005; Schwensow et al. 2007). Alleles were clustered using the amino acid sequences of codons previously identified as being under positive selection (Lighten et al. 2014). Amino acids were substituted for a set of five physicochemical properties (Sandberg et al. 1998).

3.2.4.6. Supertype clustering

The substituted amino acid sequence of the peptide-binding region of the MHC molecules can vary in functional characteristics, classified as 'supertypes'. Clusters of functionally similar MHC alleles were identified based on physicochemical properties of translated amino acids inferred to comprise the peptide-binding region using a discriminant analysis of principal components, performed using the packages *ade4* and *adegenet* (Jombart 2008). All MHC alleles identified in the current study (n = 174) and also published alleles (n = 84) were included to increase the sample size and reliability of supertype clustering. Published alleles were identified using NCBI BLAST with the search term "Poecilia reticulata MHC II NOT genome". Allele sequences were aligned using BioEdit, retaining all alleles that had all 15 peptide binding regions in the sequence.

Initially, the number of clusters (k) were identified using 5000 repeats of *find.clusters* function for k = 1-30, with arguments n.iter = 5000 and n.start = 500,

and retaining all, 100, principal components (PCs). MHC alleles are clustered into 13 supertype groups based on the amino acid sequence of the peptide-binding region where there is a drop off in rate of increase of change in the Bayesian Information Criterion (ΔBIC; (Fig. 3.1). Adding additional clusters increased the risk that iteration would not have 13 clusters and the rate of increase of ΔBIC on adding additional clusters dramatically reduced (Fig. 3.1). The discriminant analysis of principle components (DAPC) identifies the probability of an allele being in a particular supertype based on the retained discriminant functions, using 30 principle components above which little information is gained with 13 discriminant function eigenvalues due to the low cluster number. The optimal number of discriminant functions to retain was identified by performing k means clustering with k =13 PCs = 30, using the package *adegenet* and the function *optim.a.score* nine discriminant functions were determined optimal. Clusters for which the amino acid sequence of each MHC class II allele was assigned, were analysed through DAPC at 1000 iterations with the cluster names standardized between runs (Fig. 3.2). Allele sequences were assigned their model supertype cluster. An additional run of 1000 iterations of DAPC was performed and compared to the initial run of DAPC for repeatability using a mantel test in the package *vegan* (99% consistency between runs). The consistency of each individual alleles cluster membership indicates distinctiveness of the clusters. DAPC clustering was repeated 2000 times and alleles supertype classification was used when consistently in 60% or over of the 2000 runs of DAPC analysis (Fig. S3.1). Alleles that were not consistent in their supertype clustering were removed from downstream analysis (n = 33, 13%).

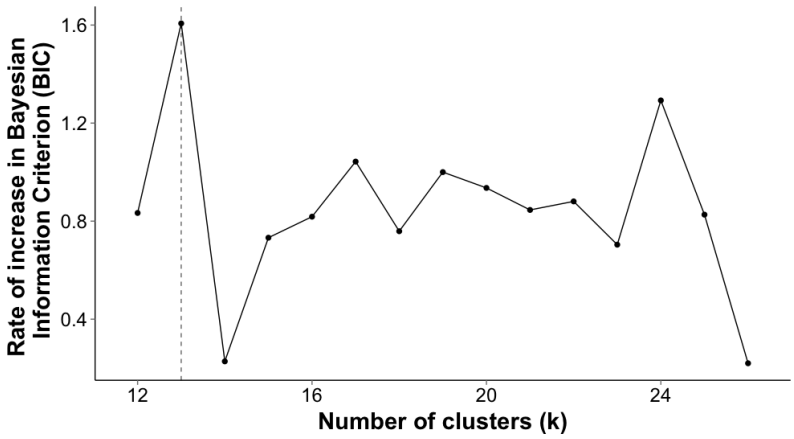

Figure 3.1: Rate of change in Δ Bayesian information criterion (BIC) with additional clusters (k) when using discriminant analysis of principle components of Major Histocompatibility Complex class II alleles from *Poecilia reticulata*. Dotted line shows the peak at 13 clusters.

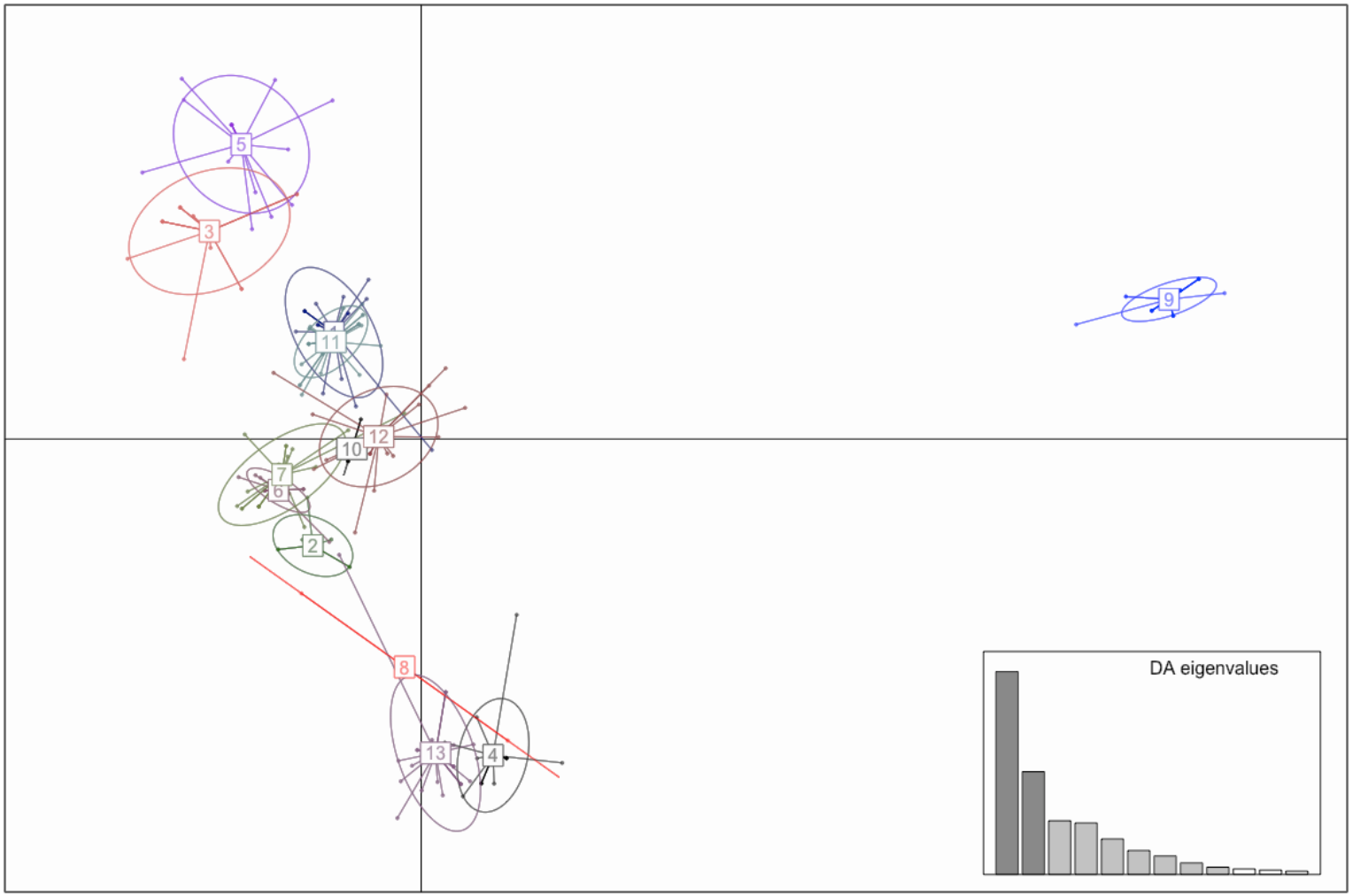

Figure 3.2: Clustering of Major Histocompatibility Complex (MHC) alleles in *Poecilia reticulata*, based on the physicochemical properties of translated amino acids inferred to comprise the peptide-binding region using Discriminant Analysis of Principle Components (DAPC). DAPC inferred the presence of thirteen clusters, or supertypes, which are visualized on nine discriminant functions. Each point represents the positioning of each MHC allele within the first nine discriminant functions. The circles represent MHC supertypes. The inset graph shows the number of discriminant functions that were used for this DAPC (9 DAs).

3.2.5. Statistical analysis

All statistical analyses were conducted using R version 2.15.1 (version 1.0.136, RStudio 2009-2016 RStudio, Inc.).

As parasite intensity, feeding lunges and time were recorded for each individual fish at different time points, 'Fish ID' was included as a random effect in all of the Generalised Linear Mixed Models (GLMMs) to avoid pseudo-replication, by incorporating repeated-measures. The residuals of all of the models were normally distributed. Only significant terms are reported.

Variation in parasite intensity, defined as the number of worms on an infected host (Bush et al. 1997), between wild type and ornamental strains of fish was analysed used a GLMM (*glmmadmb* package), with negative binomial distribution and "log" link function. The starting GLMM used parasite intensity as the response variable, with the explanatory variables: number of days post infection (day); host standard length; whether the host was a wild or ornamental strain; interaction host standard length × wild type/ornamental and the interaction day × wild type/ornamental. Model refinement using the drop1 Akaike's Information Criterion (AIC) methods suggested that the starting model was the most robust.

Variation in feeding rate between wild and ornamental fish and parasite intensity was assessed using a GLMM (*lme4* package), with the function glmer, gaussian family and "log" link function. The starting model included the explanatory variables: whether the host was wild or ornamental; host standard length; parasite intensity; and number of days from infection. The interactions day × wild type/ornamental, parasite intensity × day, wild type/ornamental × host standard length and parasite intensity × wild type/ornamental were also included in the initial model. Using the drop1 function, the starting model was deemed the most robust model and all explanatory variables remained for the analysis. A GLMM was run to assess the variation in the feeding rate between wild type and ornamental fish and infected and uninfected individuals, using the Gamma family with the "inverse" link function. The initial model included as explanatory variables: whether the host was wild type or ornamental, host standard length, infection status (treatment), and number of days from infection. The interactions day × wild type/ornamental, treatment × day,

treatment × wild type/ornamental, host standard length × wild type/ornamental and the three way interaction of treatment × wild type/ornamental × day were also included in the initial model. Using the drop1 model refinement function the starting model was identified as the most robust, leaving all explanatory variables in the final model.

Differences in MHC genotype and supertype community across type of host (wild type and ornamental) were visualised through non-metric multidimensional scaling (NMDS), using the metaMDS function (*vegan* package) (Oksanen et al. 2007). Non-metric multidimensional scaling is an ordination method used to measure dissimilarity measures (function vegdist) by running NMDS several times from a random starting configuration, comparing outputs (function procrustes) then stops after finding a similar minimum stress solution twice. The ordination was run for 1000 iterations; with stress scores of 0.04 for allele community and 0.06 for supertype community, the final solution was sufficiently low to enable reliable interpretation in two dimensions. Effect of host type on allele and supertype were assessed using the manyglm function (*mvabund* package; Wang et al. 2012). The method computes the analysis of deviance for a multivariate GLM, fitting a single GLM to each response variable with a common set of predictors. Monte-Carlo resampling tested for a significant community level response to the predictors. Analysis was conducted with (i) allele and (ii) supertype community as the dependent variable being explained by host type (wild type or ornamental) and strain of host. Model refinement was performed using the function drop1, which identified that both explanatory variables should remain in the model. To identify differences in the number of alleles and supertype per individual (*Ai* and *STi*, respectively) between wild type and ornamental fish, an Anova test (lm function in base R) was performed.

To assess the effect of supertypes on parasite intensity, a GLMM was performed using the package *glmmadmb* with the family negative binomial. The starting model included the explanatory variables experimental day, host standard length, host allelic diversity and host supertype diversity and the dependent variable *G. turnbulli* intensity. The data were subset into wild type and ornamental fish due the presence of some supertypes in one of these host types; a significant difference between these two groups of fish was identified in the previous analysis, justifying the sub-setting

(see Supplementary Material, S3.1). It was not possible to include all of the biologically relevant explanatory variables in the starting model without sub-setting, the effect of which can be examined in Supplementary Figure S3.2. Microsoft excel was used to determine the binomial distribution probability (binomdist) of MHC supertypes between wild type and ornamental fish; the results suggested a significant difference giving additional reason for data sub-setting (Fig. S3.2). The additional explanatory variables included for (1) ornamental fish: ST3, ST4, ST5, ST7 and ST12; and (2) wild fish: ST3, ST4, ST5, ST7, ST12 and ST13, the interaction between each of these supertypes and experimental day was also included in the model to identify if the supertype functional cluster elicited an immune response. A random term of "Fish ID" was also included to account for pseudo-replication. The models were refined using drop1 AIC refinement. The starting models for both analyses were the most robust and so all explanatory variables were retained for the analysis.

3.2.6. Ethical notes

All work was approved by Cardiff Ethical Committee and conducted under UK Home Office License (PPL 302876).

3.3. RESULTS

3.3.1. Parasite intensity in wild type and ornamental strains

Parasite number increased over time (glmm: $z_{1,747} = 50.53$, $p \leq 0.001$) on all 10 (8 ornamental and 2 wild type) strains (Fig. 3.3). Ornamental fish had significantly higher parasite intensity than wild type fish, over the experimental period (glmm: $z_{1,747} = -13.31$, $p \leq 0.001$; Fig. 3.3). There was an exponential increase of *G. turnbulli* on ornamental strains, but not on all individuals (Fig. 3.4a). In wild type fish, the *G. turnbulli* infections peaked at day 13, and reached a median of 31 worms. The infection on ornamental fish was higher at this point of the infection (median 128 worms), and even then, the infections continued to increase, reaching a median of 357 worms at day 17 (compared to 1 worm on wild fish on day 17; Fig 3.4b). Parasite intensity was significantly different between strains over time (glmm: $F_{9,744} = 5.50$; $p \leq 0.001$; Fig. 3.3; Table S3.1).

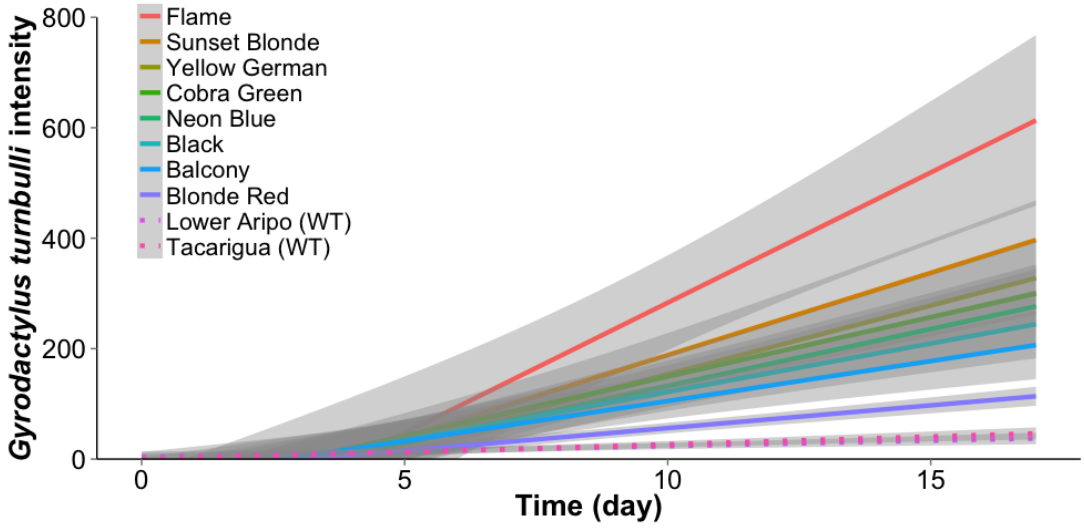

Figure 3.3: Parasite (*Gyrodactylus turnbulli*) intensity over time (days) on ten strains of guppy (*Poecilia reticulata*). Wild type (WT) strains are dotted, whilst ornamental strains are solid. Grey shading represents standard error.

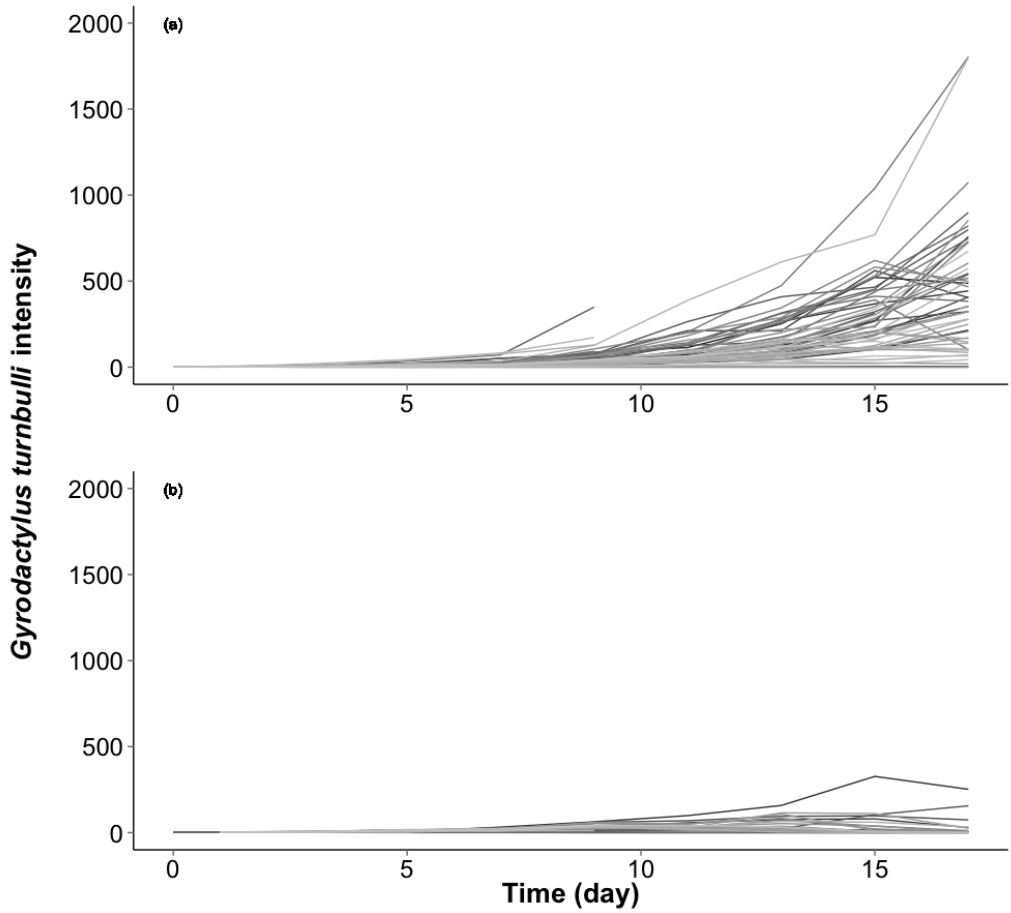

Figure 3.4: Intensity of *Gyrodactylus turnbulli* on (a) ornamental (n = 58) and (b) wild type (n = 27) individual guppies (*Poecilia reticulata*).

3.3.2. Effect of parasitic infection on host feeding ability

As parasite intensity increased over time, this significantly affected host feeding rate (glmm: $t_{1,305}$ = 2.12, p = 0.037). Host standard length did not significantly affect feeding rate (glmm: $t_{1,305}$ = -1.70, p = 0.089). Controlling for size, ornamental guppies had significantly higher feeding rate over time compared to wild type strains (glmm: $t_{1,305}$ = 4.98, p ≤ 0.001). Infection significantly reduced feeding rates of ornamentals over time, but the feeding rate of wild type guppies was not affected by infection. Uninfected ornamental fish also had significantly higher feeding rate compared both infected and uninfected wild type fish (glmm: $t_{1,305}$ = -2.64, p = 0.008; Fig. 3.5). The feeding rate of wild type guppies did not significantly differ between experimentally infected and uninfected fish (p > 0.05; see Supplementary Figure S3.3).

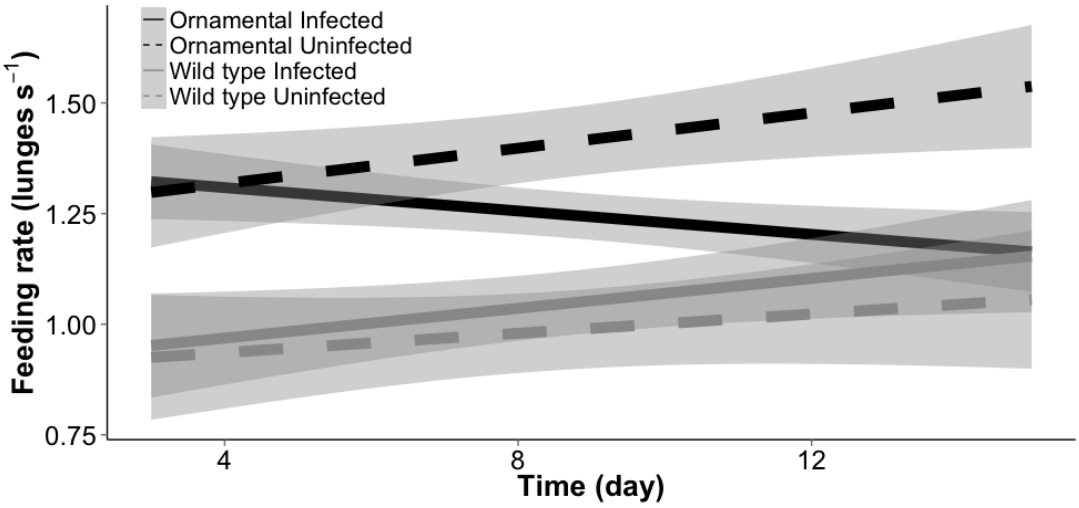

Figure 3.5: Feeding rate (lunges per second) over time for infected (solid) and uninfected (dashed) ornamental (black) and wild type (grey) guppies (*Poecilia reticulata*). Grey shading represents standard error.

3.3.3. Host MHC class II allelic and supertype similarities and differences

Wild type fish had a significantly higher number of MHC alleles per individual (*Ai*) (mean (± SE) *Ai* = 2.60 (± 0.07)) compared to ornamental fish *Ai* = 1.73 (± 0.04)) ($t_{1,736}$ = 12.01, p ≤ 0.001; Fig. 3.6a and Fig. 3.7a). The number of MHC supertypes per individual was significantly higher in wild type compared to ornamental fish (mean (± SE) *STi* = 1.52 (± 0.02) and 1.82 (± 0.05), respectively) ($t_{1,736}$= 6.74, p ≤ 0.001;

Fig. 3.6b and 3.7b). Allelic and supertype composition in a population was significantly different between host type (wild type and ornamental; Likelihood Ratio Test (LRT) $_{1,1044}$ = 2444, $p \leq 0.001$; $LRT_{1,1049}$ = 647.1, $p < 0.001$, respectively) and host strain ($LRT_{17,1028}$ = 2431, $p \leq 0.001$; $LRT_{18,1032}$ = 1529.3, $p \leq 0.001$).

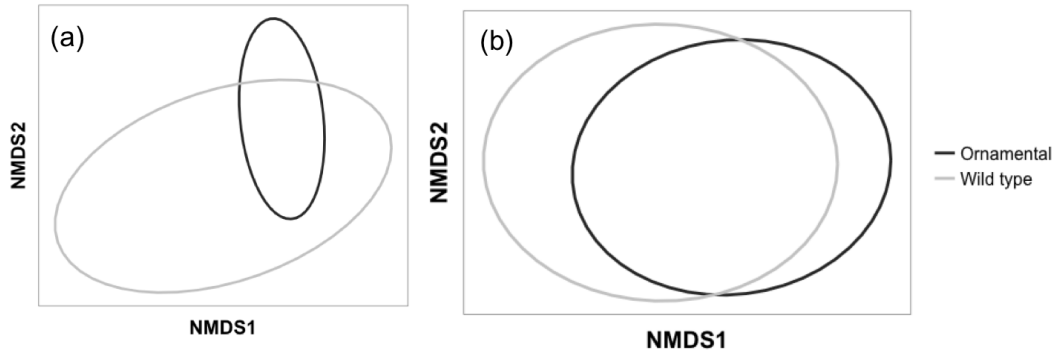

Figure 3.6: Difference in Major Histocompatibility Complex (a) allelic and (b) supertype composition between wild type (grey) and ornamental (black) guppies (*Poecilia reticulata*) based on non-metric multidimensional scaling.

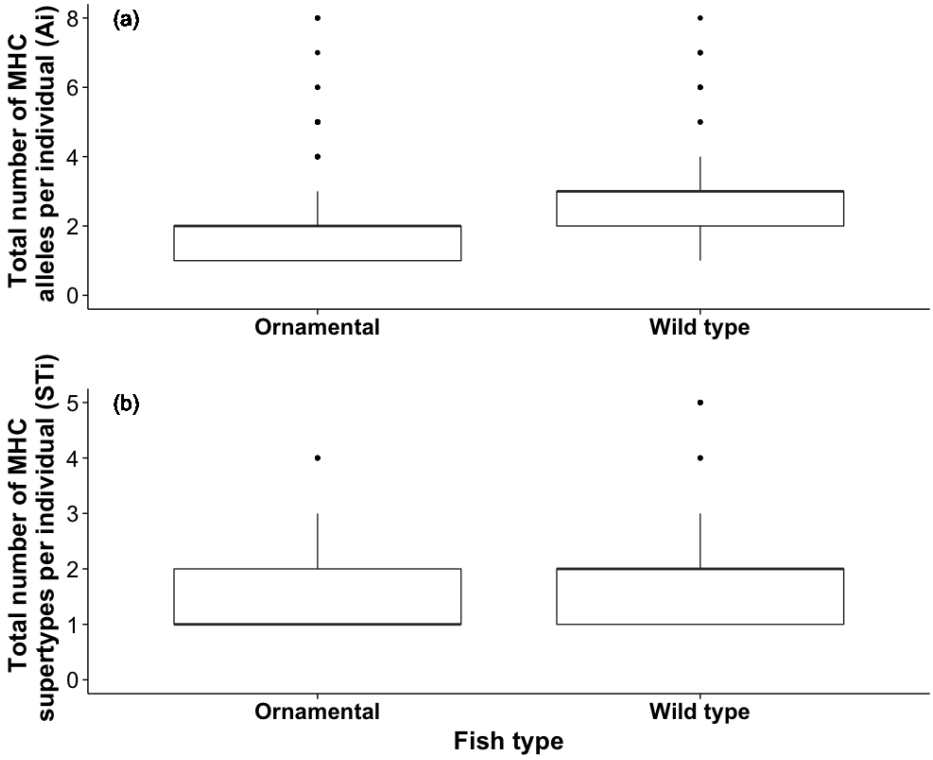

Fig 3.7: Total number of Major Histocompatibility Complex (MHC) (a) alleles (Ai) and (b) supertypes (STi) per individual for ornamental and wild type guppies (*Poecilia reticulata*).

3.3.4. Host MHC class II supertype and parasite intensity

The presence of two out of the 9 supertypes in wild type and ornamental fish had a significant effect on parasite intensity. Ornamental fish parasite intensity was significantly lower in the presence of ST5 and ST12 over time (glmm: $z_{1,421} = -4.30$, $p \leq 0.001$; $z_{1,421} = -2.11$, $p = 0.035$, respectively; Figs. 3.8a and 3.8b). Wild type fish parasite intensity was significantly lower in the presence of ST3 and ST7 (glmm: $z_{1,133} = -3.31$, $p \leq 0.001$; $z_{1,133} = 2.10$, $p = 0.036$, respectively; Figs. 3.8c and 3.8d). Allelic diversity, supertype diversity and length did not significantly affect parasite intensity in wild type or ornamental fish ($p > 0.05$).

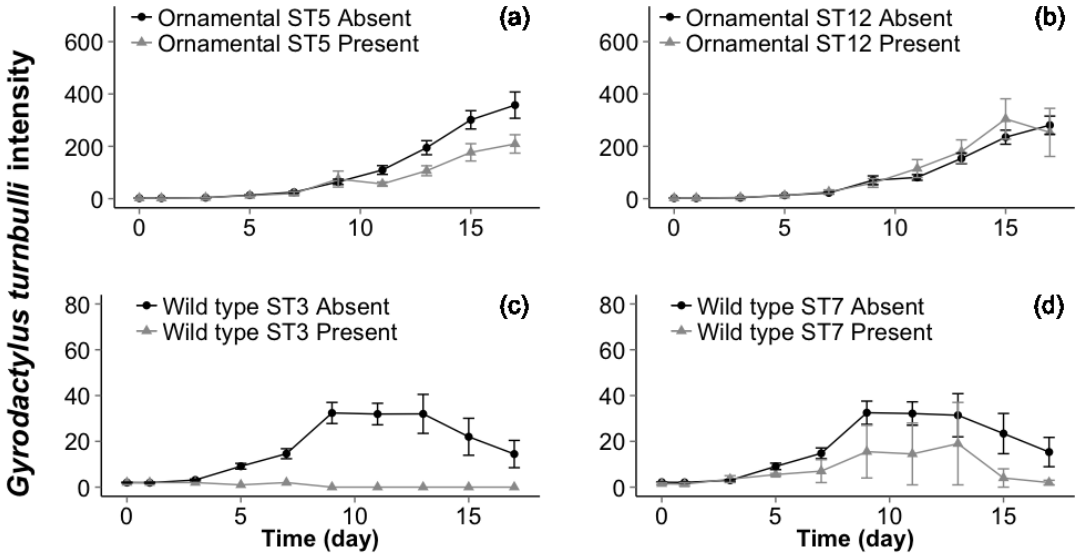

Figure 3.8: Total *Gyrodactylus turnbulli* intensity on (a and b) ornamental and (c and d) wild type guppies (*Poecilia reticulata*) over time in the presence (grey) and absence (black) of a Major Histocompatibility Complex allele from (a) supertype 5, (b) supertype 12, (c) supertype 3 and (d) supertype 7. Bars represent standard error of the mean.

3.4. DISCUSSION

The current study investigated the impact of selective breeding on the immune-genetic variation of class II MHC and immune-competence of a model fish species, the guppy *Poecilia reticulata*. MHC-genotyped fish were infected with the ectoparasite *Gyrodactylus turnbulli* in a controlled laboratory environment, monitoring the parasite intensity (number of worms per individual), and conducting

feeding experiments to examine the pathological effects of infections on guppy behaviour. Ornamental fish had a significantly reduced MHC richness, expressed both at the level of the individual (i.e. the number of MHC alleles and MHC supertypes per individual), as well as the population level (the total number of MHC alleles and supertypes found across all individuals within a population or ornamental strain). In addition, ornamental fish were significantly more susceptible to *G. turnbulli* infections, accumulating a 10 times higher intensity at the peak of the infection compared to their wild counterparts. Possibly as a result of the high infection intensity, ornamental fish also showed more severe pathology, reducing their feeding rate significantly over the experimental period. Significant variation in parasite intensity was explained by the presence of certain supertypes. This difference was so pronounced that the worm intensity differed by several orders of magnitude, depending on the supertype genotype of an individual guppy. Supertypes that offered resistance to *G. turnbulli* infections were, however, different between the ornamental and wild type strains.

The current study shows that selective breeding in farming and the pet trade can significantly increase host susceptibility to parasitism. Furthermore, the effect of parasitism on the health of wild type fish, measured as number of lunges towards prey, was negligible, whereas feeding response of ornamental fish significantly reduced during the infection. This suggests that wild fish show a moderate to high level of resistance to parasitism (albeit variable) and ornamental fish are almost invariably more susceptible, contradicting the findings of Cable and van Oosterhout (2007). Differences in ornamental host strains between studies may have caused the disparities in results. Cable and van Oosterhout (2007) used a mixed ornamental strain that had been able to naturally select for ~10 years, in parasite and treatment free conditions. In contrast, the current study used ornamental strains of fish from the pet trade, which have been artificially selected over many generations, whilst exposed to low levels of parasitism and pathogen treatment. This is likely to have impacted host immunity.

The compromised immune-competence of ornamental fish is probably the result of many generations of inbreeding (Chapter 2), which has reduced heterozygosity at the MHC loci. Unlike other fish species, guppies have not adapted to severe inbreeding in the wild, and hence, they show significant inbreeding depression (van Oosterhout

et al. 2007b). Guppies typically live in large populations in the wild, so their genetic load is not purged, which would help reduce the negative effects of inbreeding. Furthermore, the effects of inbreeding are particularly pronounced on loci with high levels of gene diversity (heterozygosity), such as the MHC. In contrast to the 2-3 MHC loci of guppies, the self-fertilizing fish, *Kryptolebias marmoratus*, has evolved robustness against inbreeding by changing the genetic architecture of the MHC. In this species, some individuals have at least six MHC class I loci (Sato et al. 2002; Ellison et al. 2012a), and alleles of distinct supertypes appear to segregate at different genetic loci (van Oosterhout 2013). Hence, even when alleles become fixed in a homozygous state at each locus, supertype diversity will not be depleted because this variation is preserved across different loci. This could explain the result, observed by Ellison et al. (2012), that even after 10 generations of selfing in inbred laboratory lines, the average MHC supertype diversity per individual was similar to that of the natural *K. marmoratus* population. In guppies, however, 13 supertypes segregate at an estimated 2 to 3 MHC class II loci (van Oosterhout et al. 2006a), implying that multiple supertypes segregate at the same genetic locus. Consequently, MHC diversity is lost because inbreeding results in fixation (and hence loss) of supertypes, which can explain the loss of supertype variation and immune-competence of fish of the highly inbred, ornamental guppy strains.

Wild type fish have a greater MHC allelic diversity compared to ornamental strains, which have been bred to be phenotypically identical to one another, increasing their inbreeding coefficient (van Oosterhout et al. 2006a). Ornamental guppies have undergone intense selective breeding (based on phenotype, from small founder populations) and intense parasite treatments, leading to lower MHC allelic diversity compared to their wild type conspecifics. Wild type fish have, however, been isolated from the wild for ~4 years and since been allowed to naturally select mating partners from a variable, yet small, population, in the absence of parasites. As well as allelic diversity, ornamental individuals have lost a significant amount of functional (supertype) MHC diversity compared to their wild type conspecifics, through generations of selective breeding. Previous studies have shown that MHC diversity is significantly associated with parasite intensity, whereby individuals with low genetic diversity are more susceptible to infection. For example, hairy-footed gerbils (*Gerbillurus paeba*) with four MHC alleles had a lower faecal egg count than those individuals with three MHC alleles (Harf and Sommer 2005).

Although not significant, parasite intensity through time in the current study was affected by the difference in MHC functional diversity between wild type and ornamental fish. Ornamental fish with reduced functional diversity are more susceptible to *G. turnbulli* infection. In wild populations, selective pressures, such as parasites and predators, maintain variation of the MHC alleles (Hedrick et al. 2001), increasing their genetic diversity. High parasite burdens are usually lethal to wild strains and are, therefore, rare in field observations (Stephenson et al. 2015). Inbreeding, however, significantly increases parasite susceptibility of wild guppies (Chapter 2). Both inbreeding and a lack of exposure to parasites are likely to lead to ornamental strains being more susceptible to infectious disease, as shown in the current study. The loss of MHC variation in domesticated fish might result in a reduction in herd immunity; individuals in a population have very similar MHC supertype genotypes, which may lead to outbreaks of infectious disease. Reduced genotypic variation in a population would mean that a pathogen would have a greater chance of a successful outbreak.

Previous studies have shown that specific MHC genotypes are associated with parasitic intensity (Paterson et al. 1998; Ditchkoff et al. 2005) and that intermediate MHC allelic diversity leads to a greater pathogen resistance than those with a low or high diversity (Wegner et al. 2003). The current study has found that the presence of alleles from particular functional clusters (supertypes) significantly affects an individual's parasite susceptibility, over the level of MHC allelic diversity; consistent with Schwensow et al. (2007), Fraser and Neff (2010) and Wang et al. (2017b). Schwensow et al. (2007) identified an association between the presence of a specific MHC supertype and reduced nematode intensity in fat-tailed dwarf lemurs (*Cheirogaleus medius*), but no overall association with MHC supertype diversity. Fraser and Neff (2010) identified 'types' of MHC allele based on unique peptide-binding regions, showing that the presence of a particular 'type' significantly reduced *Gyrodactylus* intensity. Very few of the MHC alleles identified in Fraser and Neff (2010) were present in the current study (n = 12) and those that were shared were not from the same 'type' group. Conflict between MHC diversity theories likely arises due to context specific host-pathogen interactions and/or differences in experimental design. Here, supertypes were found to confer resistance to *G. turnbulli* infections, differing between the ornamental and wild type populations. In the

ornamental strains, ST5 and ST12 offered increased resistance, whereas ST3 and ST7 were associated with an improved immune response in the wild type fish. This may be due to a difference in the T-cell repertoire between ornamental and wild type fish. Despite this contrast between wild and ornamental fish, the similarity of functional clusters ST5 with ST3 and ST12 with ST7 is high (based on physicochemical properties on translated amino acids inferred to comprise the peptide-binding region), suggesting that alleles with certain peptide-binding regions elicit a greater immune response to *G. turnbulli* infection. It is also likely that wild type and ornamental fish differ more widely across the genome, causing interaction effects not analysed during the current study. Unfortunately, it was not possible to determine the specific function of the supertypes, which have been associated with particular supertypes in the defence of *G. turnbulli*. This would require the expression of alleles to be identified. Although all alleles within a supertype are functionally very similar, each allele within a functional cluster is likely to be sufficiently different at the peptide-binding region to suggest a slight difference in peptide-binding ability.

The current study suggests that functional diversity of MHC alleles needs to be maintained to increase resistance to parasitism and resilience to change, a suggestion first made by Pilosof et al. (2014). Parasites are a driving force of MHC allelic diversity; individuals and populations with less allelic diversity, even when the main functional groups remain constant, are likely to be less resilient. Ornamental guppies are significantly more susceptible to parasitism than their wild type conspecifics, leading to a reduction in ornamental fish feeding ability. Multiple MHC supertypes have an effect on *G. turnbulli* susceptibility, suggesting that functionality (supertype) may be more important than allelic diversity. An individual with two MHC alleles of close functional similarity may not have an adaptive advantage over an individual with a single MHC allele from the functional cluster. It is important for aquaculture and related industries that use captive animals to consider the importance of allelic and functional genetic diversity, in terms of cost to profit. A reduction in genetic diversity or artificial selection of traits for breeding could lead to the loss of key functional alleles that could help protect the population from the risk of parasitic infection and effect fish growth.

ACKNOWEDGEMENTS

Data for this chapter was collected with laboratory assistance from Michael Reynolds, Owen Wright, Dayna Lea and Karan Gupta. Wild type fish were sourced from Darren Croft.

SUPPLEMENTARY MATERIAL

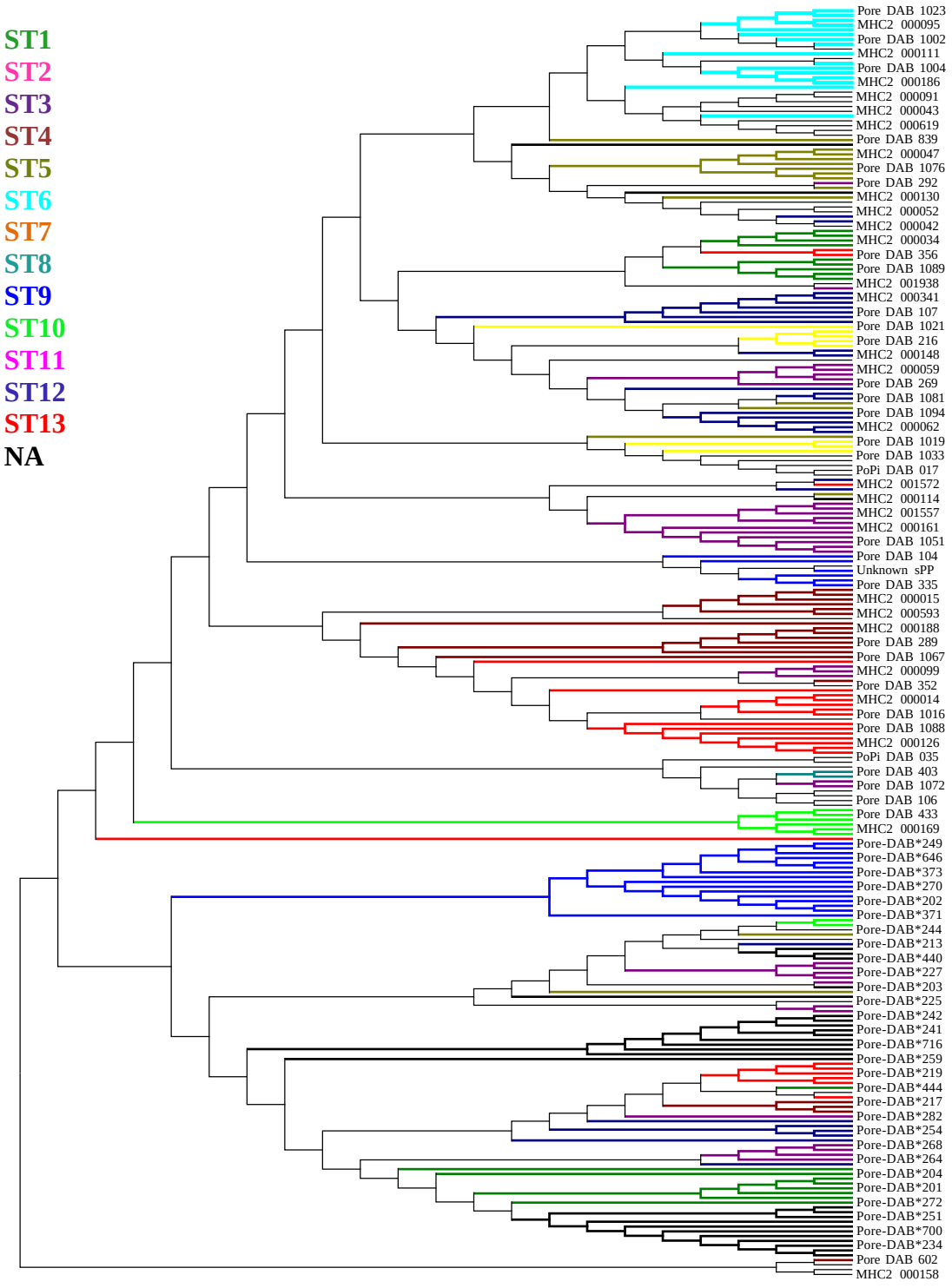

Figure S3.1: Nearest neighbour phylogenetic tree of the relationship of guppy (*Poecilia reticulata*) Major Histocompatibility Complex alleles (identified from the current study and those published as described in section 3.2.4.6). Alleles are coloured based on their inferred clustering to 13 supertypes (ST) inferred through Discriminant Analysis of Principle Components (DAPC).

S3.1: Supporting justification for data analysis. The effect of splitting data into two subsets (wild type and ornamental) compared to the entire date set. The model explained the variation in *Gyrodactylus turnbulli* intensity on guppies (*Poecilia reticulata*) using the explanatory variables time (day); host standard length; number of MHC alleles per individual (*Ai*); number of MHC supertypes per individual (*STi*); the presence of: supertype 3, supertype 4, supertype 5, supertype 7, supertype 12; and the interaction between each supertype × time (day). These variables were selected as those that were present in both subsets of data. The results of this analysis showed that the model with all fish was not significantly different from the results for wild type fish but was significantly different to the ornamental subset of data ($p = 1$; $p \leq 0.001$. respectively).

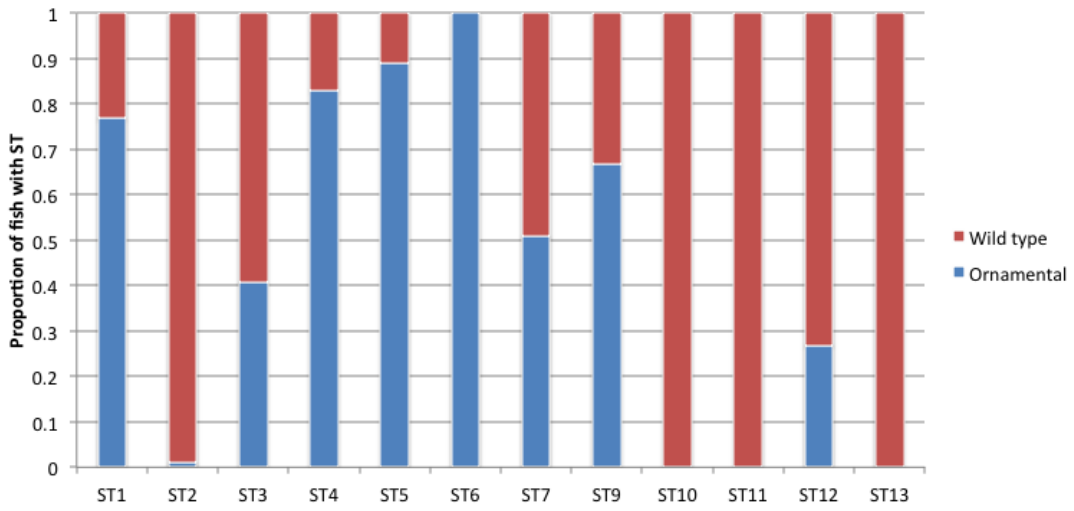

Figure S3.2: Proportion of wild type (red) and ornamental (blue) guppies (*Poecilia reticulata*) with each Major Histocompatibility Complex class II supertype (ST).

Table S3.1: Effect of the interaction between each guppy (*Poecilia reticulata*) strain and time (day) on parasite (*Gyrodactylus turnbulli*) intensity. Wild type strains of fish are reported. Significant results are highlighted grey.

Strain × Day	**Compared to**	***D.f.***	***Z***	***P***
Balcony × Day	Black	9	-0.11	0.916
	Blonde Red	9	-1.47	0.143
	Cobra Green	9	0.41	0.685
	Flame	9	1.25	0.211
	Lower Aripo (wild type)	9	-4.12	≤0.001
	Neon Blue	9	-0.07	0.947
	Sunset Blonde	9	1.12	0.262
	Tacarigua	9	-5.06	≤0.001
	Yellow German	9	0.48	0.632
Black × Day	Blonde Red	9	-1.84	0.065
	Cobra Green	9	0.68	0.495
	Flame	9	1.68	0.092
	Lower Aripo (wild type)	9	-5.03	≤0.001
	Neon Blue	9	0.05	0.959
	Sunset Blonde	9	1.69	0.091
	Tacarigua	9	-6.80	≤0.001
	Yellow German	9	0.79	0.430
Blonde Red × Day	Cobra Green	9	2.95	0.003
	Flame	9	3.65	≤0.001
	Lower Aripo (wild type)	9	-4.11	≤0.001
	Neon Blue	9	2.00	0.045
	Sunset Blonde	9	4.55	≤0.001
	Tacarigua	9	-6.41	≤0.001
	Yellow German	9	3.18	0.002
Cobra Green × Day	Flame	9	1.19	0.235
	Lower Aripo (wild type)	9	-6.11	≤0.001
	Neon Blue	9	-0.66	0.512
	Sunset Blonde	9	1.09	0.276
	Tacarigua	9	-8.68	≤0.001
	Yellow German	9	0.10	0.921
Flame × Day	Lower Aripo (wild type)	9	-6.38	≤0.001
	Neon Blue	9	-1.69	0.091
	Sunset Blonde	9	-0.39	0.697
	Tacarigua	9	-8.24	≤0.001
	Yellow German	9	-1.13	0.258
Lower Aripo (wild type) × Day	Neon Blue	9	5.24	≤0.001
	Sunset Blonde	9	7.43	≤0.001
	Tacarigua	9	-0.43	0.668
	Yellow German	9	6.33	≤0.001
Neon Blue × Day	Sunset Blonde	9	1.72	0.086
	Tacarigua	9	-7.24	≤0.001
	Yellow German	9	0.77	0.443
Sunset Blonde × Day	Tacarigua	9	-11.13	≤0.001
	Yellow German	9	-1.02	0.307
Tacarigua (wild type) × Day	Yellow German	9	9.16	≤0.001

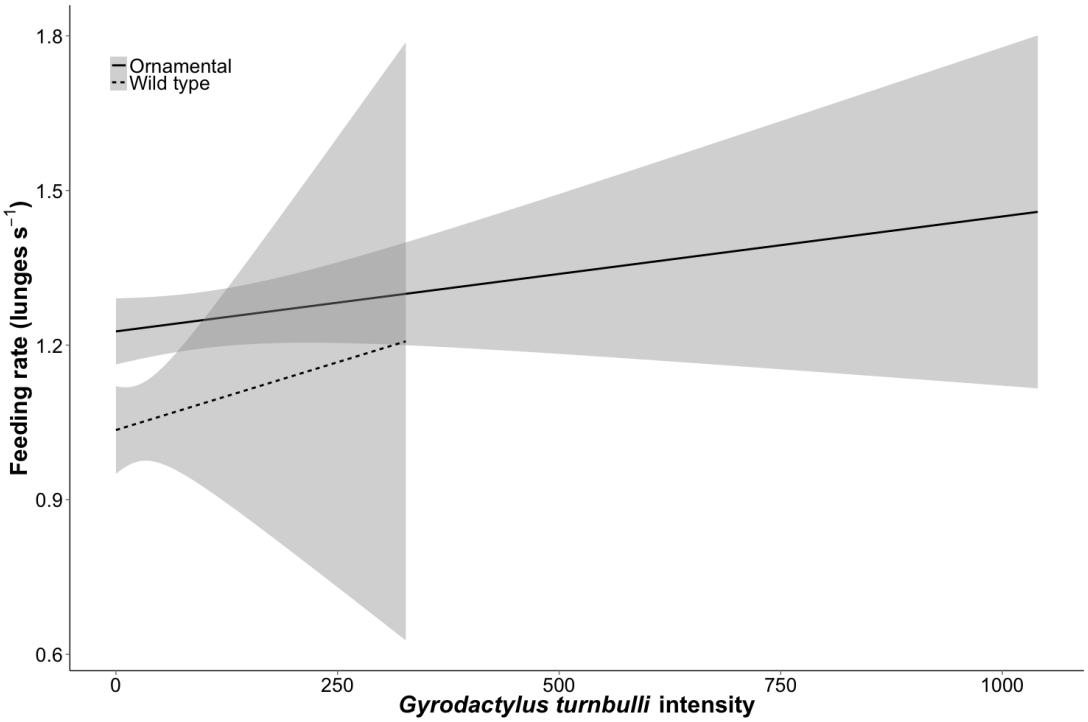

Figure S3.3: Feeding rate (lunges per second) against *Gyrodactylus turnbulli* intensity for wild type (dashed) and ornamental (solid) guppies (*Poecilia reticulata*). Grey shading represents standard error.

CHAPTER 4

Particular Major Histocompatibility Complex class II supertypes have a significant effect on the parasite community of wild guppies (*Poecilia reticulata*)

ABSTRACT

Major Histocompatibility Complex (MHC) genetic diversity plays a key role in pathogen resistance in vertebrates. An individual's MHC genotype significantly impacts on parasite susceptibility; linked to the presence of particular alleles and also MHC allelic diversity. MHC alleles are clustered according to functional characteristics into 'supertype' groups. The presence of an MHC allele within a particular supertype significantly affects parasite intensity. The overall aim of this study is to identify how MHC class II allelic and supertype composition impacts the naturally acquired parasite community of truly wild guppies (*Poecilia reticulata*). This is considered in the context of the comparative MHC composition of truly wild, wild type and ornamental stocks. Truly wild guppies had significantly greater MHC allelic and supertype richness, compared to wild type and ornamental fish. There was a significant association between the presence of particular MHC functional (supertype) groups, rather than diversity, and parasite community composition for wild fish. Replenishing cultured stocks regularly with wild fish would reduce the risk to genetic diversity and could increase host pathogen resistance. Maintaining genetic diversity in aquaculture is important, not only to increase the resistance of fish to the local parasites, but also to minimise the impact of novel pathogens following an introduction.

4.1. INTRODUCTION

The Major Histocompatibility Complex (MHC) is a highly polymorphic group of genes and plays an important role in the immune system by binding to peptide antigens derived from pathogens and displaying them on the cell surface for recognition by T-cells. Humans have over a thousand allelic variations of the genes, MHC alleles (Robinson et al. 2008). This extensive polymorphism makes it very unlikely that two individuals will have exactly the same set of MHC alleles. High levels of MHC polymorphism are thought to be maintained through selection by parasites and sexual selection (Piertney and Oliver 2006). The adaptivity of MHC is important for conferring parasite resistance (Hill et al. 1991; Hedrick et al. 1998), mate choice and mate preference (Penn 2002; Milinski 2003).

Genetic diversity of MHC plays a key part in pathogen resistance in vertebrates (Milinski 2014). Variation at the peptide-binding region of the MHC molecule influences the ability of an individual to initiate an immune response against specific pathogens (Milinski 2014). Within a single species and across populations, MHC variation is high (Málaga-Trillo et al. 1998; Figueroa et al. 2001; Reusch et al. 2001). Wild individuals tend to be exposed to a greater variety of parasites than domesticated conspecifics, leading to an increased relative fitness; specifically, high MHC class II diversity increases the repertoire of pathogen recognition (Cassinello et al. 2001; Penn et al. 2002; van Oosterhout et al. 2006b; Chapter 3). The problems associated with low MHC diversity are not trivial; a single MHC allele has been associated with a particular disease and harboring multiple different MHC alleles allows for resistance to numerous pathogens (McClelland *et al*. 2003; Evans and Neff 2009). Wild types (wild animals maintained in the laboratory for multiple generations) are often used as a model for an outbred natural population. Such wild types typically offer a greater MHC diversity relative to domesticated strains, and if the pathogens are also sourced from the wild, these wild type genotypes may have coevolved with the virulence genes of pathogens. Consequently, wild types may offer a better study system than domesticated strains to research the effects of host-parasite coevolution of immuno-genetic variation.

The prevalence of parasitic infection within host communities varies for multiple reasons, including the presence of definitive or intermediate hosts (Fredensborg et al.

2005), environmental factors (Smallbone et al. 2017) and the genetics of both host and parasite populations (Whitehorn et al. 2011). An individual's MHC genotype significantly impacts on parasite susceptibility; this has been linked to the presence of particular alleles (Lei et al. 2016) and also MHC allelic diversity (Harf and Sommer 2005). MHC alleles are clustered based on functional characteristics into 'supertype' groups based on inferred shared functional properties of the amino acids of the peptide binding region (Sette et al. 2002; Doytchinova et al. 2004; Doytchinova and Flower, 2005). The presence of an MHC allele within a particular supertype significantly affects parasite intensity, likely due to the antigen-binding properties of the supertype (Wang et al. 2017; Chapter 3). Limited research has, however, been conducted to assess how the natural parasite community of truly wild populations is linked to MHC supertypes (exceptions include Fraser and Neff 2009; Pilosof et al. 2014). Considering the entire MHC supertype composition is important in order to determine the role of each functional supertype in eliciting an immune response to a pathogen or group of pathogens.

The overall aim of this study is to identify how MHC class II allelic and supertype composition impacts the naturally acquired parasite community of truly wild guppies. Different patterns of MHC allele and supertype composition of wild-caught guppies from seven populations in Trinidad will be assessed and compared to MHC variation in two wild type captive strains and nine strains of ornamental guppies. van Oosterhout et al. (2006a) found reduced MHC diversity within ornamental strains of guppy compared to their truly wild conspecifics. It is hypothesised that (1) particular supertypes will be implicated in the absence of particular parasites; and (2) truly wild fish will have the greatest MHC allelic diversity, compared to wild type (derived from small founder population and bred in the laboratory for several generation) and domesticated (pet shop) conspecifics.

4.2. MATERIALS AND METHODS

4.2.1. Host population

Truly wild fish (n = 164) were collected for parasite screening and genetic analysis from seven sites, across four rivers, in Tobago (Reynolds et al. submitted; Table 4.1)

and processed within 2 h of collection. Wild type guppies for genetic analysis (n = 131) were randomly selected from stock populations at Cardiff University. The original fish populations came from the Lower Aripo (10°35'00"N 61°14'00"W) and Tacarigua (10°37'00"N 61°24'00"W) rivers in Trinidad and were transported to the UK in 2012 and 2007, respectively. Both wild type strains of guppy were kept in captivity at a census population size of between 200 and 1000 for 3 years without selective breeding. They originated from relatively small founder populations (estimated n = 200/strain). Ornamental guppies for genetic analysis (n = 718) were purchased from a pet shop supplier in November 2014 (strains: Black, Blonde Red, Cobra Green, Flame, Leopard, Neon Blue, Yellow German and Sunset Blonde). These ornamental fish had undergone intense selective breeding for ornamental colours for years, possibly decades, and they are phenotypically uniform within strain. These fish were regularly treated for parasites until their arrival at Cardiff University, where they were treated once and remained parasite free until use here in 2016. Another mixed ornamental strain (n = 45; balcony), obtained in 1997, originated from a Nottingham pet shop and has been maintained at Cardiff University ever since, without selective breeding and parasite treatment (after initial treatment upon arrival).

Table 4.1: A summary of the sampling locations and fish sample sizes captured across five rivers in Tobago, June 2015.

Site	Grid reference	Sample size
Dog River: DR1	0760013, 1242737	31
Dog River: DR2	0760346, 1242080	30
Golden Grove: GG1	0739713, 1235510	30
Goldsborough: G1	0757935, 1240942	27
Goldsborough: G2	0757529, 1241553	26
Goldsborough: G3	0758771, 1240639	5
Speyside: S1	0768653, 125453	21

4.2.2 Parasite community of wild-caught fish

Ectoparasite infection intensities were quantified 2 h post capture from the wild. Each individual fish was anaesthetised using 0.2% tricaine methanesulfonate

(MS222), measured (standard length, mm) and screened for ectoparasites, using a dissection microscope with fibre optic illumination. Fish were isolated in river water (250 ml; 26 ± 1 ^{o}C) until they were euthanized using MS222, fin-clipped for genetic analysis and fully dissected. Internal organs, gills and eyes were removed and dissected, the body cavity explored to record parasite fauna prevalence and intensity. Identification of parasites was to genus level, except digenean species, which were categorised to subclass taxon level.

4.2.3. Molecular methods

4.2.3.1. Fin clipping

Fish (n = 1240) were anaesthetised using 0.2% MS222 and a fin clip removed from the caudal fin. The fin clip was placed into a 2 ml Eppendorf with absolute ethanol (99.9%) and stored at -18 °C before processing. Tools and workstation were sterilised in-between individuals. In total, 763 ornamental (from 12 strains), 313 wild type (originating from 2 rivers) and 164 wild fish (caught from 7 sites across 4 rivers) were fin clipped for genetic analysis. Wild type and ornamental fish were individually injected with a Visible Implant Elastomer (VIE) on either the left or right flank, above or below the spine, for identification for future work.

4.2.3.2. MHC genotype and supertype methods

DNA extraction and MHC class II genotyping were performed using the methods described in Chapter 3 (Section 3.2.4).

4.2.4. Statistical analysis

All statistical analysis was conducted using R version 2.15.1 (version 1.0.136, RStudio 2009-2016 RStudio, Inc.).

Variation and differences in MHC genotypes and supertype community across the river of origin for wild fish and across host type (truly wild, wild type and

ornamental) were analysed through non-metric multidimensional scaling (NMDS), using the metaMDS function within the *vegan* package (Oksanen et al. 2007). Non-metric multidimensional scaling is an ordination method used to measure dissimilarity based on a distance or dissimilarity matrix. The ordination was run for 1000 iterations. Stress scores for allele and supertype composition for truly wild fish individually of 0.001 and 0.001, respectively, and for all truly wild, wild type and ornamental fish of 0.02 and 0.06, respectively, were sufficiently low to enable reliable interpretation of the final solution in two dimensions.

Multivariate abundance analysis was performed (function manyglm in the package *mvabund*) using (i) allele and (ii) supertype as the dependent variables being explained by host river of origin. A single General Linear Model (GLM) was fitted to each response variable (host type and host strain), with a common set of predictors. Monte-Carlo resampling tested for a significant community level response to the predictors. A further multivariate abundance analysis was performed to explain variation in the parasite community of truly wild populations, by including explanatory terms host origin river, number of supertypes per individual (*STi*), sex, host standard length and the presence of supertype (ST) - ST1, ST3, ST4, ST5, ST6, ST8 and ST13. Model refinement (using drop1) showed that the most robust model included the terms: host river of origin, presence of ST3, presence of ST4, presence of ST5 and host standard length in a starting model. To identify the effect of host type (truly wild, wild type or ornamental) and strain on allele and supertype composition, a multivariate abundance analysis was performed. In this case, (i) allele and (ii) supertype presence-absence were used as the community data.

4.2.5. Ethical notes

All work was approved by Cardiff Ethical Committee and conducted under UK Home Office License (PPL 302876).

4.3. RESULTS

Focusing on truly wild fish, MHC allelic and supertype composition were significantly different across origin river (Fig. 4.1a, Likelihood Ratio Test (LRT) $_{6,144}$ = 635.4 $p \leq 0.001$; Fig. 4.1b, $LRT_{3,144}$ = 305.1, $p \leq 0.001$, respectively). The river of host origin also had a significant effect on parasite community ($LRT_{3,144}$ = 220.78, $p \leq 0.001$). Furthermore, standard length had a significant effect on parasite community ($LRT_{1,140}$ = 27.47, $p \leq 0.001$). The presence of *Gyrodactylus* spp., digenean species and *Trichodina* spp. differed significantly across rivers ($LRT_{3,144}$ = 32.86, $p \leq 0.001$; $LRT_{3,144}$ = 47.28, $p \leq 0.001$; $LRT_{3,144}$ = 126.52, $p \leq 0.001$, respectively).

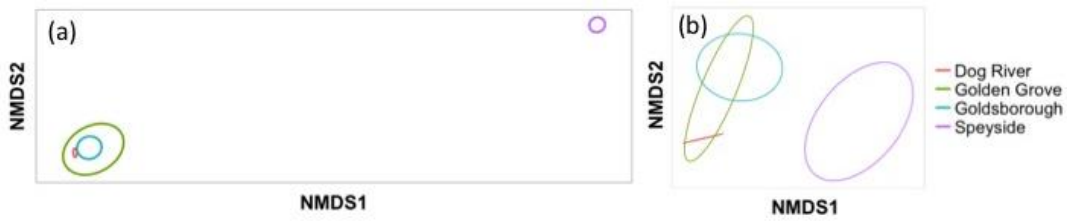

Figure 4.1: Difference in Major Histocompatibility Complex (a) allelic and (b) supertype composition for wild guppies (*Poecilia reticulata*) across four rivers in Tobago (Dog River - Red, Golden Grove - Green, Golsborough - Blue and Speyside - Pink) based on non-metric multidimensional scaling.

The presence of parasite groups differed in relation to the presence of particular supertypes (Fig. 4.2; Fig. S4.1). The presence of ST4 had a significant effect on parasite community ($LRT_{1,142}$ = 13.27, p = 0.005). Specifically, the presence of ST4 was associated with the absence of *Gyrodactylus* spp. on a host ($LRT_{1,142}$ = 8.795, p = 0.013). The presence of ST3 and ST5 had a near significant effect on parasite community ($LRT_{1,143}$ = 10.68, p = 0.057; $LRT_{1,141}$ = 10.53, p = 0.059, respectively). Specifically, the presence of ST3 was associated with the absence of *Trichodina* spp. on a host ($LRT_{1,143}$ = 6.376, p = 0.036).

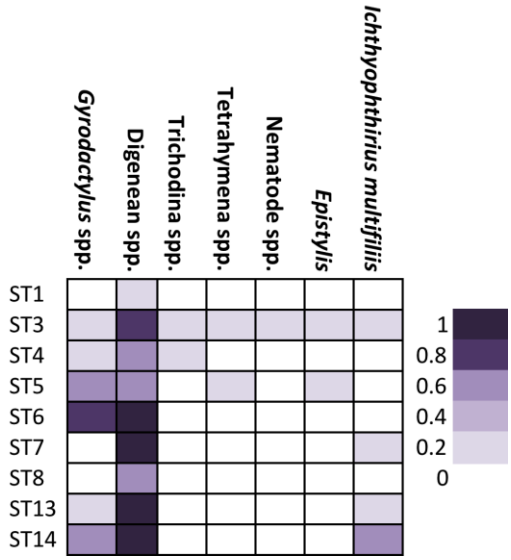

Figure 4.2: The proportion of individuals that have a particular Major Histocompatibility Complex supertype (ST) and are infected with each parasite (*Gyrodactylus* spp., digenean spp., *Trichodina* spp., nematodes, *Epistylis*, *Ichthyophthirius multifiliis*). Dark = high number of individuals have a supertype and are infected. White = no individuals have a supertype and are infected with the parasite.

There was a significant difference between allelic and supertype genotype composition across fish type (truly wild, wild type and ornamental; (Fig. 4.3a; $LRT_{3,1206} = 3895$, $p \leq 0.001$; Fig. 4.3b, $LRT_{2,1164} = 1056$, $p \leq 0.001$, respectively) and strains ($LRT_{25,1184} = 3060$, $p \leq 0.001$; $LRT_{25,1141} = 1422$, $p \leq 0.001$, respectively).

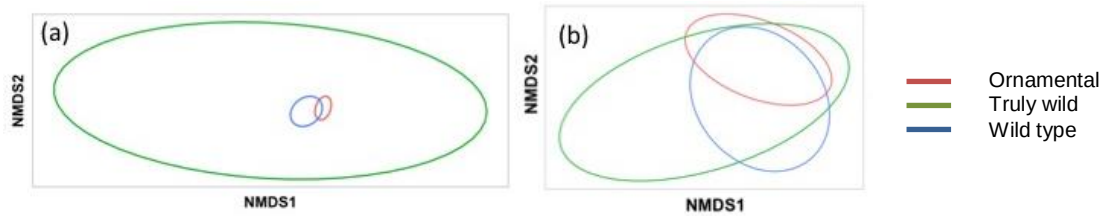

Figure 4.3: Difference in Major Histocompatibility Complex (a) allelic and (b) supertype composition between ornamental (red), truly wild (green) and wild type (blue) guppies (*Poecilia reticulata*) based on non-metric multidimensional scaling.

4.4. DISCUSSION

The current study aimed to identify whether particular supertypes were implicated in the absence of particular parasites, and to assess the differences in MHC genetic composition between truly wild, wild type and ornamental guppies (*Poecilia reticulata*). There was a significant association between the presence of particular MHC class II functional (supertype) groups, rather than diversity, and parasite community composition. Truly wild fish had significantly greater MHC allelic and supertype community compared to wild type and ornamental fish.

One evolutionary objective of parasites is to evade recognition by the host immune system, while the hosts try to ensure sufficient MHC diversity to defend themselves against pathogens. Parasite-mediated selection is the coevolution of the host and the parasites over a short time span, whereby the host maintains the particular MHC alleles that are required to defend against the local parasites (Woolhouse et al. 2002; Blanquart et al. 2016). In the current study, this appears to be reflected in the variation in parasite community and MHC supertype richness across rivers, supporting theory for complex evolutionary interplay between host and parasites. Associations between certain MHC alleles and single pathogen infection have previously been reported across multiple studies (e.g. Hill et al., 1991; Thursz et al. 1995; Langefors et al. 2001; Meyer and Thomson 2001). Several previous studies have also identified that MHC supertype diversity has a significant effect on parasite communities (e.g. Wegner et al. 2003; Pilosof et al. 2014).

Pilosof et al. (2014) demonstrated the existence of a link between particular supertypes and individual pathogen resistance in wild hosts, but no association between supertype diversity and resistance. The current study supports this finding, with a significant association between the presence of particular MHC functional (supertype) groups, rather than diversity, and parasite community composition. These associations appear species-specific; the effect of ST4 on gyrodactylid species was not detected for *Gyrodactylus turnbulli* (in Chapter 3). This is likely an indication that the wild populations are infected with a range of different gyrodactylid species and strains (Xavier et al. 2015). Whilst there may be a role for pathogen life history, and local biotic and abiotic variables, in explaining the differences in parasite community between rivers (Tomás et al. 2008; Martínez-de la Puente et al. 2009), the specificity of peptide-binding region of the alleles within each supertype cluster (Janeway 2005) could explain why the presence of particular supertypes significantly reduces the risk of being infected with specific parasites.

Truly wild, wild type and ornamental fish have significantly different MHC allelic genetic composition, but not supertype diversity. Even the wild type fish have reduced genetic diversity compared to the wild fish, showing how genetic drift erodes allelic MHC variation, but not MHC supertype variation. This highlights that results using wild type fish as a model for wild hosts should be interpreted with caution. The wild type fish used in the current study had been kept in captivity for ~4

years since they were removed from the wild. During this time they have been allowed to select mates naturally from the population, ensuring that as much allelic and functional diversity has been maintained as possible. It is unsurprising that domestication reduces genetic diversity, but in some exceptional model systems this is not the case; MHC diversity of the selfing killifish (*Kryptoblebias marmoratus*) was similar to that of wild conspecifics after 10 generations in the laboratory (Ellison et al. 2012b). The rapid loss of genetic diversity when wild fish are maintained in captivity from small founder populations has implications for aquaculture, through increased risk of pathogen related mortality. Replenishing cultured stocks regularly with wild fish would reduce the risk to genetic diversity and could increase host pathogen resistance. Maintaining genetic diversity in aquaculture is important, not only to increase the resistance of fish to the local parasites, but also to minimise the impact if a novel pathogen is accidently introduced into the stock.

ACKNOWLEDGMENTS

Data for this chapter was collected with laboratory assistance from Michael Reynolds. Wild type fish were sourced from Darren Croft.

SUPPLEMENTARY MATERIAL

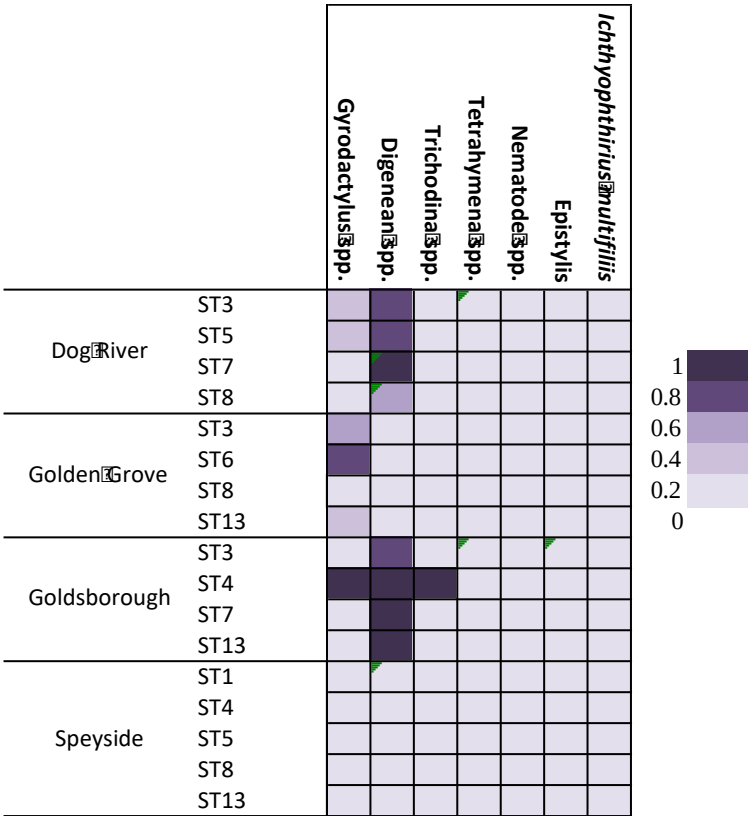

Figure S4.1: The proportion of individuals that have a particular Major Histocompatibility Complex supertype (ST) and are infected with each parasite (*Gyrodactylus* spp., digenean species, *Trichodina* spp., nematode species, *Epistylis*, *Ichthyophthirius multifiliis*) from each river (Dog River, Golden Grove, Goldsborough and Speyside). Dark = high number of individuals have a supertype and are infected. White = no individuals have a supertype and are infected with the parasite.

CHAPTER 5

Release of ornamental guppies (*Poecilia reticulata*) elevates parasite load and incidence of *Gyrodactylus turnbulli* on wild-type guppies

ABSTRACT

Animal farming and selective breeding are common practice to meet the demands of the pet trade and animal-derived protein for human consumption. The restricted gene mixing and genetic diversity characteristic of captive-bred populations can adversely affect offspring fecundity, mating and susceptibility to stressors, including parasitic infection. Understanding the risks of release of captive bred fish into wild populations on parasite transmission is one of importance for sustainable aquaculture, fisheries and fish conservation. The current study uses the popular pet and highly invasive guppy (*Poecilia reticulata*) to determine whether the release of an infected or uninfected ornamental 'invasive' individual into a wild population affects parasite abundance, prevalence and transmission. Domesticated guppies have reduced genetic diversity and increased susceptibility to *Gyrodactylus turnbulli* compared to their wild and wild type conspecifics. The introduction of an ornamental guppy increased prevalence and abundance of *G. turnbulli* in a wild type population. The introduction of an infected ornamental guppy into a wild type shoal initially significantly increased *Gyrodactylus turnbulli* abundance. Following an initial time lag, this led to significantly higher parasite prevalence. This study proposes a mechanism underlying patterns of parasite abundance and prevalence resulting from interactions between cultured and wild type fish populations. It is important for aquaculture, conservation and the ornamental pet trade to consider the risks that are associated with the release of domesticated individuals and take measures to prevent parasite spill-over between captive-bred and wild populations.

5.1. INTRODUCTION

Animal farming is common practice to meet the demands of the pet trade and animal-derived protein for human consumption, often leading to morphological, behavioural and physiological characteristic differences from wild individuals. Selective breeding of captive animal stocks to maintain commercially valuable traits, such as increased body quality (Gjedrem et al. 2012), growth rate (Cook et al. 2000), disease resistance (Calvo et al. 2003) or morphological attributes, is a fundamental practice of agriculture, aquaculture and the pet trade globally. Though economically beneficial, the restricted gene mixing and resultant increase in homozygosity (Charlesworth and Willis 2009; Hedrick and Kalinowski 2000) can adversely affect offspring fecundity (Radwan 2003), mating and susceptibility to stressors, including parasitic infection (Chapter 2).

Captive breeding can lead to increased levels of inbreeding (Miller and Hedrick, 1993; Woodworth et al. 2002). Inbred individuals tend to be more susceptible to infectious diseases, compared to genetically diverse conspecifics (Coltman et al. 1999; Cassinello et al. 2001; Chapters 2, 3 and 4), and are particularly vulnerable to novel infections (van Oosterhout et al. 2007b). The acquired immunity associated with previous pathogen exposure is reduced or lacking in captive-bred individuals (Matthews et al. 2006). As infections in cultured stocks are often maintained at low levels, through preventative medication, immune function is assumed to be negligible (van Rijn 1996). Frequent pathogen treatment in captive populations can lead to drug resistance and promote the selection of new pathogen strains, to which wild populations may be naïve (Pulkkinen et al. 2010; Aaen et al. 2015). In contrast, wild populations are typically exposed to trickle infections of natural pathogens, which boosts acquired immunity (Altizer et al. 2003; Mathews et al. 2006). The risk of parasite spill-over and spill-back between captive and wild populations is of particular concern because of the different nature of these host populations (Pulkkinen et al. 2010; Johansen et al. 2011; Kurath and Winton 2011; Chapters 3 and 4).

Fish farming has a net global value of over US$217.5 billion annually, with stocks of Silver carp, Common carp and Atlantic salmon among the most commercially valuable (Naylor et al. 2000; FAO 2010). Intensification of aquaculture has led to

individual or *en masse* release of farmed fish, both accidentally (via breaks in nets or natural disasters, such as floods; McGinnity et al. 2003; Naylor et al. 2004; Laikre et al. 2010) and intentionally (unwanted pets, conservation or fishery enhancement to supplement the natural population and increase harvest potential; Philippart 1995; Copp et al. 2006; Lorenzen 2008; Bell et al. 2008). Between 2001 and 2012, 4.6 million salmon were reported to have escaped from Norwegian fish farms (The Directorate of Fisheries, Norway 2017). The survival success of fish released into a new population or location is dependent on their health, phenotype and genotype, environmental conditions and the format of the introduction programme (Fausch et al. 2001; van Oosterhout et al. 2007b; Faria et al. 2010; Forsman 2014). These factors in turn can also influence the impact of the introduced individuals on natural populations. Escaped domesticated fish often survive sufficiently well to interact with wild fish, ecologically and genetically. Cultured animals are not as effective as wild individuals at foraging (Ersbak and Haase 1983; Stoinski et al. 2003), predator evasion (Berejikian, 1999; Fairchild and Howell 2004), and controlling their parasite burden (Chapter 3). Domesticated fish are, however, successful invaders with 288 species of ornamental fish introduced in India alone (Bijukumar 2000).

Open systems, commonly used for fish farming, provide a limited barrier between wild and captive populations (Johansen et al. 2011; Jackson et al. 2015). Furthermore, pathogen exchange between wild and captive fish is amplified by the attraction of wild fish towards the food and shelter provided by fish farms (Naylor et al. 2000; Dempster et al. 2009). Strains of *Streptococcus iniae* and *Mycobacterium marinum*, for example, were identified in wild fish 2 km from their source caged farmed fish (Diamant et al. 2000; Colorni et al. 2002; Kvitt and Colorni 2004). The risk of pathogen transmission is also increased through the movement of aquaculture stocks (Naylor et al. 2000). Movement of Atlantic salmon, for aquaculture and fishery enhancement in Europe, has facilitated pathogen outbreaks, including the notorious epidemics of furunculosis and *Gyrodactylus salaris* (see McVicar 1997). Understanding the risks of deliberate and accidental release of captive bred fish into wild populations on parasite transmission is one of importance for sustainable aquaculture, fisheries and fish conservation (Lorenzen et al. 2012).

This study uses the guppy (*Poecilia reticulata*)–monogenean ectoparasite (*Gyrodactylus turnbulli*) system as a model for parasite transmission dynamics.

Gyrodactylids increase the risk of secondary infection, and are directly transmitted, increasing the chance of pathogen transfer between wild and captive populations (Wootten 1989; Sasal et al. 2004). Guppies are small, live-bearing, sexually dimorphic tropical fish, native to the streams of Trinidad, Tobago and South America (Houde 1997), that are an important ecological and evolutionary model (Magurran 2005). They are also one of the most common home-aquaria ornamental fish, a trade estimated to be worth US$ 278 million (with approximately one billion ornamental fish exported annually). Over 100 guppy strains have been bred, selected largely on the basis of male colour patterns (FAO 1996-2005; Dykman 2012). Like other poeciliids, guppies are sold worldwide, including in their native regions, contributing to their release into the wild (Axelrod et al. 1995). They have also been widely introduced for mosquito control (Elias et al. 1995; Cavalcanti et al. 2007). The adaptability and breeding capabilities of guppies, enabling them to create viable populations from as little as one viviparous pregnant female, increases their chance of invasion success (Magurran 2005), so introduction programmes of this species must be carefully managed (Deacon et al. 2011). When they invade, they can gain the same benefits in heterospecific groups of native species as they can in a homospecific shoal (Camacho-Cervantes et al. 2014).

Guppies are genetically diverse, but selective breeding has reduced the allelic richness of domesticated strains compared to wild populations (van Oosterhout et al. 2006a; Chapter 3). Ornamental guppies have reduced immune function compared to wild types, whereby parasite (*G. turnbulli*) intensity exponentially increases on ornamental fish, with no apparent intervening immune response (van Oosterhout et al. 2006a; Chapter 3). This level of susceptibility can eventually lead to death of the fish. Wild type guppies, in contrast, often exhibit an effective immune response to infection; parasite intensity increases initially and then reduces to low levels, suggesting resistance (Chapter 3). This reduces the harm potentially caused by an infectious disease by limiting infection trajectory, through avoidance or infection clearance (Horns and Hood 2012). The primary aim of the current study is to determine whether the release of an infected or uninfected ornamental 'invasive' guppy (*P. reticulata*) into a wild guppy population affects parasite abundance, prevalence and transmission within the population. It is hypothesised that: (1) the presence of an introduced ornamental guppy (infected or uninfected) will increase parasite abundance and persistence within each population; and (2) populations in

which the introduced ornamental guppy is infected will show a higher transmission, leading to higher prevalence, than when an uninfected ornamental or wild type fish is introduced.

5.2. MATERIALS AND METHODS

5.2.1. Fish stocks

Experimental wild type guppies (*Poecilia reticulata*) originating from the Lower Aripo (10°35'00"N 61°14'00"W) and Tacarigua (10°37'00"N 61°24'00"W), Trinidad, were imported in 2002, 2004 and 2007 from Trinidad to Hull University and were transferred to and maintained at Cardiff University in 2015. Ornamental guppies were initially purchased from Frisby Aquatics in 2005 and reared at Hull University, before being transferred to Cardiff University in 2015. Fish were housed in mixed sex stock tanks, with an under-gravel filter, under 12 h light, 12 h dark photoperiod regime, at 24 ± 1 °C. They were fed on a diet of AQUARIAN® tropical fish flakes and bloodworm.

5.2.2. Experimental set-up

Groups of five female wild type guppies (n = 21), selected to represent a natural shoal size (Croft et al. 2003), were size-matched from stock tanks. These shoals were maintained in 20 L aquaria with plastic refugia, for a minimum of 2 weeks, to allow for familiarization (Griffiths and Magurran 1997). Each shoal was then moved to an experimental 70 L aquarium with an under-gravel filter, 2 cm of pea gravel and two plastic refugia. The shoals were left to acclimatise for 24 h. Additional unfamiliar fish, to be introduced to each shoal, were isolated from stock tanks in 1 L pots of dechlorinated water, 7 d prior to introduction. Each 1 L pot was 50% obscured with black opaque plastic, to ensure complete isolation from adjacent pots, while still providing sufficient light. The fish were fed twice daily and water changed every 2 d.

There were three experimental groups: (1) infected ornamentals, (2) uninfected ornamentals, and (3) uninfected, control, wild types. One fish from each of these categories was introduced to a group of 5 wild type fish (see Table 5.1). Comparing treatments (1) and (2) will test whether introducing an infection elevates the parasite

incidence and load of the resident wild type fish. The comparison between (2) and (3) will test whether ornamental fish increase the transmission efficiency of the infection (and hence the parasite load on the wild type fish) by being more susceptible to *Gyrodactylus* infections. For each of the three experimental manipulations, only one fish in each shoal was infected with 18 individuals of *G. turnbulli* (either the introduced fish or a 'resident' fish; see Table 5.1), to resemble the natural abundance in a population of this size (Supplementary Material: Table S5.1). The introduced size-matched fish was either a wild type or ornamental female guppy. For the 'infected ornamental introduced' (1) experimental manipulation, the introduced fish was infected (Table 5.1). In the case of experimental manipulations (2) and (3), the introduced fish was uninfected, and instead one of the resident fish was infected. This design will reveal whether it is purely the presence of the ornamental fish that increases parasite prevalence and abundance, or if the higher tolerance of the ornamental donor drives parasite dynamics. All fish were fed with 3 individuals of *Daphnia* sp. and left in isolation for 30 min prior to being returned to their respective experimental tanks.

Table 5.1: Experimental design: for each of the three experimental manipulations, only one fish in each shoal (six fish in total) was infected with 18 *Gyrodactylus turnbulli*. The initial resident shoal consisted of five uninfected wild type female guppies, to which a single wild type or ornamental fish was introduced. Either one fish of the resident wild type shoal or the introduced ornamental was infected.

Experimental manipulation	Origin of infected individual	Origin of introduced individual
(1) Infected ornamental introduced	Introduced	Ornamental
(2) Uninfected ornamental introduced	Resident	Ornamental
(3) Control	Resident	Wild type

5.2.3. Experimental infection and monitoring

The Gt3 strain of *Gyrodactylus turnbulli* was utilised for the experimental infections. In brief, fish were lightly anaesthetized with 0.2% MS222 and either sham infected (handled as per the infection fish) or 18 worms were naturally transferred from a donor fish to the recipient (following methods of King and Cable 2007). All uninfected fish were anaesthetized and manipulated to mimic an infection (sham-

infected). The day following infection, all fish were re-examined and we confirmed that the infection had taken on all infected fish and that no transmission had occurred to the uninfected fish. Parasite infection was monitored daily for 17 d by lightly anaesthetising each fish from the shoal and recording the absence or number of worms.

5.2.4. Statistical analysis

Statistical analyses were conducted using R statistical software (version 1.0.136, RStudio 2009-2016 RStudio, Inc.).

Generalised Linear Mixed Models (GLMMs) were used to investigate the effects of experimental manipulation group on *G. turnbulli* prevalence (as defined by Margolis et al. 1982) and abundance (total number of individuals of a particular parasite species in a sample of hosts, as defined by Bush et al. 1997) in a population of *P. reticulata*. Model 1 used a GLMM with negative binomial distribution and zero inflation, using the *glmmadmb* package, and the nbinom function with "log" link function, to identify variables affecting *G. turnbulli* abundance. The fixed terms included in the original model were experimental manipulation, *G. turnbulli* prevalence, day and the interactions between prevalence × day, experimental manipulation group × prevalence and experimental manipulation group × day (Table 5.2, model 1). Model refinement suggested the removal of the experimental manipulation group × prevalence interaction to make the model the most robust. Variables affecting prevalence were analysed using a GLMM with Gaussian distribution, using the *lme4* package in model 1. The prevalence of *G. turnbulli* was used as the dependent term with fixed explanatory terms including experimental manipulation group, *G. turnbulli* abundance and day, as well as the interaction between experimental manipulation group × day, experimental manipulation group × *G. turnbulli* abundance, *G. turnbulli* abundance × day (Table 5.2, model 2). After model refinement, using drop1, all terms were retained. As parasite prevalence and abundance was recorded for each individual shoal at different time points, 'shoal' was included as a random effect in both GLMMs to avoid pseudo-replication.

5.3. RESULTS

5.3.1. Gyrodactylus turnbulli *abundance*

The abundance of *Gyrodactylus turnbulli* in the population was significantly higher when an infected ornamental fish was introduced (1), compared to the control (3) and the introduction of an uninfected ornamental (2; Table 5.2, model 1). Parasite abundance was not significantly different between the control (3) and the introduction of an uninfected ornamental fish (2; Table 5.2, model 1). Although parasite abundance initially increased in the population with an introduced infected ornamental (1), it reduced to similar levels to the wild type control (3) population by day 17 (Fig. 5.1a). There was significantly higher *G. turnbulli* abundance when an infected ornamental was introduced (1) from day 2 to 11, compared to the control (3) and from day 4 to 9 compared to uninfected ornamental introduced (2; Table 5.2, model 1; Fig 5.1a).

5.3.2. Gyrodactylus turnbulli *prevalence*

Following the introduction of parasites, the presence of an infected ornamental fish (1) led to significantly higher *G. turnbulli* prevalence overall, compared to the infected wild type population (3) or introduction of an uninfected ornamental (2; Table 5.2, model 2; Fig. 5.1b). Initially (until day 6), however, introduction of an uninfected wild type fish into an infected population (3) increased *G. turnbulli* prevalence to a higher level than with the introduction of an infected (1) or uninfected ornamental fish (2; Fig 5.1b). During the latter half of the experimental period, *G. turnbulli* prevalence was significantly higher when an infected ornamental (1) had been introduced than the other experimental manipulation groups (infection of a resident wild type fish), and increased even as abundance reduced (Table 5.2, model 2).

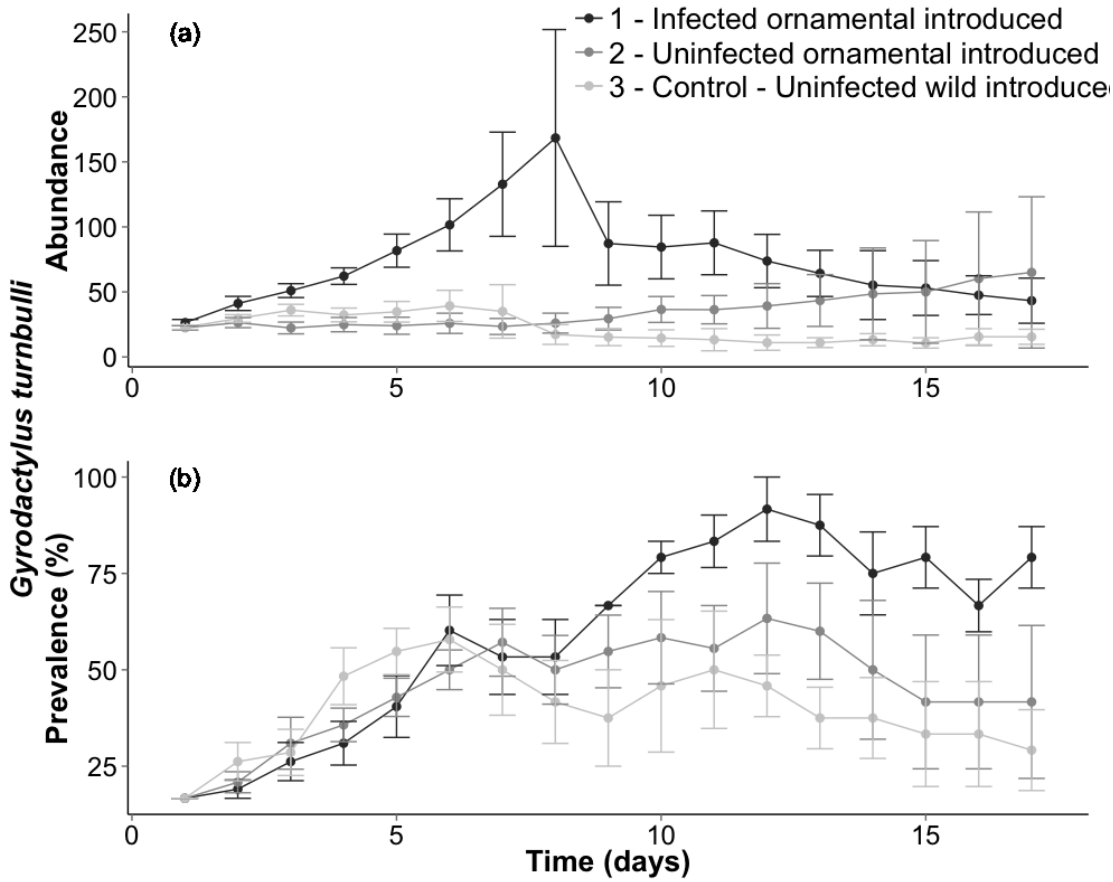

Figure 5.1: Daily (a) abundance and (b) prevalence (± standard error) of *Gyrodactylus turnbulli* infection in wild type and ornamental shoals of guppies (*Poecilia reticulata*). 1 - Infected ornamental introduced (•); 2 - uninfected ornamental introduced (•); and 3 - control - uninfected wild type introduced (•).

Table 5.2: ANOVA model output reporting the factors influencing: total (1) *Gyrodactylus turnbulli* abundance; and (2) *G. turnbulli* prevalence, in a *Poecilia reticulata* population. Highlighted predictors indicate *P* values <0.05 and are, therefore, considered significant.

Model	Dependant term	Fixed term	*D.f.*	*F*	*P*
1	*G. turnbulli* abundance	Experimental manipulation group	2	4.990	0.008
		Prevalence of *G. turnbulli*	1	19.915	≤ 0.001
		Day	1	15.135	≤ 0.001
		Prevalence of *G. turnbulli* × Day	1	10.157	0.002
		Experimental manipulation group × Day	1	4.644	0.0032
2	*G. turnbulli* prevalence	Experimental manipulation group	2	0.297	0.747
		Day	1	82.071	≤ 0.001
		G. turnbulli abundance	1	18.525	≤ 0.001
		Experimental manipulation group × *G. turnbulli* abundance	2	7.363	≤ 0.001
		Experimental manipulation group × Day	2	5.204	0.002
		Day × *G. turnbulli* abundance	1	9.585	0.002

5.4. DISCUSSION

Previous studies have used molecular identification to determine the fish farm origin of parasites (e.g. Diamant et al. 2000; Colorni et al. 2002; Kvitt and Colorni 2004). The present study is believed to be the first of its kind, using the guppy and a common gyrodactylid as a model to determine the disease impact of introducing an infected or uninfected cultured fish into a wild type host population. The introduction of an infected ornamental guppy into a wild type shoal initially significantly increased *Gyrodactylus turnbulli* abundance. Following an initial time lag, this led to significantly higher parasite prevalence. Guppies are opportunistic and highly plastic, but their success as an invader depends on individual characteristics and factors local to the site of introduction (Magurran 2005). MHC genetic diversity is reduced in ornamental fish, due to inbreeding from artificial selection, leading to increased parasite susceptibility (Chapter 3). The inability of an ornamental fish to resist

infection increased transmission to wild type conspecifics. Overall, this increased parasite abundance and prevalence within a wild type population. Although wild type fish are generally resistant to *G. turnbulli* infection, when individually housed (Chapter 4), they may not completely fight off infection when continually being re-infected by an ornamental parasite reservoir. Although not directly quantified in this study, both wild type and ornamental guppies manage to shed their *Gyrodactylus* infections by "reversing into" uninfected individuals, increasing contact and consequently transmission (Croft et al. 2011; personal observation), this behaviour would lead to continual reinfection of the wild type fish by the susceptible ornamental. To explain this in the context of epidemiological theory of directly transmitted parasites; the parasite's reproductive ratio (R_0) is the product of the transmission efficiency (b), the contact rate (c) and the duration that an infected host is contagious (d) (Lipsitch and Moxon 1997; Heesterbeek 2002; Roberts 2007; Cross et al. 2007). Hence, by increasing the transmission efficiency (b) when introducing the generally more susceptible ornamental guppies, parasite load also increased in the recipient population.

Intuitively, increased parasite abundance had a positive effect on prevalence. Experimental manipulation group, however, determined whether prevalence and abundance showed the same pattern over time. The abundance of *G. turnbulli*, when an ornamental was introduced, was significantly higher overall, compared to that of the control (wild type only). This is likely due to the presence of a susceptible ornamental fish (Chapter 3). The current study found that when an infected ornamental fish was introduced into a familiarised, uninfected wild type population, parasite abundance increased more quickly through time than when the infection had been initiated in the resident wild type population (and the introduced fish, either ornamental or wild type, was uninfected). This is likely due to wild type fish eliciting a more efficient immune response (Chapter 3). The pattern of abundance over time was, however, dependent on whether the ornamental fish was infected when introduced, or infection was transmitted from the wild type population. Once the ornamental fish became infected, parasite intensity on that individual increased, leading to the overall population parasite abundance increasing. Thus, our study supports Faria et al.'s (2010) suggestion that parasite abundance could increase with the introduction of susceptible fish, potentially promoting an epidemic.

Parasite prevalence initially increased most quickly when the infection was initiated in the wild type shoal (albeit at relatively low abundance), rather than via an introduced individual. Fish from the wild type population were familiarised, which is likely to have led to the infected wild type fish physically interacting with their conspecifics (Griffiths and Magurran 1997), facilitating direct parasite transmission. In contrast, parasite prevalence (unlike abundance) was initially slow to increase after the introduction of an infected, unfamiliar, ornamental fish to an uninfected, familiarised population. This may reflect the time taken for the ornamental fish to integrate into the social structure (shown to take 5 days to stabilise in guppies; Reynolds et al. unpublished data). In contrast to prevalence, abundance of *G. turnbulli* began to reduce between days 5-7, when an infected ornamental was introduced. This is likely due to the resistance of wild type fish to parasitism and also due to unsuccessful transmission of the parasites leading to their demise.

The current study highlights one potential mechanism for wild populations to be effected by escaped farmed individuals. Pathogen release from fish farms is reported relatively frequently (Johnsen and Jensen 1991; Johansen et al. 2011; reviewed in Costello 2009). *Gyrodactylus salaris* in Norwegian wild populations reportedly originated from Sweden, via a fish farm in Norway, and subsequently spread to salmon rivers (Johnsen and Jensen 1986, 1991). Infected escapee salmon may spread pathogens over vast distances due to their dispersal and interaction with wild conspecifics (Skilbrei et al. 2010; Madhun et al. 2015). Prevalence of gyrodactylids on juvenile tilapia from fish farms has been reported at 73% (Ellison et al. unpublished data). This suggests that, if a cultured fish escaped aquaculture pens, there is a high risk it would be carrying pathogens, easily transmitting them to the wild populations. Prevalence of the parasite is likely to increase in the population.

This study is believed to be the first to propose a mechanism underlying patterns of parasite abundance and prevalence resulting from interactions between cultured and wild type fish populations. The results could have implications for supplementary stocking of fish populations, whereby cultured, and potentially susceptible, juvenile fish are released to augment harvestable stocks. Greater susceptibility of farmed or cultured individuals to parasitic infection is likely to increase parasite abundance and prevalence within a wild population, following social integration of an introduced individual. Introduced or escaped captive individuals could lead to the dispersal of

novel parasites into naïve wild populations with potential implications for the long-term viability of the population in the absence of an effective host immune response. It is important for aquaculture and the ornamental pet trade to consider the risks that are associated with the release of domesticated individuals and take measures to prevent parasite spill-over between captive-bred and wild populations.

ACKNOWLEDGMENTS

Data for this chapter was collected with laboratory assistance from Dayna Lea. Wild type fish were sourced from Darren Croft and the Red and Black strain from Alan Smith.

SUPPLEMENTARY MATRIAL

Table S5.1: Mean intensity of *Gyrodactylus* spp. infecting *Poecilia reticulata* from 5 sampling sites across 3 rivers (Dog River, Goldsborough and Golden Grove) in Tobago, collected in 2015. These fish were collected as part of Reynolds et al. submitted and Chapter 4.

Site	*Gyrodactylus* mean intensity (range)	*Gyrodactylus* prevalence (%)	Sample size (n)
Dog River 1	3.22 (1-8)	31.03	31
Dog River 2	2.5 (1-7)	33.33	30
Goldsborough 2	1 (1-1)	4.17	26
Goldsborough 3	3 (1-4)	65.62	5
Golden Grove 1	3.21 (1-14)	289.47	30

CHAPTER 6

Identifying the effects of sexual selection, Major Histocompatibility Complex and parasitism on guppy (*Poecilia reticulata*) mate choice

ABSTRACT

Mate choice is a crucial determinant of inclusive fitness and, in vertebrates; the Major Histocompatibility Complex (MHC) has been identified as the single most important gene in sexual selection. Male phenotypic traits often provide a reliable signal about their immune-competence, the level of inbreeding and heterozygosity, and the ability to acquire and provide resources in their environment. Females are thought to prefer males with complementary MHC genotypes, expected to generate offspring with the best chance of possessing genes enabling effective response to prevailing infectious diseases. Previous studies that have focused on one aspect of mate choice may not have accounted for variation between individuals in other aspects of mate choice cues. The current study identifies the effects of sexual selection, MHC class II and parasitism on female guppy (*Poecilia reticulata*) mate choice and aims to determine which characteristics play the dominant role in a female's preference of a male. When accounting for multiple variables, the current study showed that colour interacting with shared MHC alleles, strain similarity, parasitism and host size are all important explanatory variables in female guppy mate choice. Female guppies spent more time interacting with males with higher proportion of red colouration who shared less MHC alleles. When presented with three males to choose from, a female spent significantly more time in close proximity to males: that were not infected with *Gyrodactylus turnbulli*; of the same strain as herself; with a greater standard length; and with high levels of red pigment (lower colour hue). When a female is presented with a single male, in a paired interaction, a significant preference was shown for redder males who share fewer MHC alleles with the female. This highlights that future studies need to address the complexity of partner selection.

6.1. INTRODUCTION

Mate choice is a crucial determinant of inclusive fitness, and in vertebrates, the Major Histocompatibility complex (MHC) has been identified as an important group of genes in sexual selection. As females have higher gamete investment, they tend to be choosier than males when identifying a mate. Mate choice can either have direct or indirect benefits to the female. Direct benefits result from mate selection for immediate gain, such as parental care or quality territory (Williams 1975; Alatalo et al. 1986). These material benefits reduce the energy required by the female to rear her offspring. Alternatively, mate choice may be based on 'good genes' (Trivers 1972; Milinski and Bakker 1990, 1992; Barber et al. 2001) or 'optimal' gene diversity (Eizaguirre and Lenz 2010). The 'Good Gene Hypothesis' is supported in species where there appears to be no provision for the offspring by the male. Good phenotypic condition and traits that express high genetic quality are often selected during female mate choice (Iwasa et al. 1991; Kirkpatrick and Ryan 1991; Pfennig 1998; Andersson and Simmons 2006), to increase offspring fitness (Fisher 1930; Zahavi 1975). Although evidence exists for a range of different selective forces on female mate choice, interactions between potentially important factors, such as sexual selection, genetics and parasitism, are not well understood.

Male phenotypic traits often provide a reliable signal about their immune-competence (Verhulst et al. 1999; Blount et al. 2003), the level of inbreeding and heterozygosity (van Oosterhout et al. 2003b), and the ability to acquire and provide resources in their environment (Snowberg and Benkman 2009), by indicating their overall fitness (cf. the "good genes hypothesis"). Carotenoids enhance immune function in most animals, but they must be assimilated from the environment (Milinski and Bakker 1990; Barreiro Lozano 1994). Excess carotenoids provide and augment orange colouration; thus the brighter the orange, the stronger the immune function and the more successful the animal is at exploiting the environment (Brooks and Endler 2001). In carotenoid-poor environments, male guppies that obtain carotenoids have brighter orange colouration than those that are less successful. This indicates effective foraging and predator avoidance, despite their conspicuous colour (Endler 1980). Parasite infection can also lead to changes in fish colouration (Houde and Torio 1992), as carotenoids are diverted to the host's immune response (Barreiro Lozano 1994), leading to an alteration in an individual's attractiveness to a mate

(Houde and Torio 1992).

Inbred individuals, with reduced genetic diversity, tend to be more susceptible to parasitic infection (Chapters 2, 3 and 4), and hence, females are thought to have a preference for less inbred males. An inbred male, however, may (theoretically) still be a suitable mate because alleles (rather than genotypes) are transmitted to the offspring in sexually reproducing organisms. In other words, a completely homozygous male could (again theoretically) father completely heterozygous offspring if mating with a female with complementary alleles. Hence, rather than paternal heterozygosity *per se*, females are thought to prefer males with complementary genotypes, as this is expected to generate genetically diverse offspring with the best chance of possessing genes enabling effective response to prevailing infectious disease in the local environment (Milinski 2006). Females have been reported to select mates based on resistance against parasites and pathogens (Hamilton and Zuk 1982). Individuals tend to avoid infected conspecifics by identifying chemical cues released by the infected individual (amphibians, Kiesecker et al. 1999; mammals, Kavaliers et al. 2005; fish, Stephenson and Reynolds 2016). Females have shown reduced preference for parasitised males, due to the reduced colour intensity (sticklebacks; Milinski and Bakker 1990) and lower display rate (guppies; Kennedy et al. 1987) of infected males. Parasite susceptibility has been linked to MHC (Zhang et al. 2015; Lei et al. 2016).

The highly polymorphic MHC is a large cluster of genes involved in the immune response and molecular self/non-self discrimination. Individuals with a high MHC diversity can present antigens from a greater variety of infectious diseases to the T-lymphocytes, via the MHC peptide-binding region. Mates with contrasting MHC alleles tend to be selected during mate choice experiments, increasing offspring fitness by ensuring MHC diversity (including: Yamazaki et al. 1976; Reusch et al. 2001; Aeschlimann et al. 2003; Milinski 2005, 2006; Agbali et al. 2010; Ejsmond et al. 2014; exception Pitcher and Neff 2007). A mate attractiveness mechanism related to odour has been suggested that is associated with MHC-mediated sensory discrimination (Yamazaki et al. 1979; Wedekind and Füri 1997). Females tend to choose mates with a dissimilar odour (and, therefore, MHC genotype) to themselves, increasing allelic diversity in their offspring (Milinski 2006; Eizaguirre et al. 2009; Kalbe et al. 2009). This mechanism may aid inbreeding avoidance and kin

recognition (Potts and Wakeland 1990; Penn 2002). In contrast to the suggestion that females select mates to maximise MHC genotype dissimilarity (Reusch et al. 2001; Wegner et al. 2004), the premise of the 'Optimum MHC Theory' is that an optimal level of MHC dissimilarity between mates is important in resistance to local parasitism (Eizaguirre and Lenz 2010). An excessively high MHC diversity has been predicted to deplete the T-cell repertoire and impair the immune response of individuals (Novak 1994). The optimal level of individual MHC diversity ensures that individual lifetime reproductive success is maximised (Kalbe et al. 2009).

This study uses the guppy (*Poecilia reticulata*) as a model organism to attempt to disentangle the multiple variables that influence mate choice. These small, tropical fish are sexually dimorphic livebearers with internal fertilisation. Orange and red, carotenoid, colouration in male guppies is considered an honest signal for male quality and females show active preference for males with more orange (Houde 1987). Male guppy colour is diverse and the size of a male's orange spots is heritable, but the colour saturation (chroma) is dependent on individual condition, reflecting carotenoid ingestion and parasite load (Kodric- Brown 1989; Houde and Torio 1992; Houde et al. 1997; Grether 2000; Brooks and Endler 2001). Some females also show a strong preference for males with large areas of black, melanin, colouration, but all females prefer males with larger orange patches. Carotenoid- and melanin-based male colouration, and female preference for these characteristics, can respond independently to selection and are, therefore, considered genetically uncorrelated (Brooks and Endler 2001). This suggests that black and orange colouration in male guppies serve different functions in mate signaling (Møller and Pomiankowski 1993). Male guppies perform elaborate, conspicuous courtship pursuits and displays, providing a clear mechanism for monitoring mate choice and courtship behaviours (Baerends et al. 1955; Liley 1966; Guevara Fiore et al. 2010). The red colouration on male guppies becomes paler after prolonged *Gyrodactylus turnbulli* infection and females prefer males with relatively few parasites (Kennedy et al. 1987; Houde and Torio 1992).

Most mate choice studies focus on sexual selection, parasitism or genetics; this is believed to be the first study to investigate these multiple factors simultaneously. The current study aims to explore: (1) whether a female selects and courts with a mate based on MHC or male morphology; (2) how female preference changes with

parasitic infection status; and (3) which characteristics (MHC, colour, infection status or size) play the dominant role in a female's preference of a male. The following specific hypotheses are tested: (1) females will choose a mate that is most MHC class II dissimilar to herself, to increase the genetic diversity of her offspring; (2) female mate choice will reflect male MHC genetics over morphology (colour and size) and infection status; and (3) females will show less interest in males if the males subsequently become infected.

6.2. MATERIALS AND METHODS

6.2.1. Host population

Ornamental guppies were purchased from Tartan Guppy (Black strain) and Frisby Aquatics (Red strain) in 2015 and 2005, respectively, and reared at Hull University before being transferred to Cardiff University in 2015. These strains have been in domestication for up to 300 generations (van Oosterhout pers. comm.). All fish were maintained at Cardiff University under 24 ± 1 ºC and 12 h light: 12 h dark cycle and fed twice daily with AQUARIAN® tropical fish flakes and weekly with frozen bloodworms. The current study used 32 females (Black, n = 16; Red, n = 16) and 96 males (Black, n = 48; Red, n = 48). Fish were removed from mixed sex stock tanks upon sexual maturity, to guarantee virgin females, and isolated in single sex tanks.

6.2.2. Experimental infection

To identify how mate preference varied with parasitism, male guppies (see Section 6.2.3.2), were experimentally infected with *G. turnbulli* from the Gt3 strain, which have been maintained in a laboratory culture since isolation from Nottingham aquarium shop guppy in 1997. Experimental infections, 30 worms transferred from the donor to the caudal fin of the recipient, were achieved following methods outlined in Chapter 3 (3.2.4). Males were isolated in clean water for 18 h to ensure recovery from the anaesthetic before being introduced to the experimental chamber.

6.2.3. Mate choice

Individuals were isolated in 1 L tanks for 1 week prior to introduction to the experimental chamber. Eight combinations of three males and one female were used in two mate choice set ups (Table 6.1). Each female was allowed to directly choose between three non-interacting males (Mate choice chamber) and then subsequently had the opportunity to interact with each of the same males individually, whilst excluding direct male-male competition (Paired behavioural interactions). All trials were conducted between 7:00 a.m. and 12:00 midday. Males were infected following the mate choice chamber and paired behavioural interaction trials on the first day (as described in 6.2.2.) and both trials repeated on the following day with infected males (day 2).

6.2.3.1 Mate choice chamber

To assess mate preference of a female, an experimental choice chamber was used to determine the proportion of time a female spent investigating each of three males presented simultaneously. The experimental arena consisted of a 30 × 60 cm glass tank, divided into sections (Fig. 6.1). Transparent polycarbonate sheet was used to create channels, leading to males segregated from one another by opaque tiles and restricted to the end of the channel by a plastic mesh (1 mm). The sides and bottom of both end sections were covered in white tiles to prevent the males from being able to make contact with one another and to reduce reflection. A removable partition between section 1 and 2 (Fig. 6.1) enabled the female to acclimatise to the aquarium without interacting with males. Daylight strip lights were placed above the arena and covered with white fabric to ensure an even light dispersal across the arena. A camera (Logitech c920 HD Pro Webcam) was set up directly above the arena to capture all sections of the aquarium. A white curtain surrounded the entire unit holding the experimental choice chamber, to prevent disturbance from an outside source.

For each trial, the female was introduced into section 1 and the three males into areas 3a, b and c, respectively, of the experimental arena (Fig. 6.1). After a 10 min acclimatisation period, the camera was set to record and the transparent partition

separating sections 1 and 2 was removed (Fig. 6.1). The camera recorded for 20 min before the fish were removed from the arena and isolated into 1 L tanks, where they remained for 5-20 min, until the mate choice behavioural interactions experiment (Section 6.2.2.2). The proportion of time the female spent in each section (Fig. 6.1) of the experimental choice chamber was recorded using jWatcher (Blumstein et al. 2000). The arena was emptied, wiped with ethanol, rinsed and refilled before beginning the next trial.

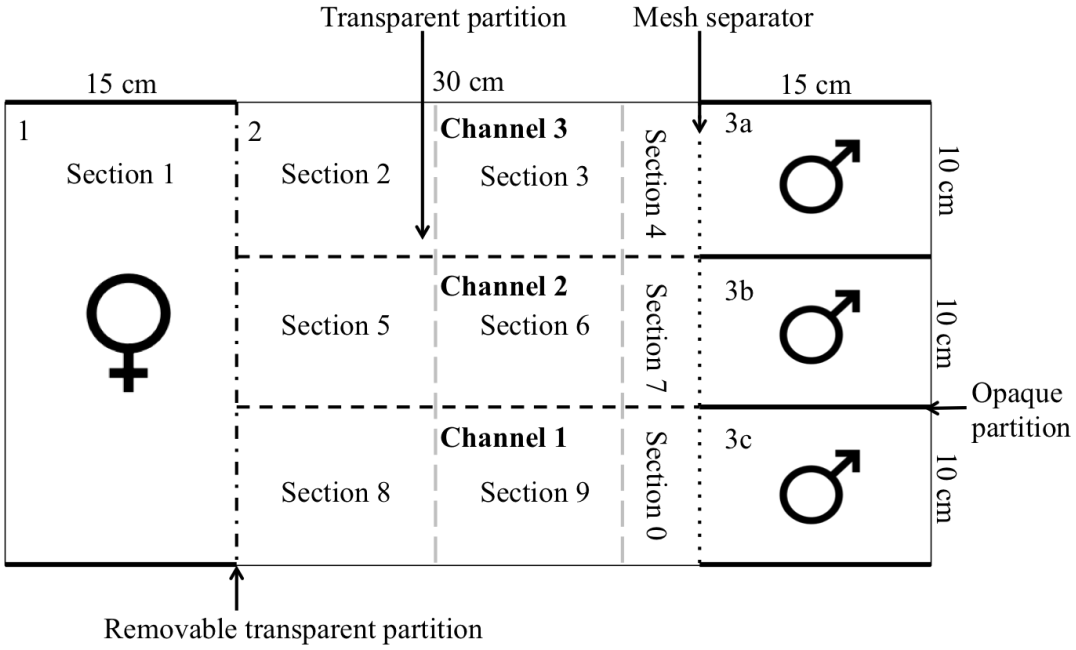

Figure 6.1: Mate choice chamber experimental aquaria used to analyse the proximity of the female to each male in each channel. Areas of the aquaria are labelled with numbers and the sections for video analysis. Male channels are labelled (bold). Measurements of the areas are recorded. Line type represents the type of material used (dot-dash = Removable transparent partition; dash = transparent partition; dotted = mesh separator; thin solid = glass; and thick solid = opaque partition).

6.2.3.2 Paired behavioural interactions

A behavioural study was used to assess how MHC, male morphology and parasitism influence female courtship behaviour when interacting with three different males, separately. An experimental glass tank (20 L; 38 × 21 × 21 cm) with the back and ends covered with white paper, was lit with two daylight strip lights (covered with white plastic sheet to dissipate the light evenly). The female was placed in the

experimental glass arena before the introduction of the male, at the opposite end of the arena to the female. The observer performed a 10 min focal follow on the male and recorded all behaviours and interactions with the female (Table 6.2; Appendix 1). All interactions between individuals, which individual initiated and ended the interaction, and the time spent performing each behaviour event, were recorded. At the end of each trial, both fish were removed from the arena and returned to their 1 L isolation tank. The arena was emptied, wiped with ethanol, rinsed and refilled before beginning the next trial, with the same female and the next male. Any copulation event was recorded for inclusion in the analysis, as female receptivity is known to change post-mating (Guevara Fiore et al. 2010). The trial was repeated for eight different combinations of red and black strains (Table 6.1) to give 4 replicates of each combination.

Following the behavioural trials, all males were anaesthetised and experimentally infected with *G. turnbulli* and the behavioural trials repeated. Once all trials were completed, each fish was lightly anaesthetised and photographed on the left and right lateral sides before being treated with levamisole to remove infection (as described in Schelkle et al. 2009).

Table 6.1: Combinations of red and black strains of guppy (*Poecilia reticulata*) used, to give a 3 male to 1 female ratio across eight experimental manipulation groups, for the mate choice chamber and paired behavioural interactions experiments.

Males		Female	Sample size
3 Black ♂	-	Black ♀	4
2 Black ♂	1 Red ♂	Black ♀	4
1 Black ♂	2 Red ♂	Black ♀	4
-	3 Red ♂	Black ♀	4
3 Black ♂	-	Red ♀	4
2 Black ♂	1 Red ♂	Red ♀	4
1 Black ♂	2 Red ♂	Red ♀	4
-	3 Red ♂	Red ♀	4

Table 6.2: Observed behaviours for interactions between male and female guppies (*Poecilia reticulata*) during the mate choice experiment. Adapted from Endler and Houde (1995).

Male behavioural response to female	Female behavioural response to male
No response; male ignores female	No response; female ignores male
Male approaches the female	Female approaches the male
Male chases the female	Female chases the male
Male nips the female	Female nips the male
Male flees from the female	Female flees from the male
Male displays to female	Female responds to display
Copulation attempt; male thrusts and make gonopodial contact	Copulation attempt; male thrusts and make gonopodial contact
Copulation; gonopodial contact followed by male jerking	Copulation; gonopodial contact followed by male jerking

6.2.4. Male morphology

Male colouration and fin size were quantified from images taken with a Panasonic DMC FZ38 camera at the end of the trial, day 2. A colour standard chart (X-Rite ColorChecker Passport - MSCCPP) was used to ensure colour consistency between photographs. Fish were left to recover in a 1 L tank of clean water. All fish were photographed under the same optical conditions and camera set-up. The camera was manually set-up with white balance calibrated to a white standard, at high-resolution (4:3), high aperture (F=8), low exposure (1/10) and images were saved as RAW files. The images were calibrated and the area of each fin and body area calculated. All fish photographs were analysed using ImageJ to determine the area and colour hue of caudal fin, posterior end of body and entire body area, excluding anal and dorsal fins for females (Fig. 6.2). In addition, the dorsal fin and gonapodium of males were measured independently (Fig. 6.2). Pelvic fins in both sexes and female dorsal and anal fins were excluded from analysis due to difficulty in distinguishing them from the photographs. Fish colouration was analysed using the 'measure RGB' package in ImageJ for the same regions of each fish. Colour hue (^{0}) was calculated from the mean red, green and blue (Hunter and Harold 1987). Hue has been shown to be more important in guppy mate choice than the size and the orange spot pigment content (Deere et al. 2012). Hue is the attribute that corresponds to whether the object is red, orange, yellow, green, blue, or violet, related to the hue circle (Fig. 6.3) and is used to determine the degree to which a stimulus can be described as similar to, or

different from, stimuli that are described as red, green, blue, and yellow (Harold 2001).

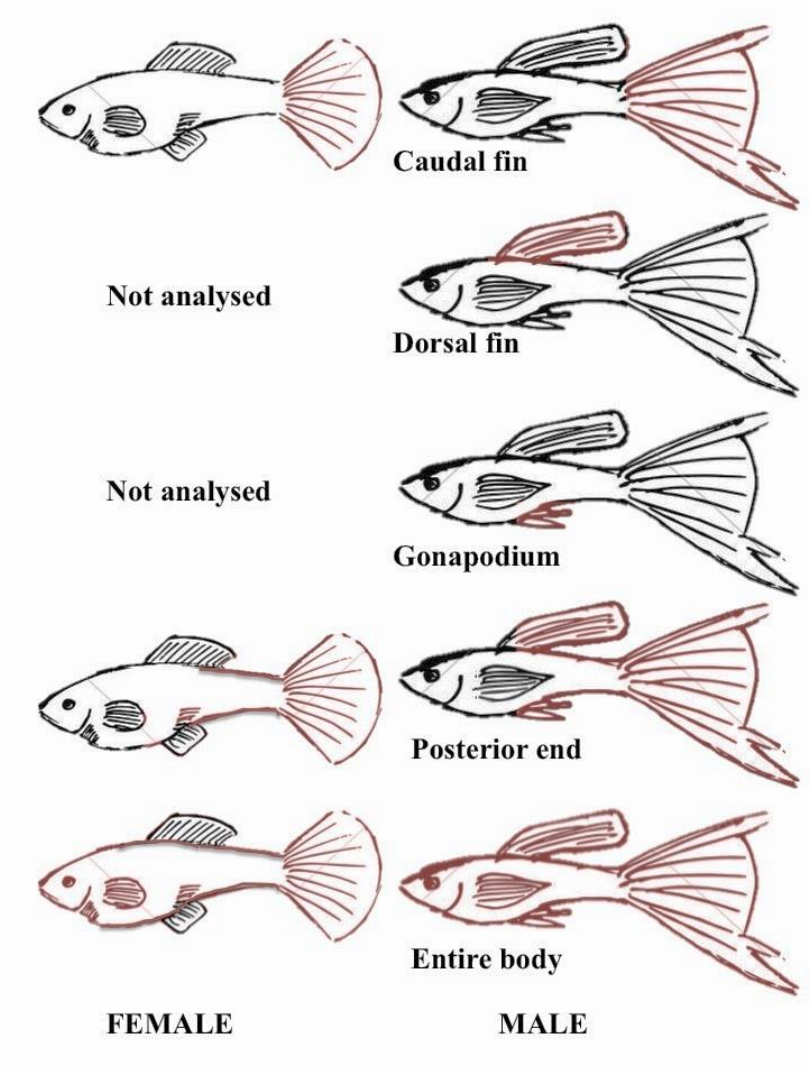

Figure 6.2: Outline (black) of female (left) and male (right) guppies (*Poecilia reticulata*) used in the mate choice experiment with the regions of the fish used for colour and size analysis highlighted (red). Female dorsal anal fins were not analysed.

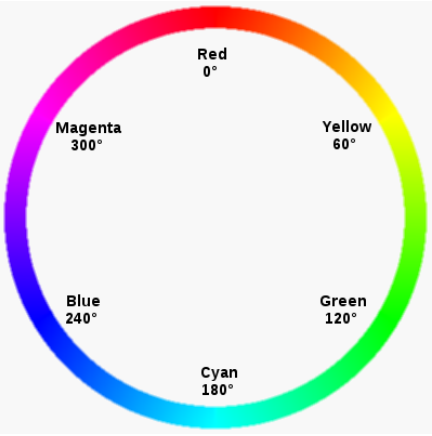

Figure 6.3: Colour hue used to determine the appearance of a colour, the degree to which a stimulus can be described as similar to or different from stimuli that are described as red, green, blue, and yellow (Harold 2001). From: https://en.wikibooks.org/wiki/Color_Models:RGB,_HSV,_HSL.

6.2.5. MHC genotype

After the mate choice trials, fish were anaesthetised using 0.2% MS222 and a fin clip removed from the caudal fin. The fin clip was placed into a 2 ml Eppendorf with absolute ethanol (99.9%) and stored at -18 °C before subsequent processing. Fin clips were MHC class II genotyped as previously described in Chapter 3 (Section 3.2.4).

The number of alleles the male and female shared was identified. A relative similarity index (from 0 to 1) of each male to the female was calculated to account for the male choice the female had in each trial, taking into account the number of shared alleles the other males had with the female:

$$\text{Relative similairity index} = \frac{\text{Number of alleles in common with female}}{\text{Maximum number of shared alleles in trial}}$$

6.2.6. Statistical analysis

Statistical analyses were conducted using R statistical software (version 1.0.136, RStudio 2009-2016 RStudio, Inc.).

Correlation analysis was performed between each of the areas of the fish that were analysed for colour and size (caudal fin, dorsal fin, gonapodium, posterior end and entire body). Posterior end of the fish was significantly ($p < 0.05$) correlated with all of the other areas, in terms of colour and size. All further analyses solely used posterior end for colour and size. An ANOVA was performed to identify whether (1) colour hue and (2) number of MHC alleles per individual (*Ai*) was significantly different between black and red strains of male guppy.

Two global models were constructed, to separately analyse the data from the mate choice chamber and paired behavioural interactions, using the same explanatory variables. The models used the Gaussian family and log error functions, and their robustness was assessed using residual plots. The dependant terms in these models were: (1) log (proportion of time female spent in close proximity to the male +1), in the choice chamber; and (2) time female spent interacting with the male after

initiating the interaction, in the paired behavioural interactions. Fixed terms included: male infection status; strain match; male standard length; treatment; number of shared alleles between female and male; male *Ai*; male colour hue; relative MHC similarity index of the male. The following interactions were also included in the model: male standard length × male infection status; number of shared alleles × infection status; male colour hue × male infection status; male relative similarity × male colour hue; number of shared alleles × male colour hue; male *Ai* × male infection status; male *Ai* × male colour hue; and relative similarity × male colour hue. Male displays were very infrequent (n = 31), so were not included in the analysis. Graphical representation of the effect size of significant results was produced using plot (effect) in the *effects* package.

During model refinement, several equally well-supported models were identified, based on comparisons of Akaike's Information Criterion (AIC). Thus, an information theoretic approach to multi-model inference was employed, to assess the relative importance of each independent term in influencing dependent variables in each General Linear Model (GLM; following methods in Burnham and Anderson 2002). As variables in each 'global' GLM were measured on different scales, model parameters were standardized to a mean of 0 and standard deviation 0.5 using the *arm* library (Gelman and Su 2013). The dredge function within the *MuMin* package (Bartoń 2014) was then used to generate a set of 'top' models, which fell within 4 corrected AIC (AICc) of the best model (see Burnham and Anderson 2002). Averaged parameter estimates from this top set of models were then calculated using the model.avg function, and the relative importance of each parameter generated by summing the Akaike weights across the models in which the parameter occurred (Burnham and Anderson 2002). The closer the importance value was to one, the greater relative importance that variable had in comparison to other model variables (Burnham and Anderson 2002). The variables were considered significant if the 95% confidence intervals did not bound zero.

6.3 RESULTS

Black male guppies had significantly less red pigment, higher colour hue ($t_{1,55}$ = -14.34, $p \leq 0.001$) and higher number of MHC alleles per individual (*Ai*; $t_{1,55}$ = 7.37, $p \leq 0.001$), compared to red strain males (Table 6.3).

Table 6.3: Summary of colouration hue (^{0}, mean (±SE)) and the number of MHC alleles per individual (*Ai*; mean (±SE)) for black and red strains of male guppies (*Poecilia reticulata*) used in a mate choice experiment. Low hue = more red pigment.

Male strain	**Hue colouration (^{0})**	***Ai***
Black	113.53 (± 2.13)	1.74 (± 0.09)
Red	68.57 (± 1.99)	1.04 (± 0.04)

6.3.1. Mate choice chamber

The most important variables explaining the amount of time a female spent in close proximity to a male were: strain match, male colour hue, male standard length, male infection status and the interaction between male colour hue and number of shared alleles between male and female (Table 6.4, model 1). Overall, females spent significantly more time in close proximity to males: that were not infected with *Gyrodactylus turnbulli* (Fig 6.4a); of the same strain as herself (Fig. 6.4b); with a greater standard length (Fig. 6.4c); and with high levels of red pigment (lower colour hue; Fig. 6.4d; Table 6.4, model 1). The significant interaction between colour hue and shared MHC alleles indicated that females spent more time in close proximity to males with a higher levels of red pigment (lower colour hue), which shared fewer MHC alleles, whereby increasing colour hue removes the effect of shared MHC alleles (Table 6.4, model 1; Fig. 6.4e).

Table 6.4: Summary of the standardised averaged model predictors used to explain variation in female guppy (*Poecilia reticulata*) mate choice, using the dependent variables (1) proportion of time female spent in close proximity to a male; and (2) amount of time the female initiated interactions with a male. Highlighted predictors indicate confidence intervals that do not bound zero and are, therefore, considered significant. CI = confidence intervals, SE = standard error.

Model	Dependant variable	Explanatory variable	Estimate	Adjusted SE	95% CI	Relative importance
1	Proportion time female in close proximity to male	Intercept	-2.014	0.015	-2.044 – -1.983	
		Strain match	-0.278	0.0485	-0.374 – -0.183	0.79
		Male colour hue	-0.167	0.019	-0.204 – -0.130	0.35
		Male allele count	-0.069	0.081	-0.228 – 0.090	0.20
		Shared alleles	-0.058	0.089	-0.232 – 0.115	0.17
		Male relative MHC similarity	-0.027	0.098	-0.218 – 0.164	0.15
		Male standard length	0.047	0.007	0.033 – 0.061	0.13
		Male infection status	-0.017	0.003	-0.024 – -0.011	0.12
		Male colour hue × Shared alleles	0.199	0.002	0.195 – 0.202	0.02
2	Time of female initiated interactions with male	Intercept	0.786	0.104	0.580 – 0.991	
		Male infection status	-0.332	0.235	-0.799 – 0.028	1
		Strain match	-1.112	0.316	-1.737 – -0.486	1
		Male colour hue	-0.911	0.237	-1.381 – -0.441	1
		Shared alleles	-0.372	0.304	-0.974 – 0.231	1
		Male infection status × Shared alleles	0.491	0.516	-0.014 – 1.651	0.86
		Male colour hue × Shared alleles	1.654	0.460	0.742 – 2.566	0.6
		Male standard length	-0.087	0.177	-0.677 – 0.206	0.37
		Male infection status × Male colour hue	-0.003	0.180	-0.886 – 0.846	0.17
		Male infection status × Male standard length	0.032	0.163	-0.402 – 1.293	0.007

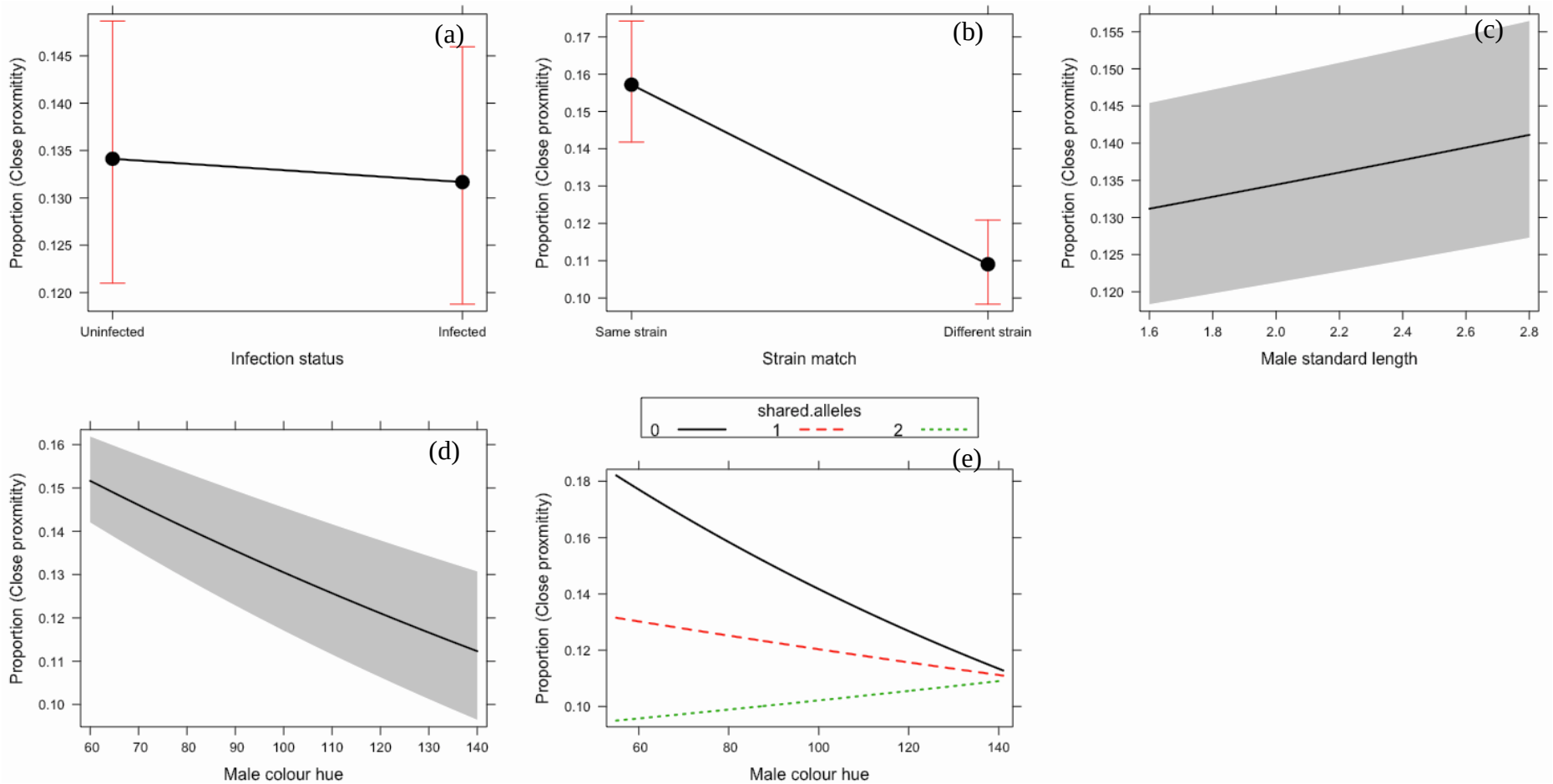

Figure 6.4: Proportion of time female guppies (*Poecilia reticulata*) spent in close proximity to (a) uninfected and *Gyrodactylus turnbulli* infected male; (b) same strain or different strain to herself; (c) males of varying standard length; (d) males of varying colour hue (low hue = more red pigmentation); and (e) males of varying colour hue with differing numbers of shared Major Histocompatibility Complex alleles between the male and female. The grey shading represents 95% confidence intervals.

6.3.2. Paired behavioural interactions

The most important variables to explain the time the female initiated interaction with the male were: strain match, male colour hue and the interaction between male colour hue and number of shared alleles between the male and female (Table 6.4, model 2). Females initiated significantly more interactions with males of the same strain as herself (Fig. 6.5a). The interaction between colour hue and number of shared alleles indicated that females were more attracted to males with higher levels of red pigmentation (lower colour hue) and who shared fewer MHC alleles (Table 6.4, model 2; Fig. 6.5b).

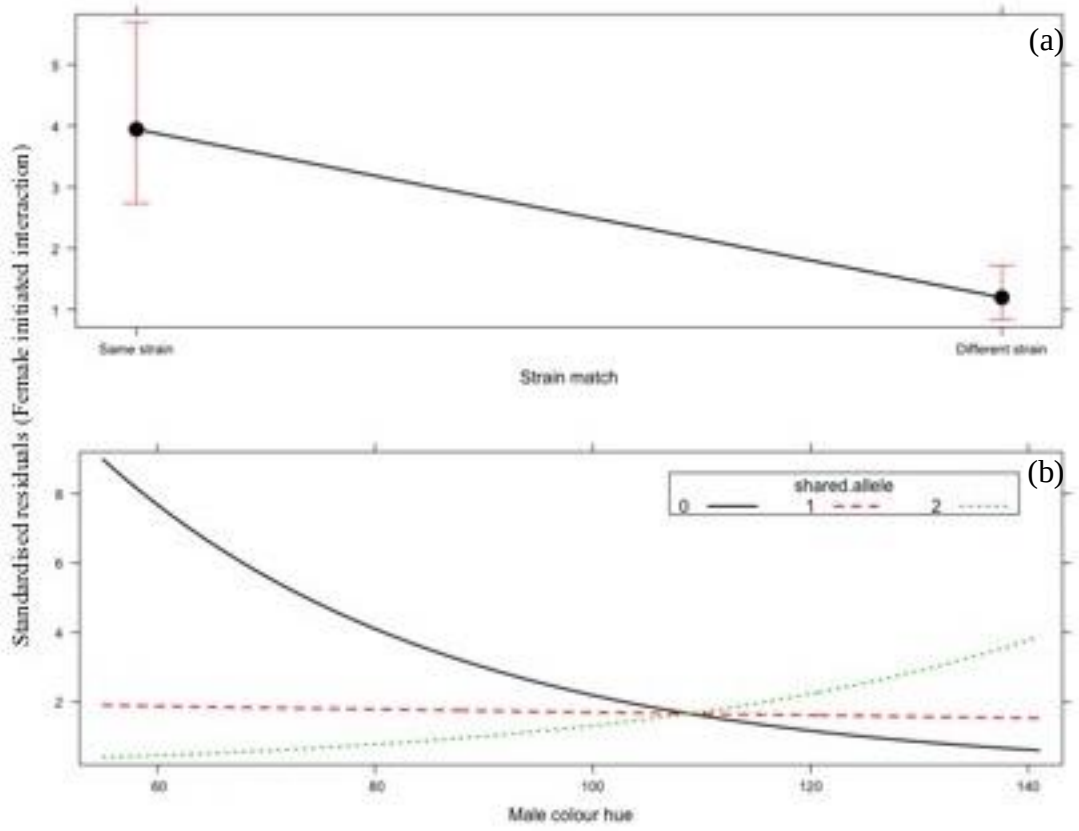

Figure 6.5: Time female guppies (*Poecilia reticulata*) spent in close proximity with three different males in a paired behavioural trial with (a) males of the same strain or different strain to herself; and (b) males of varying colour hue (low hue = more red pigmentation) with differing numbers of shared Major Histocompatibility Complex alleles between the male and female.

6.4. DISCUSSION

The current study aimed to identify the effects of sexual selection, MHC and parasitism on guppy (*Poecilia reticulata*) mate choice, showing that overall colour interacting with shared MHC alleles, strain similarity, parasitism and host size are the most important explanatory variables in explaining female guppy mate choice. The apparent preference of females for males of the same strain was not expected. Female guppies have been shown to choose males with novel colour patterns, rather than males with a colour pattern similar to themselves (Kodric-Brown 1985; Hughes et al. 1999; Zajitschek et al. 2006). The strains used in the current study were extremely different to one another, an experimental design similar to Kodric-Brown (1985), whereas other studies used the same strain and focused on 'novel' colouration (wild: Hughes et al. 1999; wild-type: Zajitschek et al. 2006). The consensus of previous studies is that guppy male colourations that are locally common are discriminated against during mate choice, which is hypothesised to reduce inbreeding (Farr 1977, 1980; Hughes et al. 1999). The contrasting results of the current study may reflect female selecting a male of the same strain as herself to ensure that her offspring were not hybrids, which could cause an "oddity effect" and increase predation risk (Landeau and Terborgh 1986). Alternatively, there may have been maternal strain imprinting leading to a mating preference for this strain, as previously shown in Lake Malawi cichlids (Svensson et al. 2017). It is also possible that female mate choice mirrors the artificial selection regime the strain has been subjected to, during selective breeding in captivity. Females that show the same male preference as the breeder are likely to show the highest inclusive fitness (i.e. reproducing offspring that are allowed to reproduce themselves in the next generation). It is hypothesised that male colour pattern is not the only trait under directional selection during artificial breeding, and that there is significant cryptic selection for female preference favouring the male colour pattern trait that is under artificial selection. In turn, this creates an artificial Fisher's Runaway model, which can explain the reason for same strain preference.

Male colour strongly influenced female behaviour and did not appear to be outweighed by MHC diversity or similarity. Females in the current study were attracted to males that had high red colouration, potentially indicating good health. Carotenoids enhance immune function in most animals, but they must be assimilated

from the environment (Barreiro Lozano 1994). Excess carotenoids provide and augment orange/red colouration; thus the brighter the orange, the stronger the immune function and the more successful the animal is at exploiting the environment (Brooks and Endler 2001). Male red colouration in guppies becomes paler after prolonged *G. turnbulli* infection (Houde and Torio 1992). Males in the wild would be parasitized by local predominant parasites; those males able to fight infection would retain their colour, thus advertising their high health status. For example, female sticklebacks show a preference for red ornamentation in males, an indicator of immune function quality (Milinski and Bakker 1990). Rather than females selecting a mate based on genetics, they might select the healthier male based on environmentally induced differences among males. Females in the current study preferred males with increased red colouration, as previously reported for guppies (Brooks and Endler 2001; Zajitschek et al. 2006).

The effect of male infection status was consistent with previous studies showing that females are less attracted to infected males (Kennedy et al. 1987; Houde and Torio 1992). The current study, in fish of ornamental lines, shows that other factors, particularly male colour, are at least as important. Although infection and colour can be linked in the longer-term, parasitic infection would not have altered male colour intensity in the current study because of the relatively short infection duration. It is more likely that odour cues specific to gyrodactylid infection detect at close range (Stephenson and Reynolds 2016), and these cues may have been used by the females, enabling females to show a preference for uninfected males, even after a short period of infection. Although females spent significantly less time in close proximity to an infected male in the choice chamber, infection status did not significantly affect the time the female initiated interactions when paired. This is likely due to the absence of direct choice during the paired behavioural interaction; a ‘bad’ infected mate is better than no mate at all. A difference is, however, shown where the female has a choice between infected and uninfected males.

Selecting the best mate should mean choosing an individual with contrasting genetics to self, thereby increasing offspring fitness (Milinski 2006; Agbali et al. 2010; Ejsmond et al. 2014). Individuals with low MHC diversity are known to be more susceptible to parasitic infection (Chapters 2, 3 and 4) and MHC genotypic discrimination on the basis of odour has been reported (Milinski 2006; Eizaguirre et

al. 2009). Females who select a male based on a high MHC diversity over the presence of locally important MHC alleles may select a male that cannot fight the locally predominant parasites; this would lead to offspring susceptible to infection (Millinski 2003, 2006). In sticklebacks, the ultimate mate choice is likely based on a combination of red colouring and optimal MHC alleles, allowing the female to identify male quality and evade local parasites via the adapted MHC alleles (Aeschlimann et al. 2003). Female sticklebacks, in lake enclosures, preferred males that were both red and offering the optimally complementary number of MHC alleles to the female (Eizaguirre et al. 2009). Similarly, the current study showed that female guppies associated more with males that had a greater prevalence of red pigment (low hue) and when they shared few or no MHC alleles.

Females spent more time interacting with larger males than small males, supporting the suggestion that larger males are assumed to be fitter (Watt et al. 2001; Skinner and Watt 2006) and the preferred mate choice for females (Zajitschek 2006). The current study showed male standard length to significantly affect the amount of time a female spent in close proximity to a male in a choice chamber. When the female was presented with males independently, however, male length did not affect the time the female initiated interactions with him. This is likely due to the lack of direct choice in the latter (paired behavioural interactions).

Previous studies that have focused on one aspect of mate choice may not have accounted for variation between individuals in other aspects of mate choice cues. Studies focusing on MHC, for example, may not account for colour or size, which have been shown by the current study to have an underlying effect on the result. Females are likely to use a combination of factors when deciding on a preferred mating partner (Eizaguirre et al. 2009). This study highlights the importance of multi-variable analysis when it comes to mate choice. Strain similarity, colour, number of shared MHC alleles, size and infection status were the most consistently important variables overall, of those previously shown to independently have a significant effect on female guppy mate choice. This emphasises the complexity of artificial breeding of animals for conservation programmes, whereby humans try select ‘important’ traits to select for to produce fit offspring. The complications are further emphasised by the inadvertent “cryptic selection” on female mate choice, which appears to mirror the artificial selection regime. The findings from the current

study suggest that there is no one trait that determines mate preference, that female preference is a highly evolvable trait that can rapidly diverge between strains.

ACKNOWLEDGEMENTS

Alan Smith provided the Red and Black strain guppies used in this study.

CHAPTER 7

Effect of parental Major Histocompatibility Complex similarity on life history traits of first generation guppies (*Poecilia reticulata*)

ABSTRACT

Selecting the right mate is essential to ensure an individual's genes continue into future generations and to maximize offspring fitness. Mate selection based on odour dissimilarity and, therefore, MHC genotype increases offspring allelic diversity. Females in the current study were given a single male, with measurable MHC similarity, to breed with, rather than being provided with a choice of male. The effect of individual MHC class II diversity (allelic and supertype) and parental MHC similarity on the life history of specific crosses of ornamental guppies (*Poecilia reticulata*) was investigated. This study found mixed evidence for the effects of parental MHC genetic similarity, across a range of offspring fitness traits. Individuals whose parents shared more alleles and supertypes were more susceptible to *Gyrodactylus turnbulli* infection. Fish from broods with a low number of siblings grew quicker than those from broods with a larger number of siblings and, as a potential consequence, were less susceptible to *G. turnbulli* infection. Intuitively, larger fish swam significantly further and had a higher critical swimming speed than smaller fish. Fish with a low number of MHC alleles per individual (*Ai*) swam significantly further than those with more *Ai*. Growth rate and parasite susceptibility are density dependent and should be considered to optimise growth rate, parasitism and profits in aquaculture.

7.1. INTRODUCTION

According to evolutionary theory, parental investment in individual offspring is based on their potential fitness (Fisher 1930). Breeding success and male attractiveness modify female investment (life history theory; Williams 1966). Inbreeding depression can affect offspring fitness-related traits (Saccheri et al. 1998; van Oosterhout et al. 2000b; Keller and Waller, 2002), including survival (Coltman et al. 1998), reproductive success (Slate et al. 2000; Spielman et al. 2004; Eszterbauer et al. 2015), sexual ornamentation and courtship behaviour (van Oosterhout et al. 2003b; Malo et al. 2017), and parasite susceptibility (MacDougall-Shackleton et al. 2005; Rijks et al. 2008; Chapter 2). A principal question in evolutionary biology concerns how mate selection on genetic variation affects offspring fitness (Falconer et al 1996; Bernatchez and Landry 2003; Ellegren and Sheldon 2008).

The Major Histocompatibility Complex (MHC) is a multi-gene family that includes most vertebrate immune genes (Jordan and Bruford 1998; Penn and Potts 1999; Ekblom et al. 2004; Consuegra and de Leaniz 2008). It is important in triggering an immune response (Hughes and Yeager 1998), parasite resistance (Trowsdale and Parham 2004; Chapter 3), and other fitness traits (e.g. Langefors et al. 2001; Arkush et al. 2002; Pitcher and Neff 2006; Eizaguirre et al. 2009). Most of these studies have considered genetic diversity as the key MHC attribute underlying reported effects. Genetic composition of the MHC, rather than diversity, has also been linked with individual fitness traits (e.g. Kalbe et al. 2009; Worley et al. 2010; Thoss et al. 2011; Chapter 3). The presence of specific MHC alleles has been linked to mortality (Soay sheep, Paterson et al. 1998; Chinook salmon, Pitcher and Neff 2006), life expectancy (Seychelles warbler, Brouwer et al. 2010) and resistance to infectious disease (Ungulates, Paterson et al. 1998; Atlantic salmon, Langefors et al. 2001; Chinook salmon, Arkush et al. 2002). MHC also affects mate choice (reviewed in Jordan and Bruford 1998; Chapter 6), with selection of mating partners based on contrasting MHC genotype to increase offspring fitness (including: Yamazaki et al. 1976; Reusch et al. 2001; Aeschlimann et al. 2003; Milinski et al. 2005, Milinski 2006; Agbali et al. 2010; Ejsmond et al. 2014; exception: Pitcher and Neff 2007). In humans, birth weight and placenta weight significantly increased with decreasing parent MHC allele similarity (Reznikoff Etievant et al. 1991).

Alleles of the MHC can be grouped according to their function (supertypes), based on physicochemical properties of amino acid sequence of the polymorphic peptide-binding region (Trachtenberg et al. 2003; Lenz 2011). Offspring fitness is likely determined by MHC functionality, rather than MHC allelic diversity, as shown with parasite susceptibility (Chapter 3). The majority of previous studies have focused on the association between MHC genetic diversity, rather than functionality, and single life history traits (e.g. Finch and Rose 1995; Lochmiller 1996; Von Schantz et al. 1996). Few studies have, however, focused on the association between MHC functionality and life history traits, and those that have shown diverse results (Radwan et al. 2012; Sepil et al. 2013). Specific functional groups of MHC have been linked to survival and reproductive success in wild populations of great tit (Sepil et al. 2013). Radwan et al. (2012) found no evidence of an association between MHC functional diversity with lifespan and lifetime reproductive success in collared flycatchers.

Few studies have assessed the effect of individual MHC genetic composition on multiple life history traits, with the exception of Fraser and Neff (2009). These authors used descendants of wild guppies (*Poecilia reticulata*) to assess fitness (growth rate, parasite load and survival) linked to the most common MHC IIB alleles in the population and homo/heterozygosity. A particular MHC allele was identified as important in reducing parasite load, and MHC heterozygosity affected fish growth rate positively. The current study also uses first generation guppies, bred from two wild type and five ornamental strains, from a selective breeding experiment to investigate the effect of MHC class II allelic and supertype genotype variation on lift history traits. Specifically, the effect of parental MHC similarity on growth rate, survival, critical swimming speed (U_{crit}), swimming distance travelled and parasite susceptibility is assessed. It is hypothesised that first generation (F1) offspring whose parents have more similar MHC genotypes will have reduced fitness, evident across a variety of traits.

7.2. MATERIALS AND METHODS

7.2.1. Parental fish stocks

Parental fish were ornamental and wild type guppies (*Poecilia reticulata*). Wild type strains of guppy originated from the wild and have been kept in captivity for 3 years, without selective breeding, but from small founder populations (n = ~ 300). Wild type guppies were collected from the Lower Aripo (10°35'00"N 61°14'00"W) and Tacarigua (10°37'00"N 61°24'00"W) rivers in Trinidad and transported to the UK, in 2012. In addition, ornamental guppies were purchased from a pet shop supplier in November 2014 (Strains: Black, Blonde Red, Cobra Green and Flame). A fifth ornamental strain originating from a Nottingham pet shop in 1997 has been maintained at Cardiff ever since. All ornamental strains of guppy have been selectively bred for a maximum of 300 generations (van Oosterhout. pers. comm). All fish were maintained at Cardiff University under 24 ± 1 ºC and 12 h light: 12 h dark cycle, and fed twice daily with AQUARIAN® tropical fish flakes and weekly with frozen bloodworms.

7.2.2. MHC genotyping and supertyping

Fin clips of the F1 generation were taken after all experimental trials (growth, survival, swimming and parasite trajectory) were conducted, were MHC class II genotyped and supertyped for each F1 generation fish (n = 221), as described in Chapter 3 (Section 3.2.4). The parents of the F1 generation were genotyped in Chapter 3 (Section 3.2.1) Parental allelic and supertype similarities were calculated as the number of alleles/supertypes shared by both parents.

7.2.3. F1 breeding crosses

Specific breeding crosses were set up by placing a male into a 5 L aerated tank, with 2 cm of gravel in the bottom and two plastic refugia, along with 3-4 females with varying numbers of similar MHC alleles present to the male (0-2 shared alleles and supertypes; from ornamental and wild type fish, genotyped in Chapter 3.2.1). The male and females were left together for 3 weeks before each female was individually

isolated in 5 L tank-aerated tank with the same aquaria set-up as during breeding. Fish were checked daily for any birthing events. All birthing events (n = 51) were recorded, as were the number of fry born (F1s) and the date of birth. Any newborn fry were removed using a net and transferred to a glass petri dish for photographing (Section 7.2.4), before being placed into their own 5 L aerated tank (as above). The females were left isolated for 6 months. After 30 weeks, all F1 generation fish were fin clipped for MHC genotyping and VIE marked for identification. Aquaria were kept at 24 ± 1 ºC and 12 h light: 12 h dark cycle and fish fed twice daily with AQUARIAN® tropical fish flakes and once daily with newly hatched *Artemia* nauplii, ensuring the fish were fed to satiation.

7.2.4. Growth rate

From the day of birth, all fry were measured weekly for 30 weeks (n = 221). Each fish was held in a petri dish, containing 10 ml dechlorinated water to restrict movement, which was placed on graph paper and photographed from above. The measurement of each fry from the brood was taken as fork length, from tip of the snout to the posterior end of the caudal fin using ImageJ. Weekly growth rate was calculated as an average for each brood and used to determine the average weekly growth rate of the broods, for the time to sexual maturity.

7.2.5. Survival

Fish survival was recorded from the time of birth as the proportion alive from each brood each week. All deceased fish were fin-clipped for MHC genotyping.

7.2.6. Swimming ability

Fish aerobic swimming endurance is commonly measured using critical swimming speed (Brett 1964; Beamish 1978). Swimming performance (U_{crit} and distance swam) of a sub-set of fish (n = 110) was assessed after the fish had reached sexual maturity (30-40 weeks), in a recirculating open channel flume, with channel length 150 cm, 16 cm depth and 20 cm height, was filled with dechlorinated water to 15 cm depth and maintained at 23 °C. A 10 cm diameter impeller was attached to a 1 horse power three phase 4-pole motor with shaft speed maximum 1500 rpm (Machine

Mart), wired to a 1.1 kW inverter (RS Components) to control motor speed. At either end of the flume was a 20 mm thick aluminum honeycomb flow straightener with 6.4 mm cell diameter. The flow straightener was covered with netting to prevent fish from entering the impeller region, restricting them to a 100 cm length section (Fig. 7.1).

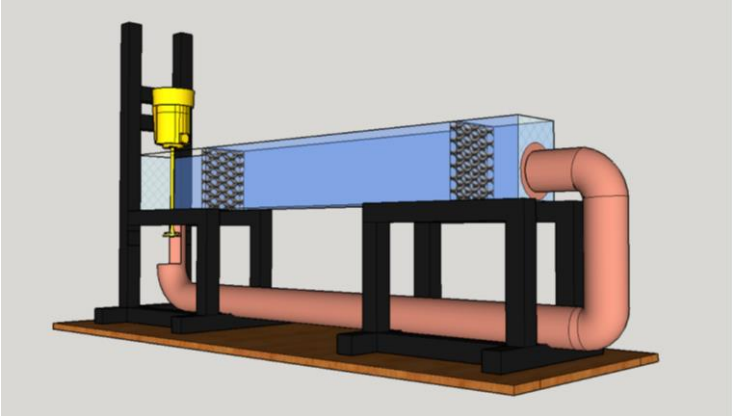

Figure 7.1: 3D schematic of the open channel flume in which *Poecilia reticulata* were placed for critical swimming performance and swimming distance trials. Image credit: Z. Smallbone.

Methods similar to those from Tierney (2011) were followed to calculate critical swimming speed (U_{crit}) and the trials were recorded using a HD camera (Logitech c920). Critical swimming speed (U_{crit}) was measured after 10 min of acclimatization at low velocity (5.8 cm s^{-1}), followed by increasing the flow by 6 cm s^{-1} every 8 min. The flow was increased until the fish could no longer swim against the current, and the fish could not move away from the downstream flow straightener of the flume (similar to Brett 1964; Beamish 1978; Kolok 1999; and Oufiero and Garland Jr 2009). Critical swimming speed was calculated as:

$$U_{crit} = U_i + U_{ii} (t_i / t_{ii})$$

Where, U_i = the area mean velocity at the last completed step, U_{ii} = step height (cm s^{-1}), t_i = duration of final incomplete step, and t_{ii} = step duration (min) (Tierney 2011).

The flume was divided into eight sections (Fig. 7.2) and the velocity recorded, using a Nixon Streamflo Velocity Meter V1.3, in the centre of each section ten times, then

an average velocity calculated. The footage of each fish trial was analysed using JWatcher video analysis software (Blumstein et al. 2000). The amount of time each fish spent in each section at each step was identified and linked to the section's velocity. This data was then used to calculate distance swam, based on the amount of time the fish spent at each velocity, and critical swimming speed (U_{crit}).

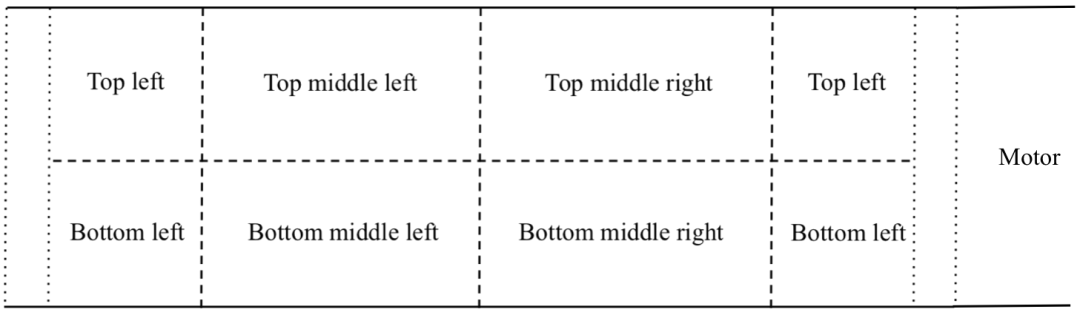

Figure 7.2: Sections of the open channel flume (side on view), in which *Poecilia reticulata* were placed to perform critical swimming speed and swimming endurance tests, used for video analysis and velocity recordings. Within the dashed lines indicates the sections and the dotted lines indicate flow straighteners.

7.2.7. Parasite trajectory

Following the swimming performance trials, fish (n = 186) were given 1 week to recover, before they were experimentally infected with two individuals of *Gyrodactylus turnbulli* and screened to determine the parasite trajectory every other day for 13 days (methods as reported in Chapter 3 Section 3.2.2. Fish were treated with levamisole to remove infection screened clear of parasites and left to recover (as described in Schelkle et al. 2009).

7.2.8. Statistical analysis

All statistical analysis was performed using R statistical software (version 1.0.136, RStudio 2009-2016 RStudio, Inc.).

7.2.8.1. Breeding success

An ANOVA was performed to assess the effect of parental shared MHC alleles and

supertypes on the total number of fry born and the number of breeding events that took place.

7.2.8.2. Growth rate

A Gaussian Generalized Linear Mixed Model (GLMM) was performed (*lme4* package) to identify whether parental MHC similarity was linked to first generation growth rate. The explanatory variables: weeks since birth, brood size, number of shared alleles between parents, number of shared supertypes between parents and the interactions weeks since birth × brood size, weeks since birth × number of shared alleles between parents and weeks since birth × number of shared supertypes between parents; were used to explain variation in growth rate. The brood number was used as a random term to avoid pseudo-replication.

7.2.8.3. Survival

An ANOVA was used to determine the difference in the proportion of F1s survived to week 30 from the time that they were born with varying number of shared parental MHC alleles and supertypes.

7.2.8.4. Swimming ability

A Gaussian General Linear Model (GLM) was performed to determine how variation in (a) the distance travelled and (b) critical swimming speed was explained by host sex, host standard length, number of alleles per individual (*Ai*), number of supertypes per individual (*STi*) and the number of MHC alleles and supertypes the parents of the individuals shared. Model refinement (drop1) suggested the most robust model was that described; all variables were retained.

7.2.8.5. Parasite trajectory

A Poisson GLMM with log link function (*lme4* package) was used to identify whether parasite trajectory was affected by day since initial infection (day), sex, host standard length, brood size, *Ai*, *STi*, number of MHC alleles and supertypes the

parents of the individuals shared. The interaction terms brood size × day, *Ai* × day, *STi* × day, number of alleles shared by parents × day and number supertypes shared by parents × day. Model refinement using the function drop1 showed the most robust model to include all variables from the initial model.

7.3. RESULTS

7.3.1. Breeding success

Parental similarity in MHC alleles and supertypes had no significant effect on the number of birthing events or the number of fry that were born ($p > 0.05$).

7.3.2. Growth rate

Across all 51 broods, mean brood size ± SE was 4.67 ± 0.52 and average growth rate ± SE 0.06 ± 0.002 (Supplementary Table S7.1). Growth rate was significantly slower through time; young F1 fish grew more quickly than older fry ($t_{1,1210} = -7.46$, $p \leq 0.001$; Fig. 7.3). F1 fish grew slower as brood size increased ($t_{1,1210} = -2.44$, $p = 0.02$), but within brood growth variation was fairly constant across brood sizes. There was no significant effect of the number of shared alleles and supertypes between parents on F1 growth rate through time ($p > 0.05$).

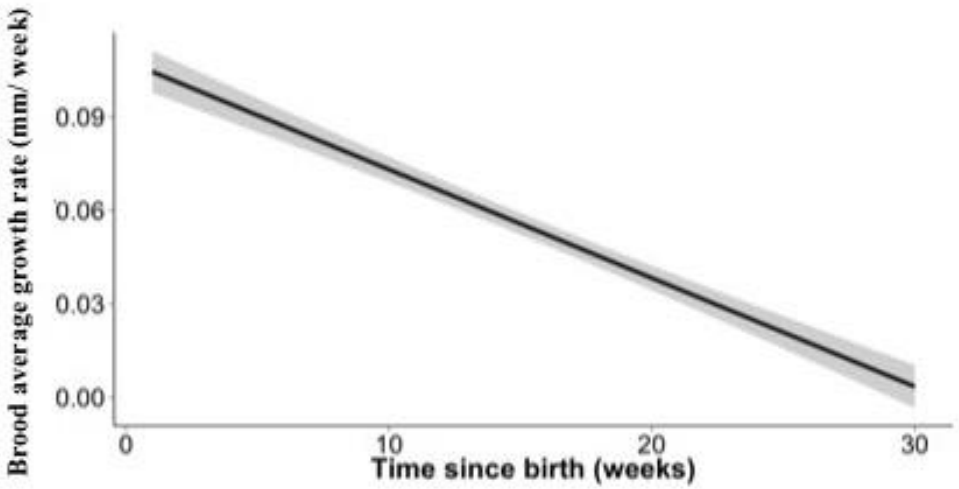

Figure 7.3: Average growth rate (mm/week) for broods of first generation guppy (*Poecilia reticulata*) crosses from the time since birth (weeks). Standard error is represented by grey shading.

7.3.3. Survival

Survival (± SE) of F1s at 30 weeks of age was 82.89% (± 4.86; Fig. 7.4; Supplementary Table S7.1). There was no significant effect of number of parental shared alleles or supertypes on F1 survival at 30 weeks since birth ($p > 0.05$).

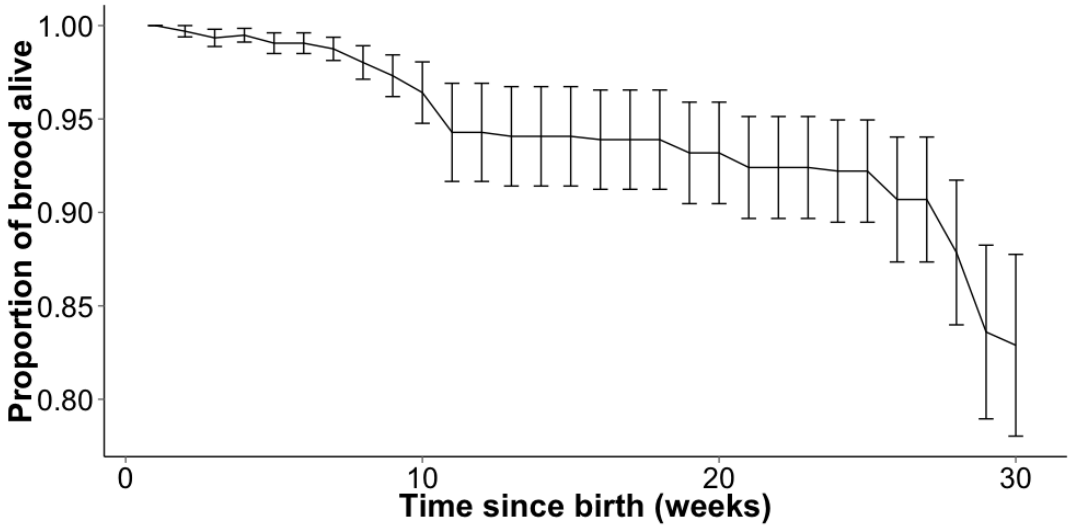

Figure 7.4: Survival of F1 guppies (*Poecilia reticulata*) in the first 30 weeks since birth.

7.3.4. Swimming ability

Increasing fish standard length significantly increased the distance swam and critical swimming speed (U_{crit}; $t_{1,68} = 3.088$, $p = 0.003$; $t_{1,53} = 2.544$, $p = 0.014$). Swimming distance increased as the number of alleles per individual (*Ai*) decreased ($t_{1,68} = -2.029$, $p = 0.005$). The number of alleles and supertypes shared between the parents of the F1 had no significant effect on the distance swam or the U_{crit} ($p > 0.05$). There was no significant effect of *Ai* on the U_{crit} ($p > 0.05$).

7.3.5. Parasite trajectory

Parasite intensity varied across broods (Supplementary Table S7.1). Individuals with higher *Ai* had reduced parasite intensity ($z_{1,1303} = -20.72$, $p \leq 0.001$). Parasite intensity was significantly higher through time with increasing number of MHC supertypes per individual (*STi*) ($z_{1,1303} = 14.56$, $p \leq 0.001$). Increasing the number of

alleles and supertypes shared by the parents of an individual significantly increased the parasite intensity through time ($z_{1,1303}$ =2.64, p = 0.008; $z_{1,1303}$ = 2.69, p = 0.007, respectively). Individuals from broods with a larger number of siblings were significantly more susceptible (increased parasite intensity through time) to *Gyrodactylus turnbulli* ($z_{1,1303}$ = 3.81, $p \leq 0.001$).

7.4. DISCUSSION

Growth rate and parasite resistance are important factors when considering lifetime fitness of *Poecilia reticulata* (see Reznick and Endler 1982; Houde and Torio 1992). The current study investigated the effect of individual MHC class II diversity (allelic and supertype) and parental MHC similarity on the life history of specific crosses of ornamental guppies (*P. reticulata*). This study found mixed evidence for the effects of parental MHC genetic similarity, across a range of offspring fitness traits. Individuals whose parents shared more alleles and supertypes were more susceptible to *Gyrodactylus turnbulli* infection. Fish from broods with a low number of siblings grew quicker than those from broods with a larger number of siblings and, as a potential consequence, fish from smaller broods were less susceptible to *G. turnbulli* infection. Intuitively, larger fish swam significantly further and had a higher critical swimming speed than smaller fish. Fish with a low number of MHC alleles per individual (*Ai*) swam significantly further than those with more *Ai*.

Maximising offspring fitness by selecting the right mate is essential for the perpetuation of an individual's genes. Offspring allelic diversity is increased by a mate being selected by the female based on dissimilarity to her odour and, therefore, MHC genotype (Milinski 2006; Eizaguirre et al. 2009; Kalbe et al. 2009). Inbreeding increases susceptibility to *G. turnbulli* (Chapter 2), so choosing a dissimilar mate will ensure reduced levels of inbreeding. The present study did not provide the female with a choice of male; instead each female was only given one option for her mating partner, with a quantifiable MHC similarity. Increasing parental MHC similarity led to their F1 offspring being more susceptible to *G. turnbulli* infection. This supports evidence that mate choice is important in ensuring offspring have high genetic diversity to ensure they can fight infectious disease (Milinski 2006; Agbali et al. 2010; Ejsmond et al. 2014). The presence of specific MHC supertypes is important in the immune defence against *G. turnbulli* (Chapter 3). The current study found a

positive association between parasite intensity and the number of MHC supertypes per individual (*STi*). Increasing *STi* does not, however, necessarily mean that an individual has the specific supertypes required to fight local infections. An individual could have only one supertype, but if it is the group that binds the antigens released from the specific pathogen that it is exposed to that this results in resistance. Of the supertypes associated with reduced parasite susceptibility in Chapter 3, only one (ST5) was present in the first generation fish from the current study.

Previously, Fraser and Neff (2009) reported that MHC heterozygosity in guppies was significantly associated with faster growth rates, but not parasite load or host survivorship. Fraser and Neff (2009) calculated growth rate between day of birth and 25 days post-birth and used the 10 day average parasite load. The current study monitored brood average growth rate every week for much longer (30 weeks) and found no effect of MHC on growth rate over time, even over the first 4 weeks from birth. This is likely due to the over-riding effect of brood size and the spatial constraint imposed by the enclosure of the brood. This result may have consequences for aquaculture, whereby the fish are maintained at high stocking density, which may limit fish growth and profit. Individuals from broods with more siblings have more competition, so may invest less into growth and development of the immune system. In the current study, parasite intensity was recorded every other day over 13 days for each individual. An association between parasite intensity through time and parental similarity was identified. The number of shared parental MHC alleles or supertypes did not significantly effect F1 survival, consistent with Fraser and Neff (2009). This result, however, contradicts previous studies that have shown that the more MHC genetically diverse an individual, the greater its chance of survival (Penn et al. 2002; Doherty and Zinkernagel 1975; Hughes and Nei 1988).

Intuitively, larger fish were significantly better at swimming than smaller fish, consistent with previous studies (Underwood et al. 2014; Remen et al. 2016; Reynolds et al. submitted). Locomotor performance can be critical for survival and is widely assumed a fitness indicator (Clobert et al. 2000; Husak et al. 2006; Wilson et al. 2010; Marras et al. 2013). Increased genetic diversity should, therefore, increase swimming performance, but the current study found that individuals with low *Ai* swam significantly further than those with high *Ai*. This suggests that increased MHC genetic diversity is not always beneficial with regards to fitness. Fish from a

broods with a smaller number of siblings grew quicker and larger fish swam better. As larger fish with better swimming ability are likely to be more successful at escaping predation and competing for food, stocking density may have been more of a limiting factor than genetics, for certain traits.

Genetics is generally predicted to affect individual fitness, notwithstanding other limiting factors. In aquaculture, however, the effect of genetics could be intensified or diminished, depending on the context and the trait concerned. High stocking density in aquaculture intensifies competition and parasite pressures, compared to the wild. The stocking density of captive fish may, as a limiting factor, over-ride any strong effect of genetics on growth or size. This has implications for other size-related fitness traits that might otherwise be determined by genetics. The intensification of parasite transmission and prevalence may, in contrast, emphasise the role of genetics and drive selection, through the survival of individuals with the MHC alleles required to fight the dominant parasites present. In conclusion, parental similarity is important to consider in relation to infectious disease susceptibility of offspring, but has little effect on growth rate or swimming ability in the presence of other limiting factors (e.g. space). Growth rate and parasite susceptibility are density dependent and should be considered to optimise growth rate, parasitism and profits in aquaculture.

ACKNOWLEDGEMENTS

The data for this chapter was collected with laboratory assistance from Dayna Lea, Karan Gupta and Dean Tulett. Darren Croft provided the wild type fish.

SUPPLEMENTARY MATERIAL

Table S7.1: Summary of life history trait data from each brood of specifically crossed *Poecilia reticulata*. Specifically includes: mother and father strain, proportion of brood alive after 30 weeks from birth, average brood growth rate over the first 30 weeks of life, average brood critical swimming velocity, average brood swimming distance and average brood parasite (*Gyrodactylus turnbulli*) intensity at day 9 and day 13. Cells highlighted in green indicate the top 10% and purple are the bottom 10% of values, to indicate the strongest and weakest brood at each life history trait.

					Average brood				
Brood	**Mother strain**	**Father strain**	**Proportion brood alive at 30 weeks**	**Average brood growth rate**	**Critical swimming velocity**	**Distance swam**	**Parasite intensity at day 9**	**Parasite intensity at day 13**	
10	Red Blonde	Leopard	0.05	39.11	45957.59	1.00	110.13	327.25	
24	Cobra Green	Sunset Blonde	0.06	NA	53478.83	1.00	229.00	617.00	Highest 10%
26	Cobra Green	Blonde Red	0.07	29.42	35407.54	1.00	140.38	486.00	Lowest 10%
28	Neon Blue	Blonde Red	0.06	37.18	49666.06	1.00	79.00	285.25	
40	Cobra Green	Leopard	0.06	36.00	25058.27	1.00	167.00	871.00	
42	Flame	Cobra Green	0.06	36.69	26814.29	0.67	185.33	581.57	
46	Cobra Green	Cobra Green	0.08	NA	NA	1.00	123.00	335.00	
48	Cobra Green	Cobra Green	0.06	34.34	24472.91	0.00	165.71	645.31	
50	Sunset Blonde	Sunset Blonde	0.06	NA	NA	1.00	270.50	663.50	
58	Cobra Green	Blonde Red	0.06	39.80	47051.98	0.67	111.00	438.50	
62	Red Blonde	Blonde Red	0.04	37.07	32871.77	1.00	97.78	285.11	
78	Balcony	Balcony	0.05	NA	NA	0.82	NA	NA	
84	Tacarigua	Tacarigua	0.05	NA	NA	1.00	80.50	97.00	
122	Neon Blue	Cobra Green	0.08	NA	NA	1.00	131.00	665.00	
128	Cobra Green	Sunset Blonde	0.07	NA	NA	1.00	87.00	466.00	
146	Tacarigua	Lower Aripo	0.05	NA	NA	1.00	53.00	88.00	
156	Tacarigua	Tacarigua	0.05	NA	NA	1.00	83.00	270.00	
158	Tacarigua	Tacarigua	0.04	35.04	23539.33	1.00	63.00	224.00	

10b	Red Blonde	Leopard	0.05	NA	NA	1.00	114.00	742.00
10c	Red Blonde	Leopard	0.06	39.13	38704.74	1.00	118.50	371.75
10d	Red Blonde	Leopard	0.06	NA	NA	1.00	153.00	638.00
10e	Red Blonde	Leopard	0.05	NA	42791.74	1.00	34.00	151.00
128b	Cobra Green	Sunset Blonde	0.04	33.74	25655.02	1.00	92.25	284.63
128c	Cobra Green	Sunset Blonde	0.05	NA	NA	0.86	126.00	894.00
146b	Tacarigua	Lower Aripo	0.06	NA	NA	1.00	92.00	295.00
156b	Tacarigua	Tacarigua	0.05	NA	NA	0.00	151.67	358.00
156c	Tacarigua	Tacarigua	0.05	32.02	19614.33	1.00	184.00	448.50
24b	Cobra Green	Sunset Blonde	0.05	NA	NA	0.00		
24c	Cobra Green	Sunset Blonde	0.06	40.58	38994.35	0.00	82.25	467.50
24d	Cobra Green	Sunset Blonde	0.04	34.65	35170.15	0.00	176.30	764.20
26b	Cobra Green	Blonde Red	0.04	36.28	35842.24	0.70	151.63	668.75
26c	Cobra Green	Blonde Red	0.08	NA	NA	1.00		
40b	Cobra Green	Leopard	0.05	33.67	23886.72	1.00	115.38	383.29
48b	Cobra Green	Cobra Green	0.07	NA	11596.47	0.80	79.00	524.00
48c	Cobra Green	Cobra Green	0.05	35.20	27373.46	1.00	83.83	402.56
48d	Cobra Green	Cobra Green	0.05	38.39	29678.81	1.00	86.60	254.00
48e	Cobra Green	Cobra Green	0.05	43.43	25134.86	1.00	124.00	284.00
48f	Cobra Green	Cobra Green	0.04	35.35	30885.02	1.00	50.20	186.71
48g	Cobra Green	Cobra Green	0.06	38.27	27046.57	0.83	36.50	222.50
50b	Sunset Blonde	Sunset Blonde	0.05	38.26	23562.24	0.00	144.25	492.75
58b	Cobra Green	Blonde Red	0.06	29.21	35651.62	0.80	167.00	329.00
78b	Balcony	Balcony	0.05	NA	33979.03	1.00	135.50	110.00
84b	Tacarigua	Tacarigua	0.05	38.17	33365.70	1.00	125.23	118.17
84c	Tacarigua	Tacarigua	0.04	36.64	31155.86	0.92	45.25	39.42
84d	Tacarigua	Tacarigua	0.05	36.79	32421.26	0.90	67.58	126.25
84e	Tacarigua	Tacarigua	0.03	34.24	29651.73	1.00	74.11	60.92

CHAPTER 8

GENERAL DISCUSSION

8.1. GENERAL DISCUSSION

The studies reported in this thesis used a combination of laboratory microcosm and field sampling experiments to understand the differences in MHC class II genetic composition between truly wild, wild type and ornamental strains of guppy (*Poecilia reticulata*) and the implications for fitness, across the entire life history of this vertebrate host. The hypothesis that inbred fish are more susceptible to parasitism was tested by monitoring parasite (*Gyrodactylus turnbulli*) trajectories on individual fish from three different breeding regimes (inbred, randomly bred control and outbred; Chapter 2). Inbred individuals had higher mean parasite intensity compared to their randomly bred and outbred counterparts (Chapter 2), supporting findings from previous studies on wild animals in their natural environment (including the host-specific ectoparasitic lice on Galapagos hawk and kestrels: Whiteman et al. 2006; Ortego et al. 2007, respectively). The parasitic infection lasted longer and reached a higher maximum burden on inbred individuals compared to the randomly bred control regime (Chapter 2). This may have implications for conservation programmes in which wild populations are supplemented with *ex-situ* bred individuals (van Oosterhout et al. 2007b). With an increased density of susceptible inbred hosts and compromised herd immunity, such programmes could intensify the risk of parasite outbreaks and increase mortality, which in turn could contribute to an extinction vortex (Gilpin and Soulé 1986). This study assessed the effect of inbreeding on parasite susceptibility, without analysing the genetic affects. A future development of this would analyse the genotypes of fish from the specific breeding regimes, in order to link the composition of relevant genes with the observed consequence for fitness of inbreeding. This was considered in subsequent chapters.

A potential mechanism behind the increased susceptibility of domesticated individuals to parasitism is reduced genetic diversity at the Major Histocompatibility Complex (MHC), as a result of inbreeding and limited exposure to pathogens (Klein et al. 1993), reflecting its importance in adaptive immunity. The effect of inbreeding

on MHC allelic and supertype composition was considered by comparing ornamental (domesticated) and wild type guppy strains, using an experimental investigation of the implications for fitness comprising parasite infection trajectories and feeding ability (Chapter 3). Ornamental fish had significantly reduced MHC richness, expressed at the level of both the individual (number of MHC alleles and MHC supertypes per individual) and population (total number of MHC alleles and supertypes found across all individuals within a strain). As a consequence, ornamental guppies were significantly more susceptible to *G. turnbulli* infection, compared to wild type fish. The feeding rate of ornamental fish was significantly reduced when infected, whereas that of wild type fish was unaffected. The presence of particular functional groups (supertypes) of MHC, rather than diversity *per se*, determined parasite intensity. The specific MHC supertypes associated with reduced parasite intensity differed between ornamental and wild type fish, potentially due to variation across the entire guppy genome. The loss of key functional MHC alleles due to reduced genetic diversity and artificial selection could have implications for host population fitness, especially in terms of parasite susceptibility. MHC functionality is, therefore, important to consider in captive breeding programmes.

The use of wild type individuals is common practice, as a model for wild populations. A comparison of MHC genetic composition between truly wild, wild type and ornamental guppies revealed that truly wild fish have the greatest MHC genetic diversity (Chapter 4). Even after few generations of a bottlenecked population, MHC allelic diversity was significantly reduced (comparing wild type to truly wild). To further investigate the importance of MHC supertypes in natural host populations, truly wild fish were sampled across 4 rivers and fully screened for both ecto- and endoparasites (Chapter 4). The presence or absence of certain parasites within the naturally occurring community was associated with specific MHC functional groups, in concordance with previous studies (Fraser and Neff 2009; Pilosof et al. 2014). These results also reflect variation in parasite community and MHC supertype richness across rivers, supporting theory for complex evolutionary interplay between host and parasites. As other regions of the genome are also important in immunity, a consideration of the entire host genome, especially non-MHC immune regions, would complement the work of Chapters 3 and 4, with respect to parasite susceptibility and community. This would enable a comparison, across levels of domestication (truly wild, wild type and ornamental), of genome

wide variation and determine whether this is associated with fitness and life history traits.

The ‘Balcony’, ornamental strain of guppy, used in Chapter 3, has been maintained and allowed to naturally select in the laboratory for ~ 20 years. This strain of guppy appears to have reverted back to near wild phenotype over this time. Samples collected from this fish stock, throughout their time in the laboratory, would provide an opportunity for future work to examine MHC composition during phenotypic reversion to wild type. This same strain of guppy has also been used to maintain the *G. turnbulli* parasite culture over the same time frame. Linking variation in host MHC genetic composition with alterations in the parasite genome, through time, would be extremely novel and provide a model for host–parasite co-evolution (collaboration with A. Ellison and M. Reynolds). Preliminary analysis has identified a reduction in host MHC allelic diversity, whilst regions of the parasite (*G. turnbulli*) genome have been lost or fixed during the last ~ 20 years. Further analysis needs to be performed to identify whether other regions of the host genome have been modified during this time (Reynolds Ph.D. thesis 2017).

Increased susceptibility to parasitism of captive bred hosts, as well as adaptation of MHC functional composition to local parasites in the wild, implies that the release of domesticated fish will negatively affect natural populations. Intensification of fish farming has led to an increase in the number of domesticated individuals released, accidentally and deliberately, into the wild (Naylor et al. 2004; Copp et al. 2006; Lorenzen 2008; Laikre et al. 2010). The hypothesis that the release of ornamental fish into a wild or wild type population would lead to increased parasite prevalence and abundance was experimentally tested, through the introduction of: an infected ornamental into an uninfected wild type population; and an uninfected ornamental or wild type fish into an infected wild type population (Chapter 5). Parasite prevalence and abundance was monitored over time, providing evidence that both are increased by the introduction of an ornamental fish (infected or uninfected). This supports the suggestion of Faria et al. (2010) that the release of susceptible fish could promote an epidemic. The results from Chapter 5 have implications for programmes supplementing fish stocks with cultured, potentially more susceptible, fish, which could lead to disease epidemics. The use of truly wild fish as the receptor population, introducing either wild type (representative of cultured fish in aquaculture) or

ornamental (characterising the pet trade industry) fish, would provide a more realistic model. This would allow consideration of the identified differences in MHC composition between truly wild and wild type fish (Chapter 4). Logistical complexities (including the time for: import license, habituation and parasite treatment) precluded the use of truly wild fish in the current study, which meant wild type fish were used as an alternative model.

The basis of artificial selection is the determination, by humans, of 'important' morphological traits to produce phenotypically desirable offspring (e.g. Cook et al. 2000; Gjedrem et al. 2012). This will typically preclude any effect of MHC, suggested as the single most important gene in sexual selection. The effect of sexual selection, MHC similarity and parasitism for mate choice were assessed using two strains of guppies of varying colour hue, size and infection status (Chapter 6). Most previous research on mate choice has focused on one aspect of selection, namely genotype (e.g. Barber et al. 2001; Andersson and Simmons 2006; Agbail et al. 2010; Ejsmond et al. 2014), phenotype (e.g. Brooks and Endler 2001; Zajitschek et al. 2006) or infection status (e.g. Kennedy et al. 1987; Milinski and Bakker 1990), but it is likely a combination of these variables that influences mate preference. The results of Chapter 6 support this; whether presented with a choice of three mates or in paired interactions, females consistently associated more with males that had redder colouration and less shared MHC alleles. These results are consistent with the promotion of offspring fitness, as male red colouration has been linked to heightened immune function (Barreiro Lozano 1994) and mate choice based on MHC dissimilarity has frequently been demonstrated (Milinski 2006; Agbail et al. 2010; Ejsmond et al. 2014).

The selection, by females, of mates of the same strain appeared to contradict the effect of dissimilarity; but may increase offspring survival by reducing the risk of predation due to the 'oddity effect', or reflect the effect of imprinting at birth (Landeau and Terborgh 1986; Svensson et al. 2017). These results demonstrate the importance of multi-variable analysis when it comes to the complexity of mate choice and offspring fitness. Humans try to select 'important' traits for profits, but tend not to consider the fitness of the offspring; thorough research is required to ensure a loss of fitness is not incurred. Considering multiple variables that influence mate choice has provided a high level overview of the importance of certain

variables, whilst controlling for others. To better control for the confounding effect of female preference for males of the same strain as herself, a future study should analyse MHC composition of individuals in advance of mate choice trials. This would allow specific interactions to be engineered, from a single strain, to provide more refined detail about the variables that have been shown to be important in multi-variable guppy mate choice. Guppies use ultraviolet vision during mate choice (Smith et al. 2002). This was beyond the scope of the current study, but would be important to consider for future research to ensure the full perception of colour in mate choice is incorporated.

In the final empirical chapter of this thesis, the implications of MHC genetic composition and mate selection for offspring fitness were investigated. Inbreeding depression can affect several offsping fittness-related traits (Saccheri et al. 1998; van Oosterhout et al., 2000a; Keller and Waller, 2002), including survival (Coltman et al. 1998), reproductive success (Slate et al., 2000; Spielman et al. 2004; Eszterbauer et al. 2015), sexual ornamentation and courtship behaviour (van Oosterhout et al., 2003b; Malo et al. 2017), and parasite susceptibility (MacDougall-Shackleton et al. 2005; Rijks et al. 2008; Chapter 2), making it important for parents to choose their mates carefully. Producing a first generation (F1) from parents with varying degrees of MHC similarity enabled an assessment of the hypothesis that parents that are genetically most dissimilar will produce the fittest offspring (Chapter 7). Parental MHC similarity influenced infectious disease susceptibility of offspring, but had little effect on growth rate or swimming ability, in the presence of other limiting factors (e.g. space). These results are not consistent with the limited previous research on multiple life history traits, whereby F1 MHC heterozygosity affected growth rate, but not parasitism or survival (e.g. Fraser and Neff 2009). Contrasting results often reflect context-dependency and complexity of studies phenomena; the density-dependence of fish growth rate, and the consequential effect on parasite susceptibility, is likely to have influenced the results of this study. Space as a limiting factor should be considered to optimise growth rate, parasitism and profits in aquaculture. The outcomes of Chapter 7 were studied over a longer time frame than previous studies and identified a potentially confounding effect of density dependence on various fitness traits. To eliminate the confounding effect and refine the results for future studies, it would be important to isolate the first generation offspring.

8.2. CONCLUSIONS

This thesis demonstrates the importance of incorporating multiple variables when assessing the effect of MHC on life history traits. Overall, MHC allelic and supertype composition has been shown to vary across different levels of fish domestication, with clear implications for some, but not all, fitness traits. MHC composition was consistently associated with susceptibility to parasitic infection, in both wild and captive bred fish stocks, and in F1 generation offspring. The results suggest that MHC functionality is at least as important as allelic and supertype diversity, with regards to individual fitness and life history traits. Aquaculture and other industries using captive animals, in particular, could benefit from considering the importance of allelic and functional genetic diversity. Identifying specific MHC allele expression would be the next step to determine which alleles are expressed when an individual is infected with a particular parasite. This would enable particular alleles to be linked directly to certain parasites and could enhance the efficacy of breeding programmes.

A reduction in genetic diversity through artificial selection of traits for breeding could lead to the loss of key functional alleles, with implications for parasite susceptibility and other fitness traits within the population. Replenishing cultured stocks regularly with wild fish could reduce the risk to genetic diversity and could increase host pathogen resistance. Maintaining genetic diversity in aquaculture is important, not only to increase the resistance of fish to the local parasites, but also to minimise the impact of novel pathogens following an introduction. Precautionary measures to reduce the release of domesticated individuals into wild populations would help prevent parasite spill-over between captive-bred and wild populations.

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APPENDIX

Appendix 1: Observed interactions between a male and female guppy (*Poecilia reticulata*) in a paried mate choice interaction. The observer performed a 10 min focal follow on the male and recorded all behaviours and interactions with the female. Each value is in seconds (s). Female/ Male intiatied behaviour is the total time the Female/ Male intiated interactions with the opposite sex. Grey highlighted cells are the total amount of time the female and male initiated interactions and the total amount of time there were no interactions occurring between the male and female.

Male ID	Male Strain	Female ID	Female Strain	Infection Status	Female Approach	Female Chase	Female Flee	Female Nip	Female initiated behaviour	Male Approach	Male Chase	Male Display	Male Flee	Male Nip	Male Reverse	Male Initiated Behaviour	No interaction
1570	Black	1573	Black	Uninfected	9.3	0	21.8	0	31.2	21.8	0	0	8.2	0	0	30.0	398.9
1571	Black	1573	Black	Uninfected	8.4	0	81.5	0	89.9	289.5	0	51.0	45.2	31.6	0	417.3	142.1
1572	Black	1573	Black	Uninfected	0	0	0	0	0	0	0	0	0	0	0	0	0
1574	Black	1577	Black	Uninfected	6.1	0	0	0	6.1	168.7	73.1	0	0	0	0	241.8	301.2
1575	Black	1577	Black	Uninfected	2.9	0	0	23.0	2.9	152.7	14.1	0	0	0	0	166.8	405.3
1576	Black	1577	Black	Uninfected	17.6	0	0	0	17.6	11.1	0	0	0	0	0	11.1	563.5
1578	Black	1581	Black	Uninfected	2.3	0	0	0	2.3	0	0	0	0	0	0	0	598.8
1579	Black	1581	Black	Uninfected	4.7	0	0	0	4.7	207.8	271.0	0	0	0	0	478.8	91.2
1580	Black	1581	Black	Uninfected	2.8	0	0	0	2.8	156.4	28.6	0	0	0	0	185.0	408.3
1582	Black	1585	Black	Uninfected	0	0	0	0	0	183.8	194.5	0	0	0.2	0	378.5	222.9
1583	Black	1585	Black	Uninfected	39.6	6.1	0	0.2	45.9	339.1	51.6	0.8	0	0	0	391.6	151.9
1584	Black	1585	Black	Uninfected	120.9	25.4	0	0.2	146.6	192.8	52.3	15.5	0	5.1	0	265.7	176.1
1586	Black	1589	Black	Uninfected	353.5	708.0	0	0	423.6	76.0	0	0	0	0	0	76.0	102.9
1587	Black	1589	Black	Uninfected	111.5	73.2	0	0.2	184.9	212.9	55.5	0	0	0	0	268.4	108.6
1588	Black	1589	Black	Uninfected	72.3	0	0	0.5	72.8	169.7	312.0	3.9	0	0.2	0	485.7	41.5
1590	Black	1593	Black	Uninfected	13.2	0	0	0	13.2	352.7	47.1	0	0	0	0	399.8	184.6
1591	Red	1593	Black	Uninfected	135.4	59.8	0	0	195.2	301.4	6.0	0	0	0	0	307.4	99.4
1592	Black	1593	Black	Uninfected	623.2	23.7	0	0	646.9	127.6	0	0.7	0	0	0	128.3	255.6

1594	Black	1596	Black	Uninfected	37.3	4.3	0	0	41.6	372.0	48.1	0	0	0.2	0	420.3	136.4
1595	Black	1596	Black	Uninfected	106.4	57.5	0	0.8	164.8	242.7	0	0	0	0.9	0	243.6	270.3
1601	Red	1596	Black	Uninfected	13.4	5.4	0	0	18.8	216.9	333.2	14.8	0	0.3	0	565.3	92.0
1597	Black	1600	Black	Uninfected	17.1	0	0	0	17.1	146.0	173.0	0	0	0.2	0	319.2	265.7
1598	Red	1600	Black	Uninfected	14.8	6.2	0	0	21.0	141.3	84.4	1.9	0	0	0	227.6	353.4
1599	Black	1600	Black	Uninfected	104.5	70.7	0	0	175.1	22.1	0	0	0	0	0	22.1	403.4
1602	Red	1605	Black	Uninfected	44.2	0	0	0	44.2	206.7	29.9	0	0	0	0	236.6	329.6
1603	Black	1605	Black	Uninfected	271.6	23.4	0	0	295.0	236.8	4.8	0	0	0.3	0	241.9	57.5
1604	Red	1605	Black	Uninfected	15.3	0	0	0	15.3	115.4	19.2	0	0	0	0	134.6	452.3
1606	Black	1609	Black	Uninfected	0	0	0	0	0	104.7	0	0	0	0.2	0	104.9	496.2
1607	Red	1609	Black	Uninfected	14.9	0	0	0	14.9	114.4	0	2.2	0	0	0	116.6	485.3
1608	Red	1609	Black	Uninfected	0	0	0	0	0	32.3	0	0	0	0	0	32.3	567.9
1610	Red	1613	Black	Uninfected	78.7	0	0	0	78.7	116.3	0	0	0	0.2	0	116.5	405.2
1611	Red	1613	Black	Uninfected	50.8	22.6	0	0	73.4	169.3	183.3	0	0	0.1	0	352.8	171.8
1612	Black	1613	Black	Uninfected	0	0	0	0	0	360.9	69.8	0	0	0.5	0	431.2	168.8
1614	Red	1617	Black	Uninfected	266.1	0	0	0	266.1	260.3	7.8	3.1	0	0	0	271.2	62.4
1615	Red	1617	Black	Uninfected	448.1	136.3	0	0.7	585.1	280.0	18.4	0	0	0	0	298.4	317.1
1616	Black	1617	Black	Uninfected	0	0	0	0	0	0	0	0	0	0	0	0	0
1618	Red	1621	Black	Uninfected	53.4	0	0	0	53.4	403.4	95.1	0	0	0.1	0	498.7	48.9
1619	Red	1621	Black	Uninfected	297.6	77.3	0	0	374.9	106.9	0	0	0	0	0	106.9	1208.0
1620	Red	1621	Black	Uninfected	33.4	0	0	0	33.4	361.5	73.4	3.8	0	8.6	0	447.4	109.2
1622	Red	1625	Black	Uninfected	2508.0	0	0	0	2508.0	0	0	0	0	0	0	0	350.3
1623	Red	1625	Black	Uninfected	173.6	0	0	0	173.6	295.2	10.9	0	0	0	0	306.1	130.5
1624	Red	1625	Black	Uninfected	60.4	0	0	0	60.4	486.5	8.6	0	0	0.3	0	495.4	42.2
1626	Red	1629	Black	Uninfected	45.4	0	0	0	45.4	436.5	39.0	0	0	0.5	0	475.9	98.8
1627	Red	1629	Black	Uninfected	74.3	0	0	0	74.3	399.6	0	0	0	1.3	0	400.9	105.9
1628	Red	1629	Black	Uninfected	5.7	0	0	0	5.7	494.8	15.5	0	0	0.3	0	510.7	84.2
1630	Red	1633	Black	Uninfected	119.7	0	0	0	119.7	319.0	21.8	0	0	0	0	340.7	142.1

1631	Red	1633	Black	Uninfected	319.4	0	0	0	319.4	241.8	0	0	0	0.2	0	242.0	50.3
1632	Red	1633	Black	Uninfected	510.8	0	0	0.2	511.0	13.1	0	0	0	0	0	13.1	68.9
1634	Red	1637	Red	Uninfected	102.5	0	0	0	102.5	346.1	48.4	0	0	0.2	0	394.7	103.2
1635	Red	1637	Red	Uninfected	42.9	0	0	0	42.9	56.6	0	1.2	0	0	0	57.8	499.7
1636	Red	1637	Red	Uninfected	107.3	0	0	0	107.3	115.4	0	0	0	0.2	0	115.6	373.7
1638	Red	1641	Red	Uninfected	44.6	15.0	0	0	59.6	381.6	0	0	0	7.8	0	389.3	131.3
1639	Red	1641	Red	Uninfected	0	0	0	0	0	408.2	0	0	0	0.2	0	408.4	194.0
1640	Red	1641	Red	Uninfected	44.1	0	0	0	44.1	329.0	0	0	0	0	0	329.0	224.2
1642	Red	1645	Red	Uninfected	113.7	0	0	0	113.7	364.9	25.1	0	0	0.3	0	390.2	96.7
1643	Red	1645	Red	Uninfected	320.5	0	0	0	320.5	111.4	0	0	0	0	0	111.4	164.6
1644	Red	1645	Red	Uninfected	10.2	0	0	0	10.2	409.4	61.5	37.1	0	0	0	508.0	41.1
1646	Red	1649	Red	Uninfected	382.3	0	0	0	382.3	47.7	0	0	0	0.2	0	47.9	171.8
1647	Red	1649	Red	Uninfected	43.9	0	0	0	43.9	444.4	0	0	0	1.0	0	445.4	105.3
1648	Red	1649	Red	Uninfected	136.4	0	0	0	136.4	257.0	0	0	0	0.2	0	257.1	155.6
1650	Red	1653	Red	Uninfected	213.3	0	0	0	213.3	213.5	0	0	0	0	0	213.5	173.9
1651	Red	1653	Red	Uninfected	1.3	0	0	0	1.3	329.6	13.8	0	0	0	0	343.4	256.6
1652	Black	1653	Red	Uninfected	0	0	0	0	0	123.1	9.9	4.0	0	0	0	137.0	464.9
1654	Black	1657	Red	Uninfected	2.2	0	0	0	2.2	155.3	135.6	0	0	0.1	0	291.0	307.2
1655	Red	1657	Red	Uninfected	222.2	0	0	0	222.2	213.8	0	0	0	0	0	213.8	174.3
1656	Red	1657	Red	Uninfected	76.9	0	0	0	76.9	479.2	0	0	0	0.5	0	479.7	43.5
1658	Red	1661	Red	Uninfected	25.5	0	0	0	25.5	399.6	8.1	0	0	0	0	407.6	166.8
1659	Black	1661	Red	Uninfected	17.7	0	0	0	17.7	207.3	111.4	0	0	0	0	318.7	264.5
1660	Red	1661	Red	Uninfected	42.1	0	0	0	42.1	286.4	0	0	0	0.2	0	286.6	271.7
1662	Black	1665	Red	Uninfected	0	0	0	0	0	147.5	0	2.0	0	0	0	149.5	450.8
1663	Red	1665	Red	Uninfected	56.9	0	0	0	56.9	283.7	0	0	0	0	0	283.7	261.6
1664	Red	1665	Red	Uninfected	27.1	0	0	0	27.1	451.5	0	23.4	0	0	0	474.9	98.6
1666	Black	1669	Red	Uninfected	0	0	0	0	0	479.2	46.6	0	0	0	0	525.8	73.4
1667	Red	1669	Red	Uninfected	905.0	0	0	0	905.0	468.6	0	0	0	0	0	468.6	36.7

1668	Red	1669	Red	Uninfected	5.2	0	0	0	5.2	350.5	4.4	0	0	0.6	0	355.5	237.1
1670	Black	1673	Red	Uninfected	0	0	0	0	0	69.4	0	0	0	0	0	69.4	530.9
1671	Black	1673	Red	Uninfected	23.9	0	0	0	23.9	80.8	0	0	0	0	0	80.8	496.2
1672	Red	1673	Red	Uninfected	61.4	0	0	0	61.4	284.0	0	0	0	0	0	284.0	224.4
1674	Red	1677	Red	Uninfected	117.8	0	0	0	117.8	431.5	0	0	0	0	0	431.5	52.7
1675	Black	1677	Red	Uninfected	53.7	0	0	0	53.7	317.7	60.4	0	0	0	0	378.0	176.5
1676	Black	1677	Red	Uninfected	0	0	0	0	0	348.3	52.5	0	0	0	0	400.8	199.1
1678	Black	1681	Red	Uninfected	0	0	0	0	0	67.4	0	0	0	0	0	67.4	506.4
1679	Red	1681	Red	Uninfected	0	0	0	0	0	560.6	0	10.7	0	0.2	0	571.5	29.3
1680	Black	1681	Red	Uninfected	0	0	0	0	0	173.5	226.5	0	0	0	0	400.0	172.1
1682	Black	1685	Red	Uninfected	0	0	0	0	0	562.9	0	0	0	0	0	562.9	37.3
1683	Black	1685	Red	Uninfected	61.3	0	0	0	61.3	64.4	0	0	0	0	0	64.4	405.7
1684	Red	1685	Red	Uninfected	0	0	0	0	0	455.7	0	0	0	0.2	0	455.9	139.0
1686	Black	1689	Red	Uninfected	0	0	0	0	0	193.8	0	0	0	0.2	0	194.0	405.9
1687	Black	1689	Red	Uninfected	2.9	0	0	0	2.9	216.0	4.5	0	0	0	0	220.5	377.7
1688	Black	1689	Red	Uninfected	0	0	0	0	0	171.7	6.0	0	0	0	0	177.7	424.9
1690	Black	1693	Red	Uninfected	7.6	0	0	0	7.6	25.1	0	0	0	0	0	25.1	568.2
1691	Black	1693	Red	Uninfected	0	0	0	0	0	206.3	11.9	0	0	0	0	218.2	382.1
1692	Black	1693	Red	Uninfected	0	0	0	0	0	228.0	0	0	0	0	0	228.0	372.1
1678	Black	1681	Red	Uninfected	28.1	0	0	0	28.1	67.4	0	0	0	0	0	67.4	506.4
1570	Black	1573	Black	Infected	270.2	0	62.5	0	332.7	35.9	0	0	0	0	0	35.9	231.6
1571	Black	1573	Black	Infected	144.0	0	52.1	1.0	197.1	139.1	4.4	6.0	0	0.6	0	150.1	252.6
1572	Black	1573	Black	Infected	181.2	0	33.5	4.1	218.8	98.6	0	0	0	0	0	98.6	316.8
1574	Black	1577	Black	Infected	20.8	0	3.1	0	23.8	23.2	21.5	0	0	0	0	44.6	374.3
1575	Black	1577	Black	Infected	25.8	0	38.5	0.7	64.9	159.7	22.4	14.0	0	2.0	0	198.1	321.6
1576	Black	1577	Black	Infected	30.6	0	118.4	0.4	149.4	0	0	3.8	0	0	0	3.8	447.3
1578	Black	1581	Black	Infected	12.6	0	0	0	12.6	172.7	114.6	0	0	0	0	287.3	338.7
1579	Black	1581	Black	Infected	5.5	0	0	0.3	5.8	187.2	210.9	1.1	0	0.2	0	399.3	196.7

1580	Black	1581	Black	Infected	58.1	0	6.8	0	64.9	83.1	14.1	4.4	0	0.2	0	101.8	437.6
1582	Black	1585	Black	Infected	55.7	0	226.6	5.3	287.5	29.3	65.0	0	0	9.1	0	103.4	189.5
1583	Black	1585	Black	Infected	116.5	0	241.1	1.3	358.9	24.5	20.2	0	0	3.3	0	48.0	193.1
1584	Black	1585	Black	Infected	157.1	0	146.7	0	303.8	101.4	14.9	0	0	0	0	116.3	180.5
1586	Black	1589	Black	Infected	272.5	0	41.3	0	313.8	40.0	4.7	0	0	0.2	0	44.8	231.4
1587	Black	1589	Black	Infected	135.0	0	33.0	0	168.0	132.5	43.9	0	0	0	0	176.4	255.8
1588	Black	1589	Black	Infected	60.1	0	2.3	0	62.4	63.8	96.4	6.3	0	0	0	166.5	371.1
1590	Black	1593	Black	Infected	40.8	0	28.8	0	69.6	421.6	0	0	0	0.7	0	422.3	109.4
1591	Red	1593	Black	Infected	174.8	0	16.4	0	191.2	141.3	0	0	0	0	0	141.3	268.1
1592	Black	1593	Black	Infected	121.1	19.2	23.4	0	163.7	98.0	0	0	0	0.2	0	98.2	335.0
1594	Black	1596	Black	Infected	149.9	0	14.7	0	164.6	208.3	66.2	0	0	0	0	274.5	164.1
1595	Black	1596	Black	Infected	24.1	0	0	0.2	24.3	184.3	0	8.7	0	0	0	193.0	388.0
1601	Red	1596	Black	Infected	27.8	0	0	0	27.8	348.5	18.9	0	0	0.2	0	367.6	198.1
1597	Black	1600	Black	Infected	0	0	0	0	0	263.7	25.2	0	0	0.6	0	289.5	301.1
1598	Red	1600	Black	Infected	0	0	0	0	0	60.0	0	0.8	0	0.2	0	61.0	501.0
1599	Black	1600	Black	Infected	177.2	0	22.1	0.2	199.5	8.3	0	0	0	0	0	8.3	392.9
1602	Red	1605	Black	Infected	47.5	0	0	0	47.5	376.1	23.7	0.7	0	0.1	0	400.6	153.4
1603	Black	1605	Black	Infected	0	0	0	0	0	255.7	0	0	0	0.3	0	256.0	126.6
1604	Red	1605	Black	Infected	0	0	0	0	0	174.8	15.4	0	0	0.2	0	190.4	344.6
1606	Black	1609	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1607	Red	1609	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1608	Red	1609	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1610	Red	1613	Black	Infected	19.9	0	0	0	19.9	96.7	0	0	0	0	0	96.7	483.2
1611	Red	1613	Black	Infected	57.0	0	0	0	57.0	216.7	27.8	0	0	0	0	244.5	299.0
1612	Black	1613	Black	Infected	0	0	0	0	0	278.8	0	0	0	0	0	278.8	322.6
1614	Red	1617	Black	Infected	177.3	0	23.8	0	201.1	186.5	0	0	0	0	0	186.5	212.7
1615	Red	1617	Black	Infected	78.7	0	0	0	78.7	111.2	66.3	0	0	1.0	0	178.4	342.4
1616	Black	1617	Black	Infected	35.0	0	0	0	35.0	265.9	91.8	0	0	0.8	0	358.5	206.6

1618	Red	1621	Black	Infected	4.7	0	0	0	4.7	306.6	13.6	0	0	0	26.3	346.6	257.2
1619	Red	1621	Black	Infected	92.4	0	0	0	92.4	106.0	19.3	0	0	0	2.9	128.1	389.8
1620	Red	1621	Black	Infected	210.6	0	0	0.2	210.8	143.9	0	0	0	0	1.6	145.5	244.0
1622	Red	1625	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1623	Red	1625	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1624	Red	1625	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1626	Red	1629	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1627	Red	1629	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1628	Red	1629	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1630	Red	1633	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1631	Red	1633	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1632	Red	1633	Black	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1634	Red	1637	Red	Infected	0	0	0	0	0	548.9	0	0	0	0.2	18.0	567.0	33.1
1635	Red	1637	Red	Infected	0	0	0	0	0	153.2	6.4	0	0	0.2	0	159.8	295.3
1636	Red	1637	Red	Infected	7.1	0	0	0	7.1	222.0	0	0	0	0.3	0	222.3	371.1
1638	Red	1641	Red	Infected	12.5	0	0	0	12.5	125.1	0	0	0	0	0	125.1	402.5
1639	Red	1641	Red	Infected	801.0	0	0	0	801.0	195.9	0	0	0	0	0	195.9	325.0
1640	Red	1641	Red	Infected	0	0	0	0	0	417.6	0	0	0	0	5.3	422.9	183.0
1642	Red	1645	Red	Infected	69.7	0	18.2	0	88.0	301.7	0	0	0	0	4.7	306.4	206.0
1643	Red	1645	Red	Infected	55.8	0	0	0	55.8	334.4	0	0	0	0.1	1.8	336.3	207.9
1644	Red	1645	Red	Infected	0	0	0	0.1	0.1	448.4	7.5	10.2	0	0.1	0	466.2	132.7
1646	Red	1649	Red	Infected	185.0	0	0	0	185.0	379.0	0	9.9	0	0.7	0	389.6	29.1
1647	Red	1649	Red	Infected	108.2	0	0	0	108.2	155.4	0	3.7	0	0	0	159.1	325.5
1648	Red	1649	Red	Infected	43.3	0	0	0	43.3	196.3	0	0.9	0	0.5	0	197.7	359.0
1650	Red	1653	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1651	Red	1653	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1652	Black	1653	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1654	Black	1657	Red	Infected	0	0	0	0	0	217.6	6.7	0	0	0	0	224.3	365.6

1655	Red	1657	Red	Infected	107.8	0	0	0	107.8	284.7	0	0	0	0	0	284.7	191.9
1656	Red	1657	Red	Infected	180.4	0	0	60.2	240.6	127.9	0	0	0	0.2	2.8	130.9	188.8
1658	Red	1661	Red	Infected	91.4	0	0	0	91.4	351.3	0	3.0	0	0	7.2	361.5	147.3
1659	Black	1661	Red	Infected	0	0	0	0	0	149.1	252.6	0	0	0	0	401.7	198.5
1660	Red	1661	Red	Infected	23.1	0	0	0	23.1	250.2	25.7	0	0	0.2	15.9	291.9	282.0
1662	Black	1665	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1663	Red	1665	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1664	Red	1665	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	0
1666	Black	1669	Red	Infected	0	0	0	0	0	444.5	73.6	0	0	0	2.7	520.8	79.2
1667	Red	1669	Red	Infected	0	0	0	0	0	448.9	0	0	0	0	0	448.9	181.4
1668	Red	1669	Red	Infected	0	0	0	0	0	289.9	7.0	0	0	0	19.5	316.3	284.0
1670	Black	1673	Red	Infected	0	0	0	0	0	39.7	0	0	0	0	0	39.7	561.7
1671	Black	1673	Red	Infected	0	0	0	0	0	8.1	0	0	0	0	0	8.1	592.2
1672	Red	1673	Red	Infected	167.4	0	0	0	167.4	103.0	0	0	0	0	7.9	110.9	321.9
1674	Red	1677	Red	Infected	0	0	0	0	0	534.9	6.5	15.4	0	0	0	556.9	43.4
1675	Black	1677	Red	Infected	61.0	0	0	0	61.0	101.6	0	0	0	0	0	101.6	437.5
1676	Black	1677	Red	Infected	0	0	0	0	0	268.8	42.0	0	0	0	0	310.9	289.3
1678	Black	1681	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	600.0
1679	Red	1681	Red	Infected	0	0	0	0	0	451.5	0	0	0	0	2.6	454.1	146.2
1680	Black	1681	Red	Infected	0	0	0	0	0	178.2	239.0	0	0	0.2	0	417.5	183.3
1682	Black	1685	Red	Infected	7.9	0	0	0	7.9	527.9	0	0	0	0	0	527.9	83.0
1683	Black	1685	Red	Infected	1.3	0	0	0	1.3	79.9	0	0	0	0	0	79.9	509.1
1684	Red	1685	Red	Infected	19.2	0	0	0	19.2	313.8	8.4	0	0	0	5.4	327.6	237.2
1686	Black	1689	Red	Infected	0	0	0	0	0	76.1	0	0	0	0	0	76.1	524.1
1687	Black	1689	Red	Infected	0	0	0	0	0	7.9	0	0	0	0	0	7.9	595.4
1688	Black	1689	Red	Infected	0	0	0	0	0	123.9	0	0	0	0	0	123.9	479.6
1690	Black	1693	Red	Infected	0	0	0	0	0	6.3	0	0	0	0	0	6.3	597.9
1691	Black	1693	Red	Infected	0	0	0	0	0	31.5	0	0	0	0	0	31.5	569.4

1692	Black	1693	Red	Infected	0	0	0	0	0	31.2	0	0	0	0	0	31.2	568.8
1678	Black	1681	Red	Infected	0	0	0	0	0	0	0	0	0	0	0	0	600.0

Appendix 2: Guppy (*Poecilia reticulata*) Major Histocompatibility Complex alleles from the current study and those published (3.2.4.6) with their supertype (ST) and sequence.

Allele	ST	Sequence
Pore-DAB*602	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCATTTATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCTGTCTGACCACCGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*308	1	AGCTGAAGGACATCCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAAC GGCTCAACAAAGACACGTCATTTATCGCTGGGCTGAGAGCTCAGAGGGAGACCTACTGCCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*287	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA GGTGGAACAAAGACACGTCACTGATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACGCTCTGGA
Pore-DAB*1094	12	ACCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACG GTTCAACAACGACCCGTCAATTATTGGAGCGAGGAGAGCTCTGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*452	3	AGCTGAAGGACATCCAGTATGTCCTCTCCTATTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACTTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCAGAACG CTGGAACAACGATCCGTCAATTATTGGAGGGAGGAGAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
Pore-DAB*429	3	AGCTGAAGGACATTCAGTACATCGAGTCCTATTATTACAACAAGCTGGAGCTCGCCAGGTTTGACAGCAACGTGGGTAGATATGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACACGTCACTGATCTCTATGAGGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
Pore-DAB*839	5	AGCTGAAGGACATCCAGTACAGCAGGTCCTTCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAGCCTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAAAGACACGTCACAGATCGCTGCGATGAACGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACATTCTGGA
Pore-DAB*1069	7	AGCTGAAGGACATTCAGTATGTTGACTCCTACTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGACACGTCACTGATCGCATCAGAGGCGGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*363	12	AGCTGAAGGACATTCAGTACGTCTACTCCATGTATTACAACAAGCTGGAATACATGAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGACGTATGGAGTGAAGAACGCTGAGC ACTGGAACAAAGACACGTCACAGATCGCTGCGAGGAAAGCTCAGAGTGAGACCGTCTGTCTGCACAACGTCGGTATCGACTACCAGGCGGCTCTGGA
Pore-DAB*403	8	AGCTCAAAGACATCGAGTTCATCAGGTCTGACTTCTACAACAAGCTGGAGATCTTCAGGTTCAGCAGCAGTTTGGGCAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGTA CAGGAACAACAACCCGTCATTTATTGCTGGGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTAACTGGTACCCAAATATGCTGAC
Pore-DAB*1082	5	AGCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCAGAGC GGTTCAACAACGACCCGTCAATTATTGGAGCAAGGAGAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCGAAACGCTCTGGA
Pore-DAB*120	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCAACTATTACAACAAGCTGGAGCTGTACAGGTTCAGCAGCAGTTTGGGCAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGT ACTTCAACAGCGACCCGTCAATTATTGGAGCAATGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*133	2	AGCTCAAAGGCATTCAGTACGTCTACTCCATGTATTACAACAAGCTGGAATACATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGACGTTTGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCATCAGAGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCTGGTACGGGAATCCACTGGC
Pore-DAB*1080	5	AGCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCAGAGC GGTTCAACAACGACCCGTCAATTATTGGAGCAAGGAGAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTAACGACTACCGAAACGCTCTGGA
Pore-DAB*1079	12	AGCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCAGAGC GGTTCAACAACGACCCGTCAATTATTGGAGCAAGGAGAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*1071	7	AGCTGAAGGACATCGAGTTCATCGACTCATATTATTACAACAAGCTGGAGCTGTACAGGTTCAGCAGCAGTTTGGGGAGGTTTGTTGGATACACAGAGTATGGAGTGAAGCAGGCCGAGTA CTTCAACAGCATCCCGTCATTTATCGCTGCGATGAGAGCTCAGAGGGAGGCCTACTGCCTGAACAACATCGGCGTCTACTACCGAACCGCTCTGGA
Pore-DAB*1083	NA	AGCTGAAGGACATCCAGTACATCGACTCCTATTGTTACAACAAGCTGGAGATGATCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA CCTGGAACAACAACCCGTCACTGATCGCATCAGAGATGGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACGGGAATCCACTGGC
Pore-DAB*829	NA	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCATATATTGGAGTGCTGAACGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTAACTGGTACCCAAATATGCTGAC
Pore-DAB*107	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCGTCAGAGATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTGTCGACTACCAAACCGCTCTGGA
Pore-DAB*296	4	AGCTCAAAGACATCCAGTACGTCTTCTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACGTGGGTAGATATGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACT ACTGGAACAACAACCCGTCAATTATTGGATCAAGGAAAGCTCAGAGGGAGGCCTACTGTCTGACCACCGTCGGTATCGACTACCAGGCGGCTCTGGA

MHC2-000023	4	AGCTGAAGGACATCCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTCGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAGCAACCCGTCATATATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1092	13	AGCTCAAAGACATCCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAATGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACCCGTCAGAGATCTCTGGGAGGAGAGCTCAGAGGGAGACCGTCTGCCTGCACAACATCGGTATCTGGTACGGGAATCCACTGGC
Pore-DAB*330	NA	AGCTCAAAGACATTCAGTACATCTACTCTACATTCTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACAACCCGTCAGAGATCGCTAGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*445	13	AGCTGAAGGACATCCAGTACGTCTACTCTGAGTTTTACAACAAGAAGGAGTTCATCAGGTTTGACAGCAACCTCAGGGAGTATGTTGGATTCACAGAGTACGGAGTGAAGAACGCTGAAC GGCTCAACAAAGATCAGTCATTTATATCTGTGCTGAGAGCTCGGAAGAAGAGCTACTGCCAACGCAACATTGATATCTGGTACGGGAATCCACTGGC
Pore-DAB*1067	4	AGCTGAAGGACATTCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAACAACCCTTCATTTATCTCTGGGCTGAAAGCTCAGAAGGAGGCCTACTGCCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*1070	1	AGCTGAAGGACATTCAGTATGTCGAGTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGTAAGTTTGTTGGATACACGGAGTACGGAGTGAAGAACGCTGAGC GCTGGAACAAAGACACGTCACTGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*269	3	AGCTGAAGGACATTCAGTACGTCCAGTCCTATTATTACAACAAGCTGGAATACATCAAGTTTGACAGCAGCCTGGATAAATATGTTGGATACACAGAGTATGGAGTGAAGAACGCTGAACG GCTCAACAACGACCCGTCAATTATTGGAGGGAGGAAAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
Pore-DAB*1075	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA GGTGGAACAAAGACACGTCACTGATCGCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACGCTCTGGA
Pore-DAB*411	7	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGACACGTCACTGATCGCTGCGCTGAACGCTCAGAGGGAGGCCTACTGCATGAACAACATCGGTGTCTACTACCGAACCGCTCTGGA
Pore-DAB*138	10	AGCTGAAGGACATTCAGTTCATCAGGTCCATCTATTACAACAAGCTGGAGATCATCAGGTTCACCAGCAGCCTGGGCAGATTTGTTGGATACACAGAGTATGGAGTGATGAACGCTGAGCG CTGGAACAAAGACACGTCAATTATTGGAGCAATGGCAGCTCAGAGGGAGGGTTACTGCATGAACAACATCGGTATCGACTACGAAACCGCTCTGGA
MHC2-000142	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCGTCAGAGATCGCTGCGAGGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTGTCGACTACCAAACCGTTCTGGA
Pore-DAB*106	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCAACTATTACAACAAGCTGGAGCTGTACAGGTTCAGCAGCAGTTTGGGCAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGT ACTTCAACAGCGACCCGTCAATTATTGGAGCAATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*422	5	AGCTGAAAGACATTGAGTACATCAGGTCCTATTGTTACAACAAGCTGGAGGTCACCAGGTTTGACAGCAACGTGGGCAGATTTATTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGT ACTTCAACAACAACCCGTCATATATCGCATCAGAGAGAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTAACGACTACCGAAACGTTCTGGA
Pore-DAB*1081	12	AGCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCAGAGC GGTTCAACAACGACCCGTCAATTATTGGAGCAAGGAGAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*1087	13	AGCTCAAAGACATTCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAATGTGGGGAAGTTTGTTGGATACACAGAGTATGGAGTGAAGCAGGCAAACTA CTGGAACAGCAACCCGTCAGAGATCGCTGGGAGGAGAGCTCAGAGGGAGACCGTCTGCCTGCACAACATCGGTGTCTGGTACGGGAATCCACTGGC
Pore-DAB*396	4	AGCTGAAGGACATCCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTCGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACACGTCATATATCGCTGGGCTGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1089	1	AGCTCAAAGACATTCAGTACATCGAGTCGTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAGC GCTGGAACAAAGACACGTCACTGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*131	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCGTCAGAGATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTGTCGACTACCAAACCGTTCTGGA
Pore-DAB*289	4	AGCTGAAGGACATCCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTCGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACACGTCATATATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*386	4	AGCTGAAGGACATCCAGTTCATCAGGTCCAACTATTACAACAAGCTGGAGGTTGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAACATCCCGTCATATATCGCATCGATGAAAGCTCAGAGGGAGGCCGTCTGTCTGCACAACGTTGATATCAACTACCAAACCGCTCTGGA
Pore-DAB*406	12	AGCTCAAAGACATTCAGTACATCTACTCCAACTATTACAACAAGCTGGAGTTCGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGACACGTCATATATCGCTGCGAGGAGAGCTCAGAGGGAGGCCTACTGCCTGAACAACATCGGTAACGACTACCAGGCGGCTCTGGA
Pore-DAB*292	3	AGCTCAAAGACATCCAGTATGTTGACTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCAGAGAT CTGGAACAACAACCCGTCATATATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACGTCGGTCTCTGGTACCCAAATATGCTGAC
Pore-DAB*1084	12	AGCTGAAGGACATCCAGTACATCGACTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGAAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA CCTGGAACAACAACCCGTCACTGATCGCATCAGAGAGAGCTCAGAGGGAGGCCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCTCTGGA

Pore-DAB*1015	13	AGCTCAAAGACATCCAGTACGTCTACTCTGCATTCTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACAACCCGTCAGAGGTCTCTGTGAGGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
Pore-DAB*1085	11	AGCTGAAGGACATCCAGTACATCAGGTCCTATTGTTACAACAAGCTGGAGATGATCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGTTCAACAAAGACACGTCAGATATCTCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*352	NA	AGCTGAAAGACATTCAGTACATCGACTCCAGCTATTATAACAAGCTGGAGCTCTTCAGGTTTGACAGCAACCTGGGCAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGTA CAGGAACAACAACCCTTCAATTATTGGATCAAGGAAGGCTCAGAGGGAGGCCTACTGCCTGACCAACATCGGTATCGACTACCAGGTGGCTCTGGA
Pore-DAB*216	2	AGCTCAAAGGCATTCAGTACGTCTACTCCATGTATTACAACAAGCTGGAATACATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGACGTTTGGAGTGAAGAACGCTGAACG GTTCAACAGCAACCCATCAGAGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCTGGTACGGGAATCCACTGGC
Pore-DAB*103	9	AGCTGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGAATGCTGAACG GTGGAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACGTTAGTATAGACTACCAGAATGTTCTGAC
Pore-DAB*221	13	AGCTGAAAGACATTCAGTACGTCTTCTCCAACTATTACAACAAGCTGGAGATCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACATCCCGTCAGAGATCGCTAGGAGGAGAGCTCAGAGGGAGACCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
MHC2-000139	13	AGCTCAAAGACATCCAGTACGTCTACTCTGCATTCTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACAANCCGTCAGAGATCTCTGTGAGGATGGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAAACGCTCTGGA
Pore-DAB*286	12	AGCTGAAGGACATCCAGTACATCGACTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGAAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA TCTGGAACAACAACCCGTCACAGATCGCATCAGAGAGAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1072	7	AGCTGAAGGACATCGAGTTCATCGACTCATATTATTACAACAAGCTGGAGCTGTACAGGTTCAGCAGCAGTTTGGGGAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGT ACTTCAACAGCATCCCGTCATTTATCGCTGCGATGAGAGCTCAGAGGGAGGCCTACTGTCTGAACAACATCGGCGTCTACTACCGAACCGCTCTGGA
Pore-DAB*1002	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCAACACAACATCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*1077	7	AGCTGAAGGACATCCAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGAAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGACACGTCATTTATCGCATCAGAGGCGGCTCAGAGGGAGGTCTACTGCATGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1021	2	AGCTCAAAGACATTCAGTACGTCTTCTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAACG GCTCAACAACATCCCGTCAGAGATCGCTGCGATGAGAGCTCAGAGGGAGACCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
Pore-DAB*356	13	AGCTGAACGACATCCAGTTCGTCTACTCTGAGTTTTACAACAAGAAGGAGTTCATCAGGTTTGACAGCAACCTCAGGGAGTATGTTGGATTCACAGAGTACGGAGTGAAGAACGCTGAGCG GCTCAACAAAGATCAGTCATTTATATCTGTGCTGAGAGCTCGGAAGAAGAGCTACTGCCAACGCAACATTGATATCTGGTACGGGAATCCACTGGC
Pore-DAB*1019	2	AGCTCAAAGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCACCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGTA CTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
Pore-DAB*1076	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA GGTGGAACAAAGACACGTCACTGATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACATTCTGGA
Pore-DAB*433	10	AGCTGAAGGACATTCAGTTCATCAGGTCCATCTATTACAACAAGCTGGAGATCATCAGGTTCACCAGCAGCCTGGGCAGATTTGTTGGATACACAGAGTATGGAGTGATGAACGCTGAGCG CTGGAACAAAGACACGTCAATTATTGGAGCAATGGCAGCTCAGAGGGAGACCTACTGCATGAACAACATCGGTATCGACTACGAAACCGCTCTGGA
MHC2-000042	NA	AGCTGAAGGACATCCAGTACATCGACTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGAAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA TCTGGAACAACAACCCGTCACAGATCGCATCAGAGAGAGCTCAGAGGGAGGCCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*310	13	AGCTCAAAGACATCCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGTTCGCCAGGTTTGACAGCAACCTGGGGAGGTTTGTTGGATACACGGAGTACGGAGTGAAGCAGGCAAACTA CTGGAACAACATCCCGTCATATATCTCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCAACACAACATCGGTATCTGGTACGGGAATCCACTGGC
MHC2-000066	1	AGCTCAAAGACATTCAGTACATCGAGTCGTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAGGTTTGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAACG CTGGAACAAAGACACGTCACTGATCGCTGGGAGGAAAGCTCAGAGGGAGGGTTACTGTCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*1088	13	AGCTCAAAGACATTCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAATGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACTA CTGGAACAACAACCCGTCAGAGATCTCTGGGAGGAAAGCTCAGAGGGAGACCGTCTGTCTGCACAACATCGGTGTCTGGTACGGGAATCCACTGGC
MHC2-000022	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCATTTATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCNNTCTGACCACCGTCNGTATCGACTACCAAACCGCNCNGNA
MHC2-000015	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCATTTATCGCTGCGCTGAACGCTCNGAGGGAGGCCGTCTGTCTGACCACCGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1086	NA	AGCTGAAAGACATTGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGCTGTACAGGTTCAGCAGCAGTTTGGGCAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGT ACTTCAACAGCGACCCGTCAATTATTGGAGCAATGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA

MHC2-000016	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCNTTTATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCTGTCTGACCACCGTCGGTATCGACTACCAAACCGCTCTGGA
MHC2-000017	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGANACGTCATTTATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCTGTCTGACCACCGTCGGTATCGACTACCAAACCGCTCTGGA
MHC2-000072	5	AGCTGAAGGACATTCAGTTCATCAGGTCCTATTGTTACAACAAGCTGGAGCTCACCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA CCTGGAACAAAGACACGTCAGATATCGCTGCGATGAACGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAATTGGTACAGGAGTCCACTGGC
MHC2-000034	1	AGCTGAAGGACATCCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAAC GGCTCAACAAAGACACGTCATTTATCGCTGGGCTGAGAGCTCNGAGGGAGACCTACTGCCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000043	NA	AGCTCAAAGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCNCAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000035	NA	AGTGCTTTATGGATTTGACAGAATAAAATCACAGAAATATGAAACTAACTACTTTTTTTGCATTGTTGTTTACATTATTGTTTTAGGCTTAACCAGAATGTTTTTTCCTATCGTAGAAACTGCT GTGTAGTTCTGTATTGAAATGTGTGAAGCATACGGA
Pore-DAB*335	9	AGCTGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACG GTGGAACAGAGATCCTTCATCTATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACGTTAGTATAGACTACCAGAATGTTCTGAC
Pore-DAB*381	NA	AGCTGAAGGACATCCAGTACGTCTTCTCCATGTATTACAACAAGCTGGAATTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAACG GTTCAACAACGACCCGTCAATTATTGGAGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTAACTGGTACGGGAATCCACTGGC
MHC2-000190	NA	AGCTGAAGGACATCCAGTACATCAGGTCCTATTATTACAATAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGAT CTGGAACAACATCCCGTCACAGATCGCATCAGAGATGGCTCAGAGGGAGGTCTACTGTCTGACCAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-001557	7	AGCTCAAAGACATCCAGTACATCGAGTCCTATTGTTACAATAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGAAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGATACGTCATTTATCGCTGGGAGGAGAGCTCAGAGGGACACCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGA
Pore-DAB*256	9	AGCTGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACG GTGGAACAGAGATCCTTCATATATAGCATTGATGAGAGCTCAGAAGGAGACCTACTGCCAGCACAACATTGGTGTCGAGTACCAGAATGTTCTGAC
MHC2-000311	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCAACTATTACAACAAGCTGGAGCTGTACAGGTTCAGCAGCAGTTTGGGCAGGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGT ACTTCAACAGCGACCCGTCAATTATTGGAGCAATGAAAGCTCNGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000029	1	AGCTGAAGGACATCCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAAC GGCTCAACAAAGACACGTCNTTTATCGCTGGGCTGAGAGCTCAGAGGGAGACCTACTGCCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*1023	6	AGCTGAAAGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*329	1	AGCTCAAAGACATTCAGTACATCGAGTCGTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAGGTTTGTTGGATACACGGAGTTTGGAGTGAAGAACGCAGAGC GCTGGAACAAAGACACGTCACTGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000039	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGA ACGCTGAGA GGTGGAACAAAGACNCGTCACTGATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACGCTCTGGA
MHC2-000059	3	AGCTGAAGGACATCCAGTATGTCCTCTCCTATTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACTTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCAGAACG CTGGAACAACGATCCGTCNATTATTGGAGGGAGGAGAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
MHC2-000036	1	AGCTGAAGGACATCCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAAC GGCTCAACAAAGACNCGTCATTTATCGCTGGGCTGAGAGCTCAGAGGGAGACCTACTGCCTGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000445	2	AGCTCAAAGGCATTCAGTACGTCTACTCCATGTATTACAACAAGCTGGAATACATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGACGTTTGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCATCAGAGATCGCTGGGAGGAAAGCTCNGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCTGGTACGGGAATCCACTGGC
MHC2-000004	NA	AGCTCAAAGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000014	13	AGCTCAAAGACATCCAGTACGTCTACTCTGCATTCTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACAACCCGTCAGAGATCTCTGTGAGGATGGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAAACGCTCTGGA
Pore-DAB*1066	6	AGCTGAAAGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTGTCTACTACCAAAACGCTCTGGA
Pore-DAB*1065	12	AGCTGAAAGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAATACATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAACG GTTCAACAGCGACCCGTCAATTATTGGAGTGAGGAGAGCTCAGAGGGAGGCCTACTGCCTGACCAACATCGGTATCGACTACCGAACCGCTCTGGA

Pore-DAB*1064	6	AGCTCAAAGACATCCAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCAACACAACATCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*275	8	AGCTGAAGGACATCGAGTTCATCAGGTCCAACTTCTACAACAAGCTGGAGCTGTTCAGGTTCAGCAGCAGTTTGGGCAGGTTTGTTGGATACACAGAGTATGGAGTGAAGCAGGCCGAGTA CAGGAACAACATCCCGTCATTTATTGCTGCGATGAAGGCTCAGAGGGAGGCCTACTGTCTGAACAACATCGGTGTCTACTACCGAACCGCTCTGGA
Pore-DAB*273	11	AGCTCAAAGACATCCAGTTCATCAGGTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAACG CTGGAACAACAACCCGTCACAGATCGCATCAGAGAGAGCTCAGAAGGAGGCCTACTGCCTGAACAACGTCGGCATCGACTACCAAACCGCTCTGGA
Pore-DAB*1005	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAGCG GCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCAACACAACATCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*1051	7	AGCTGAAGGACATTCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGATACGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAACCGCTCTGGA
Pore-DAB*1004	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTGTCTACTACCAAACCGCTCTGGA
MHC2-000052	NA	AGCTGAAGGACATCCAGTACATCGACTCCTATTGTTACAACAAGCTGGAGATGATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGCTGGGAGTGAAGAACGCTGAGA CCTGGAACAACAACCCGTCACTGATCGCATCAGAGATGGCTCAGAGGGAGACCTACTGTCAACACAACATCGGTAACTGGTACGGGAATCCACTGGC
Pore-DAB*1063	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTGTCTACTACCAAAACGCTCTGGA
Pore-DAB*1062	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTGTCTACTACCAAACCGCTCTGGA
Popi-DAB*017	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGT ACTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTATCGACTACCGAACCGCTCTGGA
Pore-DAB*615	1	AGCTGAAGGACATTCAGTACATCGAGTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTACGGAGTGAAGAACGCTGAAC GCTGGAACAAAGACACGTCAATTATCGCTATGAGGAGAGCTCAGAGGGAGGCCTACTGTCTGTACAACATCGGTATCGACTACCCAAACGCTCTGGA
Pore-DAB*1024	6	AGCTGAAAGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*1031	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGGTCACCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGT ACTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTATCGACTACCGAACCGCTCTGGA
MHC2-000095	6	AGCTGAAAGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTGTCTACTACCAAAACGCTCTGGA
MHC2-000087	5	AGCTCAAAGACATTCAGTACATCAGGTCCTATTGTTACAACAAACTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACAGAGCTGGGAGTGAAGAACGCAGAGA TCTGGAACAAAGACCCGTCACAGATCGCTGCGATGACGGCTCAGAGGGAGGGTTACTGTCTGAACAACGTCGGTAACGACTACCGAAACATTCTGGA
Pore-DAB*1009	6	AGCTCAAAGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCAACACAACATCGGTATCGACTACCAAAACGCTCTGGA
MHC2-000089	2	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCACCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
Pore-DAB*1003	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCAACACAACATCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*1027	7	AGCTGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGATACGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAACCGCTCTGGA
MHC2-000086	2	AGCTCAAAGGCATTCAGTACGTCTACTCCATGTATTACAACAAGCTGGAATACATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGACGTTTGGAGTGAAGAACGCTGAACG GTTCAACAGCAACCCATCAGAGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCTGGTACGGGAATCCACTGGC
Pore-DAB*1018	NA	AGCTCAAAGACATCCAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000111	6	AGCTGAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*1040	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGT ACTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTATCGACTACCGAACCGCTCTGGA

Pore-DAB*1046	2	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCACCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGTA CTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
MHC2-000114	11	AGCTGAAGGACATCCAGTTCATCAGGTCCTATTGTTACAACAAGCTGGAGCTCACCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA CCTGGAACAAAGACACGTCAGATATCGCTGCGATGAGAGCTCAGAGGGAGGCCTACTGTCTGTACAACGTCGGTATCGACTACCAAACCGCTCTGGA
MHC2-000130	5	AGCTGAAGGACATCCAGTACATCAGCTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAAC GCTGGAACAACATCCCGTCACAGATCGCATCAGAGATGGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTAACGACTACCAGGTGGCTCTGGA
MHC2-000129	13	AGCTCAAAGACATTCAGTACATCTTCTCCATGTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAATATGTTGGATACACAGAGTATGGAGTGAAGCAGGCAAACTA CTGGAACAGCAACCCGTCAGAGATCTCTGGGAGGAGAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTAAC
Pore-DAB*1033	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGGTCACCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGT ACTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTATCGACTACCGAACCGCTCTGGA
MHC2-000148	12	AGCTCAAAGACATTCAGTACGTCTTCTCCTATTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGCAGATTTATTGGATACACGACGTTTGGAGTGAAGAACGCTGAGCG CTGGAACAAAGACCCATCAGAGATCGCTAGGAGGAAAGCTCAGAAGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000161	7	AGCTGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGATACGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTGTCTACTACCAAACCGCTCTGGA
Pore-DAB*1001	NA	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000144	6	AGCTGAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAAACGCTCTGGA
Pore-DAB*1000	NA	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGGTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Pore-DAB*1016	13	AGCTCAAAGACATCCAGTACGTCTACTCTGCATTCTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACAACCCGTCAGAGGTCTCTGTGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCACTGGC
MHC2-000186	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1028	7	AGCTGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAACCGCTCTGGA
Pore-DAB*1098	7	AGCTGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAACCGCTCTGGA
Pore-DAB*1039	NA	AGCTGAAGGACATCGAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCCGAGT ACTTCAACAACAACCCGTCAGAGATCTCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACGTCGGTATCGACTACCAAAACGCTCTGGA
MHC2-000158	NA	AGTGCTTTATGGATTTGACAGAATAAAATCACAGAAATATGAAACTAACTACTTTTTTTGCATTGTTGTTTACATTATTGTTTTAGGCTTAACCAGAATGTTTTTTCCTATCGTAGAAACTGCT GTGTAGTTCTGTATTGAAATGTGTGAAGCACACGGA
Popi-DAB*009	NA	AGCTGAGAGACATCCAGTTCATCAGCTCCTCCATTTACAACAAGATGGAGTATCTCAGGTTCGACAGCAACCTGGGGAAATATGTTGGATACACAGAGTATGGAGTGAAGAATGCTGAACG ATGGAACCAAGATCCTTCAATAATAGCATCGATGAGAGCTCAGAAGGAAAACTACTGCCAGCGCAACATTGGTGTCGAGTACGAGAAAGTTCTGAC
Pore-DAB*1060	6	AGCTCAAAGACATCCAGTACATCAGGTCCATCTATTACAACAAGCTGGAGGTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCAACACAACATCGGTATCGACTACCAAAACGCTCTGGA
MHC2-000265	7	AGCTGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGATACGTCACAGATCGCTGGGTTGAACGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAACCGCTCTGGA
MHC2-000055	NA	AGCTCAAAGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGANCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000057	NA	AGCTCAAAGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACNCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000040	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA GGTGGAACAAAGANACGTCACTGATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACGCTCTGGA
MHC2-000044	12	ACCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACG GTTCAACAACGACCCGTCNATTATTGGAGCGAGGAGAGCTCTGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA

MHC2-000189	NA	AGTGCTTTATGGATTTGACAGAATAAAATCACAGAAATATGAAACTAACTACTTTTTTTTGCATTGTTGTTTACATTATTGTTTTAGGCTTAACCAGAATGTTTTTTCCTATCGTAGAAACTGC TGTGTAGTTCTGTATTGAAATGTGTGAAGCATACGGA
Pore-DAB*274	7	AGCTCAAGGACATTCAGTACATCAACTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGCAGCAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGACACGTCACTGATCGCTATGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000099	3	AGCTGAAGGACATTCAGTACATCGAGTCCTATTATTACAACAAGCTGGAGCTCGCCAGGTTTGACAGCAACGTGGGTAGATATGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACT ACTGGAACAGCGANACGTCACTGATCTCTATGAGGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
MHC2-000101	3	AGCTGAAGGACATTCAGTACATCGAGTCCTATTATTACAACAAGCTGGAGCTCGCCAGGTTTGACAGCAACGTGGGTAGATATGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACNCGTCACTGATCTCTATGAGGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
MHC2-000024	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA GGTGGAACAAAGACACGTCNCTGATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACGCTCTGGA
MHC2-000061	12	ACCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACG GTTCAACAACGANCCGTCAATTATTGGAGCGAGGAGAGCTCTGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000062	12	ACCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACG GTTCAACAACGACNCGTCAATTATTGGAGCGAGGAGAGCTCTGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000078	12	ACCTGAAGGACATCCAGTACATCTACTCCATGTATTACGACAAGCTGGAGTACATCAGGTTTGACAGCAACGTGGNTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACG GTTCAACAACGACCCGTCAATTATTGGAGCGAGGAGAGCTCTGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000091	NA	AGCTCAAAGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGNGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGC GGCTCAACAACGACCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
Popi-DAB*035	NA	AGCTGAACGACATTCAGTTCATCTGGTCTGTGTATTACAACAAGCTGGAGATCTACAAGTTCGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAATATGGAGTGAAACAGGCTGAATA CTTCAACAGCGACTCGTCATATATCGGAGCAATGAGAGCTCAGAGGGGGACCTACTGCCTGAACAACGTTGGTAACGACTACCGAACCGCTCTGAC
Popi-DAB*033	NA	AGCTCAACGACATCCAGTACATCTACTCTGCATATTACAACAAGCTGGAGATCTACAAGTTCGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAATATGGAGTGAAACAGGCTGAATA CTTCAACAGCGACTCGTCATATATCGGATCACTGAGAGCTCAGAAGGAGACCTACTGCCAACACAACATTGGTATCGACTACCGAACCAAACTGAC
MHC2-000094	10	AGCTGAAGGACATTCAGTTCATCAGGTCCATCTATTACAACAAGCTGGAGATCATCAGGTTCACCAGCAGCCTGGGCAGATTTGTTGGATACACAGAGTATGGAGTGATGAACGCTGAGCG CTGGAACAAAGACACGTCNATTATTGGAGCAATGGCAGCTCAGAGGGAGGGTTACTGCATGAACAACATCGGTATCGACTACGAAACCGCTCTGGA
MHC2-000127	4	AGCTGAAGGACATCCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTCGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACACGTCNTATATCGCTGGGCTGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Unknown_spp	9	AGCTGAGAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGATCAGGTTTGACAGCAACGTGGGGAAATATGTTGGATACACAGAGTTTGGAATGAAGAATGCTGAAC GATGGAACCAAGATGCTTCAATAATAGCATCGATGAGAGCTCAGAAGGAAAACTACTGCCTGAACCGCGTTGGTTTAGACTACCAGAATGTTCTGAC
MHC2-000224	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACGGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCATTTATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCTGTCTGACCACCGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*1096	NA	AGCTGAAGGACATTCAGTTCATCAGGTCTACATTCTACAACAAGCTGGAGCTTTTCAGGTTCAGCAGCAGTTTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAACAGGCTGAACA CTTCAACAGCAACCCGTCATATGTAGCATCAATGAGAGCTCAGAGGGAGACCTACTGCCTGCACAACATTGATATCGACTACCGAACCGCTCTGAC
MHC2-001428	12	AGCTGAAGGACATTCAGTACATCTACTCTGCCTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGCAGAAGGAGTGAGACAGGCTGAGA TCTGGAACAAAGACTCGTCACAGATCGCTGCGAGGATGGCTCAGAGGGAGACCTACTGCCTGAACAACATTGGTATCGACTACCAGGTGGCTCTGGA
MHC2-000150	10	AGCTGAAGGACATTCAGTTCATCAGGTCCATCTATTACAACAAGCTGGAGATCATCAGGTTCACCAGCAGCCTGGGCAGATTTGTTGGATACACAGAGTATGGAGTGATGAACGCTGAGCG CTGGAACAAAGACNCGTCAATTATTGGAGCAATGGCAGCTCAGAGGGAGGGTTACTGCATGAACAACATCGGTATCGACTACGAAACCGCTCTGGA
MHC2-001572	13	AGCTGAAGGACATTCAGTACATTTACTCCGACTTCTACAACAAGCTGGAATTTGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGCAGCAGGAGTGAGACAGGCTGAGAT CTGGAACAAAGACACTTCAATCATCGCTGCGAGGATGGCTCAGAAGGAGACCTACTGCCTGCATAACATTGGTATCTGGTACGGGAATCCACTGAC
MHC2-000169	10	AGCTGAAGGACATTCAGTTCATCAGGTCCATCTATTACAACAAGCTGGAGATCATCAGGTTCACCAGCAGCCTGGNCAGATTTGTTGGATACACAGAGTATGGAGTGATGAACGCTGAGCG CTGGAACAAAGACACGTCAATTATTGGAGCAATGGCAGCTCAGAGGGAGGGTTACTGCATGAACAACATCGGTATCGACTACGAAACCGCTCTGGA
MHC2-000147	10	AGCTGAAGGACATTCAGTTCATCAGGTCCATCTATTACAACAAGCTGGAGATCATCAGGTTCACCAGCAGCCTGGGCAGATTTGTTGGATACACAGAGTATGGAGTGATGAACGCTGAGCG CTGGAACAAAGANACGTCAATTATTGGAGCAATGGCAGCTCAGAGGGAGGGTTACTGCATGAACAACATCGGTATCGACTACGAAACCGCTCTGGA
MHC2-000188	4	AGCTGAAGGACATCCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTCGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAGCGACNCGTCATATATCGCTGGGCTGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
MHC2-000185	4	AGCTGAAGGACATCCAGTTCATCAGATCCAACTATTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAACCTCGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAGCGANACGTCATATATCGCTGGGCTGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA

MHC2-000126	13	AGCTCAAAGACATTCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAATGTGGGGAAGTTTGTTGGATACACAGAGTATGGAGTGAAGCAGGCAAACTA CTGGAACAGCAACCCGTCNGAGATCGCTGGGAGGAGAGCTCAGAGGGAGACCGTCTGCCTGCACAACATCGGTGTCTGGTACGGGAATCCACTGGC
MHC2-000047	5	AGCTGAAGGACATCCAGTTCATCAGGTCGTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGNTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGA GGTGGAACAAAGACACGTCACTGATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAACGACTACCAAAACGCTCTGGA
MHC2-000593	4	AGCTGAAGGACATCGAGTACATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGATTTGACAGCAGCCTGGGGAGGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACT ACTGGAACAAAGACACGTCATTTATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCTGTCTGACCACCGTCGGTATCGACTACCAAACCGCTCTGGA
MHC2-001938	NA	AGCTGAAGGACATTCAGTTCATCAGGTCCTTATATTACGATAAGAGGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCAGAAC GATTCAACAAAGACACGTCAATCATCGCTGGGCTGATGGCTGAGAAGGAGAGATACTGCCAACACAACATTGGTATCGACTACCAGGGCTTTCTGAC
MHC2-000639	3	AGCTGAAGGACATTCAGTACATCAACTCCTACTATTACAACAAGCTGGAATGGGCCAGATTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGAAGTTCGGAGTTTATAATGCAGAACG ATGGAACAAAGACCCGTCACAGATCGCTGTGAGGATGGCTCAGAGGGAGACCTACTGCCAACACAACATTGGTCTCTGGTACCCAAACATGCTGAC
Pore-DAB*375	9	AGCTGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACG ATGGAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACGTTAGTATAGACTACCAGAATGTTCTGAC
MHC2-000081	3	AGCTGAAGGACATCCAGTATGTCCTCTCCTATTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACTTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCAGAACG CTGGAACAACGANCCGTCAATTATTGGAGGGAGGAGAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
MHC2-000082	3	AGCTGAAGGACATCCAGTATGTCCTCTCCTATTATTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACTTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCAGAACG CTGGAACAACGATNCGTCAATTATTGGAGGGAGGAGAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATGCTGAC
Pore-DAB*104	9	AGCTGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACG ATGGAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTTCTGAC
MHC2-000145	13	AGCTCAAAGACATCCAGTACGTCTACTCTGCATTCTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGTTTGGAGTGAAGCAGGCAAACTA CTGGAACAACAACNCGTCAGAGATCTCTGTGAGGATGGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTGTCTACTACCAAAACGCTCTGGA
MHC2-000619	NA	AGCTCAAAGACATCCAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GCTCAACAACGANCCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-001726	NA	AGCTCAAAGACATCCAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GCTCAACAACGACCCGTCNCAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000629	NA	AGCTCAAAGACATCCAGTTCATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGGAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GCTCAACAACGACNCGTCACAGATCGCTGGGTTGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCTCTGGA
MHC2-000176	13	AGCTCAAAGACATTCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAATGTGGGGAAGTTTGTTGGATACACAGAGTATGGAGTGAAGCAGGCAAACTA CTGGAACAGCAACNCGTCAGAGATCGCTGGGAGGAGAGCTCAGAGGGAGACCGTCTGCCTGCACAACATCGGTGTCTGGTACGGGAATCCACTGGC
MHC2-000073	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCGTCNGAGATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTGTCGACTACCAAACCGCTCTGGA
MHC2-000178	13	AGCTCAAAGACATTCAGTACATCTACTCTGAGTTTTACAACAAGCTGGAGATCGCCAGGTTTGACAGCAATGTGGGGAAGTTTGTTGGATACACAGAGTATGGAGTGAAGCAGGCAAACTA CTGGAACAGCAANCCGTCAGAGATCGCTGGGAGGAGAGCTCAGAGGGAGACCGTCTGCCTGCACAACATCGGTGTCTGGTACGGGAATCCACTGGC
MHC2-000106	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAANCCGTCAGAGATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTGTCGACTACCAAACCGCTCTGGA
MHC2-000100	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACNCGTCAGAGATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTGTCGACTACCAAACCGCTCTGGA
MHC2-000341	12	AGCTCAAAGACATTCAGTTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCG GGCCAACAGCAACCCGTCAGAGATCGCTGGGCTGAAAGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCTCTGGA
Pore-DAB*444	1	TGAAGGACATCCAGTACATCTACTCTGCATTCTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTACTG GAACAGCAACCCGTCAGAGATCGCTAGGAGGAAAGCTCAGAGGGAGGGTTACTGTCTGAACCATGTTACTGCTTTCTACCCAAACATT
Pore-DAB*387	12	TGAAGGACATCCAGTACATCTTCTCCATGTATTACGACAAGCTGGAGGTCGCCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*373	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTG GAACAGGGATCCTTCATCTATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*371	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGAATGCTGAACGGTG GAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACGTTAGTATAGACTACCAGAATGTT

Pore-DAB*282	3	TCAAAGACATCCAGTACATCTTCTCCATGTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTACTG GAACAGCAACCCGTCAGAGATCTCTGGGAGGAAAGCTCAGAGGGAGACCTACTGCCTGCACAACGTCGGTAACTGGTACCCAAATATG
Pore-DAB*277	3	TCAAAGACATTCAGTTGATCAACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCGGTT CAACAAAGACACGTCACGGATCGCTGCGATGAACGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAATTGGTACAGGAATCCA
Pore-DAB*276	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGATGTTTGTTGGATACACGGAGTTTGGAGTGAAGAATGCTGAACGGTG GAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACGTTAGTATAGACTACCAGAATGTT
Pore-DAB*272	1	TCAAAGACATCCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAGAGGTG GAACAAAGACACGTCACTGATCGCTGCGCTGAACGCTCAGAGGGAGACCTACTGCATGAACAACGTCGGTATCAACTACCCAAACATT
Pore-DAB*271	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGATGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTG GAACAGAGATCCTTCATCTATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*270	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGATGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTG GAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*268	3	TGAAGGACATTCAGTACGTCCAGTCCTACTATTACAATAAGCTGGAATACATCAAGTTTGACAGCAGCCTGGATAAATATGTTGGATACACAGAGTATGGAGTGAAGAACGCTGAACGGCT CAACAACGACCCGTCAATTATTGGAGGGAGGAAAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATG
Pore-DAB*267	13	TGAAGGACATCCAGTTCGTCTACTCTGAGTTTTACAACAAGAAGGAGTTCATCAGGTTTGACAGCAACCTCAGGGAGTATGTTGGATTCACAGAGTACGGAGTGAAGAACGCTGAACGGCT CAACAAAGATCAGTCATTTATATCTGTGCTGAGAGCTCGGAAGAAGAGCTACTGCCAACGCAACATTGATATCTGGTACGGGAATCCA
Pore-DAB*265	1	TGAAGGACATCCAGTATGTCGAGTCCTATTGTTACGACAAGCTGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGAACGCAGAGCGCT GGAACAAAGACACGTCACTGATCGCTGTGAGGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCT
Pore-DAB*264	3	TGAAGGACATCCAGTACGTCCTGTCCTATTATTACAACAAGCTGGAATACGCCCGGTTTGACAGCAACTTGGGTAAATATGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACGGCT CAACAACGACCCGTCAATTATTGGAGGGATGAAAGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATG
Pore-DAB*263	3	TGAAGGACATCCAGTACGTCCAGTCCTATTATTACAACAAGCTGGAATACATCAAGTTTGACAGCAGCCTGGATAAATATGTTGGATACACAGAGTATGGAGTGAAGAACGCTGAACGGCT CAACAACGACCCGTCAATTATTGGAGGGAGGAAAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATG
Pore-DAB*262	3	TGAAGGACATCCAGTACGTCCAGTCCTACTATTACAATAAGCTGGAATACATCAAGTTTGACAGCAGCCTGGATAAATATGTTGGATACACAGAGTATGGAGTGAAGAACGCTGAACGGCT CAACAACGACCCGTCAATTATTGGAGGGAGGAAAGCTCAGAAGGAGACCTACTGTCTGCACAACATCGGTAACTGGTACCCAAATATG
Pore-DAB*261	NA	TGAAGGACATCCAGTACATCGACTCCTATTGTTACAACAAGCTGGAGATGATCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGACCT GGAACAACAACCCGTCACTGATCGCATCAGAGACGGCTCAGAGGGAGGCCTACTGCCTGCACAACGTTGGTATCTGGTACGGGAATCCA
Pore-DAB*260	7	TGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGACGTTTGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGATCT GGAACAAAGACACGTCACTGATCGCATCAGAGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACGTTGGTATCTGGTACGGGAATCCA
Pore-DAB*259	11	TGAAGGACATCCAGTACATCAGGTCCATCTGTTACGACAAGAGGGAATACGCCAGGTTTGACAGCAGCCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGAACGCTGAGCGCT GGAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*258	4	TGAAAGACATCCAGTTCATCAGGTCCAACTATTACAACAAGCTGGAGATCGCCAGGTTCACCAGCAGCCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTACTG GAACAGCAACCCTTCATTTATCTCTGGGCTGAAAGCTCAGAGGGAGGCCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*257	7	TCAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGATCT GGAACAAAGACACGTCACTGATCGCATCAGAGGCGGCTCAGAAGGAGGCCTACTGCCTGAACAACGTCGGTATCGACTACCAGGTGGCT
Pore-DAB*255	12	TCAAAGACATCCAGTACATCTTCTCCATGTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGAGGAAAGCTCAGAGGGAGACCTACTGCCTGCACAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*254	12	TCAAAGACATCCAGTACATCTTCTCCATGTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAGCAACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCGTCTGCCTGCACAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*253	12	TCAAAGACATCCAGTACATCTTCTCCATGTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAGCAACCCGTCAGAGATCTCTGGGAGGAAAGCTCAGAGGGAGACCGTCTGCCTGCACAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*252	12	TCAAAGACATCCAGTACATCTTCTCCATGTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGAGGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*251	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAACGGTT CAACAAAGACACGTCACTGATCGCTGGGCTGAACGCTCAGAAGGAGGCCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGCT
Pore-DAB*250	NA	TGAAGGACATTCAGTACATCTACTCTACATTCTACAACAAGCTGGAGCTCGCCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGTACGGAGTGAAGCAGGCAAACTACTG GAACAGCAACCCGTCAGAGATCGCTAGGAGGAGAGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTATCGACTACCAGGTGGCT

Pore-DAB*249	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGACTGCTGAACGGTG GAACAGAGATCCTTCATATATAGCATTGATGAGAGCTCAGAAGGAGACCTACTGCCAGCACAACATCGGTGTCGAGTACCAGAATGTT
Pore-DAB*248	13	TGAAGGACATTCAGTACATCTACTCTACATTCTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGTACGGAGTGAAGCAGGCAAACTACTG GAACAGCAACCCGTCAGAGATCGCTAGGAGGAGAGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTATCTGGTACGGGAATCCA
Pore-DAB*247	10	TGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCACTGATCGCATCAGAGACGGCTCAGAGGGAGGTCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*246	10	TGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGATCTG GAACAACAACCCGTCACTGATCGCATCAGAGACGGCTCAGAGGGAGGTCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*245	5	TGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGATCTG GAACAACAACCCGTCACTGATCGCATCAGAGACGGCTCAGAGGGAGGTCTACTGTCTGACCAACGTCGGTAACGACTACCAGGTGGCT
Pore-DAB*244	NA	TGAAGGACATCCAGTACATCAGGTCCTATTATTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGATCTG GAACAACAACCCGTCACTGATCGCATCAGAGACGGCTCAGAGGGAGGTCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGCT
Pore-DAB*243	11	TGAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*242	11	TGAAAGACATTCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*241	11	TGAAAGACATCCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*240	11	TGAAAGACATCCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*239	11	TGAAAGACATCCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGTT
Pore-DAB*238	11	TGAAAGACATCCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGTT
Pore-DAB*237	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*236	12	TCAAAGGCATTCAGTACGTCTACTCCATGTATTACAACAAGCTGGAATACATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACGACGTTTGGAGTGAAGAACGCTGAACGGTT CAACAGCAACCCATCAGAGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAGGTGGCT
Pore-DAB*235	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGGTCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGTT
Pore-DAB*234	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*233	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGCT
Pore-DAB*232	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAAGGAGGCCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGCT
Pore-DAB*231	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAACGGTT CAACAAAGACACGTCACTGATCGCTGGGCTGAACGCTCAGAAGGAGGCCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGCT
Pore-DAB*230	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*229	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGAACGCTGAACGGTT CAACAAAGACACGTCACTGATCGCTGGGCTGAACGCTCAGAAGGAGGCCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGTT
Pore-DAB*228	11	TCAAAGACATCCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTG GAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*227	7	TGAAGGACATTCAGTACATCGAGTCCTATTATTACAACAAGCTGGAGCTCAACAGGTTTGACAGCAACGTGGGAAAATATGTTGGATACACGGCAGCAGGAGTGAGACAGGCTGAGATCT GGAACAAAGACACGTCAATTATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCT

Pore-DAB*226	3	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCAGAGATCTG GAACAACAACCCGTCATATATCGCTGCGATGAAAGCTCAGAGGGAGACCTACTGTCTGCACAACGTCGGTCTCTGGTACCCAAATATG
Pore-DAB*225	NA	TGAAGGACATCCAGTTCAGCAGGTCCTTTTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCGGCT CAACAAAGACCCGTCACAGATCGCTGCGATGACGGCTCAGAGGGAGGGTTACTGTCTGCACAACGTCGGTAACTGGTACGGGAATCCA
Pore-DAB*223	11	TGAAGGACATCCAGTACATCAGGTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGCAGATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGCGGTT CAACAAAGACACGTCAGATATCTCTGCGATGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCGACTACCAAACCGCT
Pore-DAB*222	13	TGAAAGACATTCAGTACGTCTTCTCCAACTATTACAACAAGCTGGAGATCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTACTG GAACAACATCCCGTCAGAGATCGCTAGGAGGAGAGCTCAGAGGGAGGGTTACTGCCAACACAACATTGATATCTGGTACGGGAATCCA
Pore-DAB*220	13	TGAAAGACATTCAGTACGTCTTCTCCAACTATTACAACAAGCTGGAGATCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACAGAGTACGGAGTGAAGCAGGCAAACTACTG GAACAACATCCCGTCAGAGATCGCTAGGAGGAGAGCTCAGAGGGAGACCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCA
Pore-DAB*219	13	TGAAAGACATTCAGTACGTCTACTCCAACTATTACAACAAGCTGGAGATCATCAGGTTTGACAGCAACCTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAAACTACTG GAACAACATCCCGTCAGAGATCGCTAGGAGGAGAGCTCAGAGGGAGACCTACTGTCTGCACAACGTTGATATCTGGTACGGGAATCCA
Pore-DAB*218	4	TGAAAGACATCCAGTTCATCAGGTCCAACTATTACAACAAGCTGGAGGTCGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGCAGGCAA ACTACT GGAACAGCAACCCTTCATTTATCTCTGGGCTGAAAGCTCAGAGGGAGGCCTACTGTCTGAACCATGTTACTGCTTTCTACCCAAACGCT
Pore-DAB*217	4	TCAAGGACATCGAGTTCATCAGGTCTGCATTCTACAACAAGCTGGAGTTCGCCAGGTTTGACAGCAACCTGGGCAGGTTTGTTGGATACACGGAGTACGGAGTGAAGCAGGCAAACTACTG GAACAAAGACACGTCATATATCGCTGCGCTGAACGCTCAGAGGGAGGCCGTCTGTCTGAACAACGTCGGTATCGACTACCGAACCGCT
Pore-DAB*215	1	TCAAAGACATTCAGTACATCGAGTCGTATTGTTACGACAAGAGGGAGTACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGCGTATGGAGTGAAGAACGCTGAACGCT GGAACAAAGACACGTCACTGATCGCTGGGAGGAAAGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTATCTACTACCAGGTGGCT
Pore-DAB*214	5	TCAAAGACATTCAGTACATCAGGTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAACGCTG GAACAACAACCCGTCACAGATCGCTGCGATGACGGCTCAGAGGGAGGCCTACTGTCTGAACAACGTCGGTAACGACTACCGAAACATT
Pore-DAB*213	12	TCAAAGACATTCAGCTCATCGACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAACGCTG GAACAACAACCCGTCACTGATCGCATCAGAGAGAGCTCAGAGGGAGACCGTCTGTCTGAACAACGTCGGTATCGACTACCGAACCGCT
Pore-DAB*212	13	TCAAAGACATTCAGTACATCTACTCCAACTATTACAACAAGCTGGAGATCTTCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGTACAG GAACAACGACCCGTCAATTATTGCATCAAGGAGAGCTCAGAAGGAGGCCTACTGTCAACACAACATCGATATCTGGTACGGGAATCCA
Pore-DAB*211	1	TGAAGGACATTCAGTATGTCGAGTCCTATTGTTACGACAAGAGGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGCGTTTGGAGTGAAGAACGCAGAGCGCT GGAACAAAGACACGTCACAGATCGCTGTGAGGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCT
Pore-DAB*210	1	TGAAGGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAATATGCCAGGTTTGACAGCAACCTGGGAAAATATGTTGGATACACGGAGTACGGAGTGAAGAACGCTGAACGCT GGAACAAAGACACGTCACAGATCGCTGTGAGGAGAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCT
Pore-DAB*209	7	TGAAGGACATTCAGTACATCAAGTCCTATTATTACAACAAGCTGGAGACCAACAGGTTTGACAGCAACGTGGGGAAGTATGTTGGATACACAGCAGCAGGAGTGAGACAGGCTGAGATCT GGAACAAAGACACGTCAGATATCGCTGCGATGAGAGCTCAGAGGGAGGCCTACTGTCTGCACAACATCGGTATCGACTACCAAACCGTT
Pore-DAB*208	11	TCAAAGACATCCAGTTCATCAGGTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCAGAGATCTG GAACAGCAACCCGTCACAGATCGCATCAGAGAGAGCTCTGAGGGAGACCTACTGCCTGCACAACGTCGGCATCGACTACCAAACCGCT
Pore-DAB*207	1	TGAAGGACATTCAGTATGTCGAGTCCTATTGTTACGACAAGAGGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGCGTTTGGAGTGAAGAACGCAGAGCGCT GGAACAAAGACACGTCACAGATCGCTGTGAGGAGAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCT
Pore-DAB*206	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTG GAACAGAGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*205	13	TCAAAGACATTCAGTACATCTACTCCAACTATTACAACAAGCTGGAGATCTTCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGTACGGAGTGAAGCAGGCCGAGTACAG GAACAACGACCCGTCAATTATTGCATCAAGGAGAGCTCAGAAGGAGACCTACTGTCAACACAACATCGATATCTGGTACGGGAATCCA
Pore-DAB*204	1	TGAAGGACATCCAGTACGTCTACTCCATGTATTACGACAAGCTGGAATATGCCAGGTTTGACAGCAACCTGGGAAAATATGTTGGATACACGGAGTACGGAGTGAAGAACGCTGAACGCT GGAACAAAGACACGTCACTGATCGCTGTGAGGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCT
Pore-DAB*203	11	TGAAGGACATCCAGTATGTTGACTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGTGAATTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAGACCTG GAACAAAGACACGTCAAAGATCGCTGTGATGAACGCTCAGAGGGAGGCCTACTGCATGAACACCGTCGATATCGACTACCAAAACGCT
Pore-DAB*202	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTG GAACAGAGATCCTTCATCTATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACATTAGTATAGACTACCAGAATGTT
Pore-DAB*201	1	TGAAGGACATTCAGTATGTCGAGTCCTATTGTTACGACAAGAGGGAATACGCCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGCGTTTGGAGTGAAGAACGCAGAGCGCT GGAACAAAGACACGTCACTGATCGCTGTGAGGAAAGCTCAGAGGGAGGCCTACTGCATGAACCATGTTACTGCTTTCTACCCAAACGCT

Pore-DAB*130	3	TCAAAGACATTCAGTTGATCAACTCCTATTATTACAACAAGCTGGAGTTCCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCTGAACGGTTCAACAAAGACACGTCACGGATCGCTGCGATGAACGCTCAGAGGGAGACCTACTGTCTGAACAACGTCGGTAATTGGTACAGGAGTCCA
Pore-DAB*641	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGACTGCTGAACGGTGGAACAGAGATCCTTCATATATAGCATTGATGAGAGCTCAGAAGGAGACCTACTGCCAGCACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*646	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTGGAACAGGGATCCTTCATATATAGCATTGATGAGAGCTCAGAAGGAGACCTACTGCCAGCACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*790	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGATGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGATGGAACAGAGATCCTTCATCTATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*796	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTGGAACAGGGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*820	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGAATGCTGAACGGTGGAACAGGGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCTGAACCACGTTAGTATAGACTATCAGAATGTT
Pore-DAB*716	11	TGAAAGACATTCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTGGAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*638	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTGGAACAGAGATCCTTCATCTATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*640	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTATCTGAGGTTTGACAGCAACCTGGGGATGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTGGAACAGAGATCCTTCATCAATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTCGAGTACCAGAATGTT
Pore-DAB*667	11	TCAAAGACATCCAGTTCATCAGGTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACAGAGCTGGGAGTGAAGAACGCTGAGATCTGGAACAGCAACCCGTCACAGATCGCATCAGAGAGAGCTCTGAGGGAGACCTACTGTCTGCACAACGTCGGCATCGACTACCAAACCGCT
Pore-DAB*700	11	TCAAAGACATTCAGTATGTTGACTCCTATTGTTACGACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAACGCTGGAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGCT
Pore-DAB*715	11	TGAAAGACATTCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAATACATCAGGTTTGACAGCAACGTGGGTAAATATGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTGGAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCAGTACAACATCGGTATCGACTACCAAACCGTT
Pore-DAB*862	11	TGAAGGACATCCAGTACATCAGGTCCATCTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACAGAGTTTGGAGTGAAGAACGCTGAGCGCTGGAACAAAGACCCGTCAGAGATCTCTGGGATGAGAGCTCAGAGGGAGACCTACTGTCTGACCAACGTCGGTATCGACTACCAAACCGTT
Pore-DAB*440	11	TCAAAGACATCCAGTTCATCAGGTCCTATTGTTACAACAAGCTGGAGTTCATCAGGTTTGACAGCAACGTGGGGAAGTTTGTTGGATACACGGAGCTGGGAGTGAAGAACGCAGAGATCTGGAACAGCAACCCATCACAGATCGCATCAGAGAGAGCTCTGAGGGAGACCTACTGCCTGCACAACGTCGGCATCGACTACCAAACCGCT
Pore-DAB*318	9	TGACAGACATCCAGTTCATCAGCTCCTACATTTACAACAAGATAGAGTGGGCCAGGTTTGACAGCAACCTGGGGAAGTTTGTTGGATACACGGAGTTTGGAGTGAAGACTGCTGAACGGTGGAACAGGGATCCTTCATATATAGCATCGATGAGAGCTCAGAAGGAGACCTACTGCCAATACAACATTGGTGTTGAGTACCAGAATGTT