ENEMY WITHIN THE GATES: REASONS FOR THE INVASIVE SUCCESS OF A GUPPY POPULATION (*POECILIA RETICULATA)* IN TRINIDAD

Caya Sievers

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews

2011

Please use this identifier to cite or link to this item:
http://hdl.handle.net/10023/1865

Enemy within the gates:
Reasons for the invasive success of a guppy population (*Poecilia reticulata*) in Trinidad

Caya Sievers

Thesis submitted for the degree of Doctor of Philosophy,
University of St Andrews

October 2010

Declaration

I, Caya Sievers, hereby certify that this thesis, which is approximately 51,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in October 2007 and as a candidate for the degree of Ph.D. in October 2008; the higher study for which this is a record was carried out in the University of St Andrews between 2007 and 2010.

Date: Signature of candidate:

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Ph.D. in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date: Signature of supervisor:

In submitting this thesis to the University of St Andrews we understand that we are giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. We also understand that the title and abstract will be published, and that a copy of the work may be made and supplied to any *bona fide* library or research worker, that my thesis will be electronically accessible for personal or research use unless exempt by award of an embargo as requested below, and that the library has the right to migrate my thesis into new electronic forms as required to ensure continued access to the thesis. We have obtained any third-party copyright permissions that may be required in order to allow such access and migration, or have requested the appropriate embargo below.

The following is an agreed request by candidate and supervisor regarding the electronic publication of this thesis:

Access to Printed copy and electronic publication of thesis through the University of St Andrews.

Date:
Signature of candidate: Signature of supervisor:

Abstract

The invasion of individuals into new habitats can pose a major threat to native species and to biodiversity itself. However, the consequences of invasions for native populations that are not fully reproductively isolated from their invaders are not yet well explored. Here I chose the Trinidadian guppy, *Poecilia reticulata*, to investigate how different population traits shaped the outcome of Haskins's introduction, a well-documented invasion of Guanapo river guppies into the Turure river. I especially concentrated on the importance of behaviour for invasive success. I investigated if the spread of Guanapo guppies is due to superiority in behaviour, life-history and/or genetics, or if the outcome of this translocation is due to chance.

Despite the fact that by today the invasive front has passed the Turure's confluence with the River Quare many kilometres downstream of the introduction site, and the original genotype only survives in small percentages, as was revealed by genetic analysis in this and other studies, no obvious differences between invasive and native populations could be detected in any of the tested behavioural, life-history and genetic traits. When tested for mate choice, neither Guanapo nor Oropuche (Turure) males seemed to be able to distinguish between the population origin of females, but courted and mated at random. At the same time, females did not prefer to school with individuals of the same population over schooling with more distantly related females. The formation of mixed schools after an invasive event is therefore likely. Because female guppies showed a very low willingness to mate, even after having been separated from males for up to six months, sperm transfer through forced copulations will become more important. Taken together, these behaviours could increase the speed of population mixing after an invasion without the need for behavioural superiority of the invasive population. When tested for their schooling abilities, offspring of mixed parentage, in contrast to pure bred s, displayed a large amount of variety in the time they spent schooling, a circumstance that can potentially influence survival rates and therefore the direction of gene pool mixing. Guanapo fish did not show reproductive superiority in a mesocosm experiment, where both populations were mixed in different proportions. On the contrary, in two out of three mixed treatments, the amount of Oropuche (Turure) alleles was significantly higher than expected from the proportion of initially stocked fish. The almost complete absence of distinguishable traits other than genetic variation between the examined populations that belong to different drainage systems, opposes the recent split of the guppy into two different species following drainage system borders, as is argued in this thesis. However, the successful invasion of the Turure by Guanapo guppies and the nearly entire disappearance of the original population can be explained in absence of differing population traits.

Here I demonstrate how behavioural and genetic interactions between subspecies influence the outcome of biological invasions and second, how factors other than population traits, such as the geographic situation, can produce an advantageous situation for the invader even in the absence of population differences.

Acknowledgements

I want to thank...

...first of all my parents. Without their help and support I would not have been able to finish this thesis. I am always looking forward to come home, even if it is only twice a year.

Anne Magurran, for being such a good and helpful supervisor, for encouraging and pushing me in the right moments, critical and mind opening discussions, the opportunity to go to many excellent conferences and for turning me into a better scientist.

Indar Ramnarine, for co-supervising me in Trinidad, organising lab space, mesocosms, fish catching trips and a hundred other things and for always being at hand when I needed help. Also thanks a lot for taking me and Amy out to the Caroni swamps for some amazing bird watching.

Raj Mahabir and Kharren Deonarinesingh, who helped me catching my fish all over Trinidad, sometimes fed them while I was away, always provided me with all the names of birds, insects and plants I could wish for and once crawled with me through a two metre long rock tunnel into one of the Tamana caves to stand in the middle of tens of thousands of bats (and equally many bugs).

Christine Dreyer and her group at the MPI in Tübingen, especially Margarete Hoffmann, for helping me with my genetic analysis, answering all my questions and for preventing me from making too many time consuming mistakes.

Amy Deacon, who was my one constant office mate during my time in St Andrews, travelled the world and shared half of my time in Trinidad with me, including some crazy jungle hiking trips and visits to the market, proofread chapter after chapter of my thesis, and very patiently listened to uncounted complaints about non-significant data throughout my PhD. Thanks also for the invention of DABPs, where half of my annual birthday and Christmas presents were produced.

Renée Cozier, for turning my time in Trinidad into something special, spending the lunch breaks at UWI campus together with me under the big trees, and taking me along for a wonderful weekend with her family in Icacos.

Ben, Meli, Fatima, Regina, Nik, Sunita and LaDana, my other friends from Trinidad, for lots of chatting and laughing, a proper introduction to Trinidadian food and language, crazy trips to Port of Spain, some excellent dinners, a day collecting data (and watching birds) in the Caroni swamps and a very nice hike to the Mermaid Pools.

Franzi Göhringer, for writing and answering hundreds of e-mails and letters, and hour long phone calls on Skype (thanks to them as well, I guess). And, together with Steffi, for housing me for a month on their living room floor while I analysed my fish in Tü.

My various house mates in DRA, Fife Park and Fleming Place, most of all Milena Elsinger and René Swift, for uncounted cooking and baking events, good chocolate and films, and braving the cold in our house at winter. I always felt at home when I came back from work in the evenings.

My other office mates Tess, Steph, Thomas, Silvana, Miguel (and Humphrey, of course), for good discussions, hilarious stories, some gossip and an enormous amount of cup cakes and other things containing lots of sugar at least once every week.

Bill Anderson, my book selling friend, who supplied me with what felt like a ton of books (I must know, because in the end I all had to carry them all back home), thereby ensuring that not only my scientific skill improved over time, but also my knowledge of Scottish and English literature.

Jeff Graves and Siân Griffiths, my examiners, for some hours of good discussion and a very interesting and pleasant time during my viva.

So many others who made my time in St Andrews special and unforgettable. Because I am sure, I would forget somebody, instead of names a big hug to everybody. Thank you, folks!

Donald Malone, simply for turning up, right in the end.

Table of Contents

Chapter 1 General introduction

For a long time now invasive species have spread around the world and colonised even the remotest places. They now represent one of the greatest threats to biodiversity and play an important role as agents for global change, alongside with human-caused releases of greenhouse gases and oceanic alterations (Mack et al., 2000). In this thesis, invasive species are defined as non-native individuals that have a negative impact on their new environment. These impacts can include predation on native species, competition for resources or the alteration of ecosystem variables such as water availability or soil stability. Humans have intentionally and unintentionally assisted species to invade new territories for thousands of years, but the dramatic recent increase of immigrants correlates with growing transport and international commerce (Elton, 1958). Ecologists distinguish between natural and human-mediated range expansions. Like species extinctions, species invasions are natural events, but compared to natural range expansions, humans have massively increased the rate and magnitude at which species invade new areas as well as the distances over which invasions occur, and they have substantially changed the geographic patterns of invasion (Cassey et al., 2005). In their analysis of invasion events in the San Francisco Bay, Cohen & Carlton (1998) found that the rate of invasion accelerated during the last 150 years, increasing from an average of one new species established every 55 weeks from 1851 to 1960 to an average of one new species every 14 weeks from 1961 to 1995.

Once established as an invader, introduced species can cause many problems in their new habitats. These include the disruption of the evolutionary processes, for example by mixing species from once separated habitats, which now are able to produce hybrid offspring and cause a loss of distinctiveness between different geographical regions (Olden et al., 2004). Radical alterations in the species composition of certain ecosystems are also common, sometimes leading to the extinction of native species (Rhymer & Simberloff, 1996). While the term extinction describes the complete disappearance of a species, extirpation signifies the local extinction of a population. Clavero and García-Berthou (2005) pointed out that invasive species are the most important cause of bird extinctions and the second most important cause of fish and mammal extinctions worldwide. For instance, the release of the Nile perch (*Lates nilotica*) into Lake Victoria in the 1950s has led to the extinction or extirpation of more than a hundred endemic cichlid species so far (Goldschmidt et al., 1993). A botanical example is the introduction of the Australian paperbark tree (*Melaleuca quinquenervia*) to the southern parts of Florida that resulted in the replacement of several native species like pond cypress (*Taxodium ascendens*) and sawgrass (*Cladium jamaicensis*). The high density at which this tree grows excludes many native animals by providing a poor habitat (Serbesoff-King, 2003).

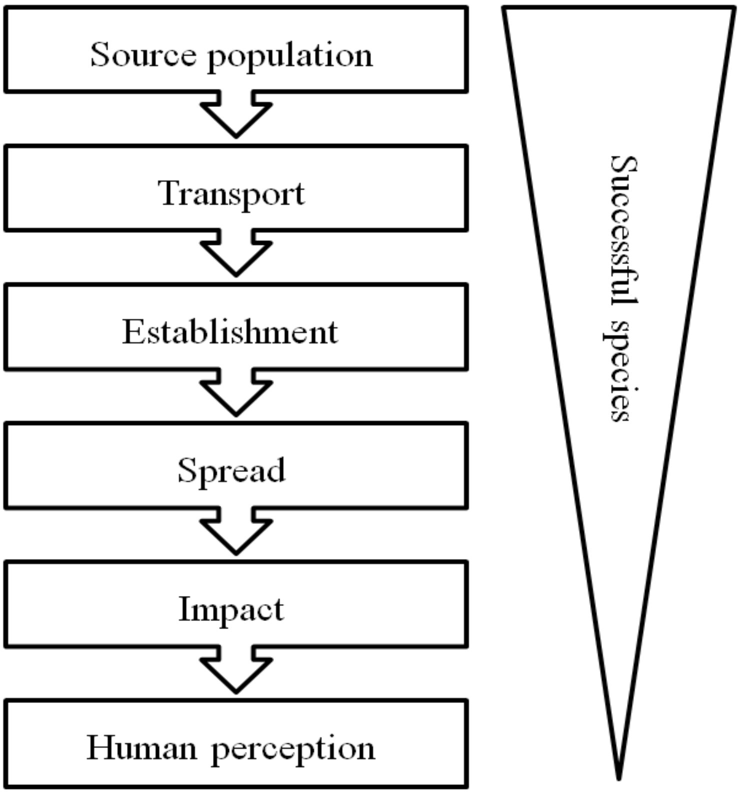


Figure 1: A species that is transported out of its native range has to successfully run through several steps before it will be called invasive. The risk of failure at any one of these stages is high and only a limited amount of species will turn into invaders in the end. Adapted from Lockwood et al. (2007).

Steps to becoming an invader

Transport

Before individuals of a species are called invaders, they have to pass several stages along the way. First of all, members of a species have to be transported out of their natural range, either intentionally, for example as ornamental plants, crop species and pets, or unintentionally as hitch-hikers, when other products are transferred (Figure 1). One of the most striking examples of an unintentional mode of transport is the ballast water of ships. Carlton (1999) estimated that up to 10,000 species might be transported around the world within ballast water each day. Compared to natural colonisation events, human-mediated transport is faster and often operates on a larger scale. Depending on the mode of transport, the conditions faced by involved individuals can be rough and many will not survive long enough to reach their destination or arrive in poor condition.

A set of individuals that is released outside their native habitat is referred to as a propagule. Propagule pressure is a combination of the number of individuals per propagule, the number of release events and the physiological condition of individuals within a propagule and can be used to explain significant differences in the establishment success of populations, even within the same species. It helps to understand the large amount of variation in the outcome of invasions independent of taxon and location (Lockwood et al., 2005)

Establishment

After arriving in a new habitat, the surviving individuals have to establish a new, self-sustaining population. The success of this establishment depends on the ability of the introduced species to survive the ecological conditions it encounters, escape predation, find enough food in a new environment and to successfully breed. The time a population of newly arrived individuals needs to adapt to local biotic and abiotic factors can cause a delay in population growth. Many populations of non-native species experience at least some sort of lag time between the initial establishment in a new habitat and a rapid range expansion by spread. Daehler (2009) reported that the lag time between introduction and spread of invasive tropical plants was approximately 14 years for woody plants and 5 years for herbaceous plants and therefore a lot shorter than lag times normally observed in introduced plants of temperate regions. A potential cause for lag times are Allee-effects. This term describes the reduction in population growth rates due to low population density and therefore difficulties of individuals to find mating partners, reduced anti-predator defence (Lewis & Kareiva, 1993) and less efficient feeding in low densities (Way & Banks, 1967). But when populations overcome Allee-effects, they can rapidly start spreading into new locations. Because weak Allee-effects are hard to detect, it is difficult to predict which non-native population is going to stay local and which population is likely to quickly spread across the landscape as soon as a certain population density is reached. Another reason for a lag time between establishment and spread might simply be the threshold of detection by human observers who fail to discover individuals from newly arrived species due to their initial low concentration (Lockwood et al., 2007).

Spread

As soon as a population of non-native individuals has reached a certain density and has adapted to local conditions, it can start to spread out across its new habitat, establish further populations within the non-native range, thereby encountering new native species and competing with them for resources. In their analysis of 30 recorded cases of successful invasions, Arim et al. (2006) found that spread dynamics often follow a regulated process that is consistent between different invasions. Because the colonisation of new sites will deplete their limited resources, these sites are not available for new invasions of the same species. Furthermore, new sites will only be colonised after local population growth and the production of new propagules of invading individuals. It is therefore common to find a non-reproductive line leading the invasive front that decreases and therefore regulates the speed of further spread.

An important mode of travelling long distances during the dispersal stage is once again human-mediated transport which is not likely to stop after a population became displaced from its native habitat to a new location. Hastings et al. (2005) proposed that one key to understanding the spread of an invasive species in a new habitat is measuring human transport processes. It is possible for an expanding non-native population to have two different dispersal curves, one for the spread under its own power and one for dispersal as a hitch-hiker. A good example for the two modes of spread is the colonisation pattern of the zebra mussel (*Dreissena polymorpha*) in North America. After first appearing in the Great Lakes in the 1980s, it quickly spread downstream and colonised both the Hudson and the St. Lawrence River. This process is thought to have occurred naturally as larvae were distributed by the water current. But zebra mussels also appeared in various other lakes and rivers, unconnected to the water system of the Great Lakes. It was later found that the first introduced individuals most likely reached these places as adults while connected to the boats of recreational fishers (Johnson & Carlton, 1996).

Impact

But to finally be called an invasive species, the newcomers need to have a negative ecological or economical effect on native species or ecosystems. Parker et al. (1999) argued that the total impact of an invader includes range, abundance and per-capita/per-biomass effect of the invader and that these impacts can be measured at five different levels. These consist of effects on individuals, genetic effects, population dynamic effects, community effects and, finally, effects on ecosystem processes. Impacts can be direct, such as competition for resources, predation on native species, herbivory of native plants and parasitism or indirect by changing existing ecosystem variables, like water filtration and soil stabilisation. The threat of extirpations or extinctions of native species seems to be greatest in species with high energy requirements, especially endothermic vertebrates (Vermeij, 1991). At the same time this group of animals is also more likely to cause extinctions when invading new habitats. The impact an invasive species will have on other members of its new habitat is likely to vary with the role it plays in the system. Predators and pathogens impose a greater threat on native species than do competitors, which do not commonly cause extinctions of long-term residents (Davis, 2003). The reduction of genetic diversity, local extirpation of natives, niche displacement and genetic homogenisation are often the results of an invasion process and in the end increase the risk of extinction for native species (Olden & LeRoy Poff, 2003).

During each of the aforementioned stages, many non-native species are likely to fail and therefore never turn invasive at all (Figure 1). Williamson & Fitter (1996) proposed that only around 10% of introduced species proceed at each stage of the invasive process, a calculation known as the Tens Rule. More important than an actual percentage, which is likely to vary between different stages of an invasion event or between different species, is the fact that this rule highlights how difficult it is to predict which species of the large number of species that are translocated every day will become invasive in the end. However, even if an invasion does not directly lead to the extinction of species, is it capable of altering the environment in many different ways.

Biotic homogenisation

Biotic homogenisation, or the replacement of native biota by locally expanding, non-native and often cosmopolitan species, is leading to a loss of distinctiveness of formerly separated regions. Two distinct processes influence the process of biotic homogenisation: the extinction of native species and the invasion of species into new areas (Olden & LeRoy Poff, 2003). Genetic homogenisation reduces the spatial component of genetic variability between different populations within a species. Today, scientists expect homogenisation to be the most important cause of the impoverished biodiversity the earth is facing (Olden et al., 2004). As an example, Galil (2007) uses the Mediterranean Sea, where hundreds of biotic invaders contributed to the decline of native species and local extinctions of some of them. Two of many examples are the replacement of the oyster *Spondylus gaederopus* by the Erythrean spiny oyster (*S. spinosus*) in Haifa Bay, Israel, within only ten years (Mienis et al., 1993) and the quick spread of *Caulerpa taxifolia*, a tropical green alga, throughout the western Mediterranean Sea. This organism is responsible for a 25-55% decline in species richness of native hart substrate algae (Verlaque & Fritayre, 1994). The functional diversity of a ecosystem includes a variety of biological processes like metabolic and photosynthetic pathways or trophic levels that make an ecosystem work. Changing this diversity can result in functional homogenisation and often involves the replacement of highly specialized species by the same set of widespread generalists. Functional homogenisation of several or all communities within an ecosystem can therefore increase the vulnerability to large-scale environmental events by synchronising local biological responses across individual communities (Olden et al., 2004).

Next to environmental and climatic changes, for example the release of enormous amounts of CO_2 and other greenhouse gases into the atmosphere, ecological modifications by invasive species are a leading factor of global change produced by humans. The effects of invasive species on ecosystems and biodiversity often have direct economic consequences and can cause pronounced losses in agriculture, the fishing industry and forestry (Mack et al., 2000). The invasion of several extremely water-consuming tree species into the Cape Province, South Africa, completely changed the region's water supply. Consequently, numerous regional rivers dried out. These changes now threaten many endemic plant species and local agriculture (van Wilgen et al., 1996). Pimentel et al. (2000) points out that it costs the US economy around $137 billion (£67.5 billion) a year to pay for losses in goods and services and environmental damages caused by invaders as well as to control invasive species, an amount that is likely to increase dramatically during the next few decades.

Genetic consequences and constraints of invasions

Normally the number of individuals founding a new population during an invasive event is small, representing only a fraction of the source population's gene pool. Therefore the population can go through a ‘bottleneck’ or severe reduction in its demographic size. Changes in gene frequencies are likely to occur: a phenomenon known as the ‘founder effect’. If a population is going through a narrow bottleneck, the loss of genetic variability can be very high (Sakai et al, 2001). Chance events will be responsible for the loss of low frequency alleles from the population’s gene pool. Main heterozygosity and allele frequency per locus can decline fast and therefore eliminate a lot of genetic variation. Due to new mutations, genetic variation will slowly increase once the population starts growing again. However, while the population size is small, genetic drift can further eliminate low frequency alleles (Nei et al., 1975).

Genetic variation is important for rapid evolution and adaptation to a new environment, hence a loss of variability can have negative consequences for the adaptability of a founder population. A species arriving in a new habitat is likely to face new environmental conditions it is not adapted to. Not being able to evolve fast due to reduced genetic variability could therefore prevent the arrivals from establishing a viable population. However, Roman & Darling (2007) recently stated that there is increasing evidence that reduced genetic diversity in invasive species in not as widespread as previously thought. High numbers of introduced individuals as well as multiple introductions of members of the same source population or species reduced the risk of founder effects in most of 43 examined successful aquatic invasions. Thus the loss of genetic variability through narrow bottlenecks is decreased and the potential for rapid adaptations to the new environment remains high (Sakai et al., 2001). For example, the ballast water of ships is an effective way to transport many individuals at the same time and repeatedly from the same original population to distant places. Ten out of 15 studies investigating the genetic diversity of ballast-water-mediated invasions showed similar levels or even an increase of genetic diversity compared to the source populations (Roman & Darling, 2007). Interbreeding between formerly separated populations or between species does not necessarily have to be detrimental by destroying local adaptations, as it can also produce novel combinations of genes or an increase of overall genetic and phenotypic variability and can therefore create a broad basis for selection to work on (Bossdorf et al., 2005). Hence hybridisation can enhance the invasiveness of a non-native population through the creation of novel genotypes and high genetic variation that allow individuals to survive under various circumstances.

Behaviour as a reason for successful invasion

To understand, effectively control, and prevent invasions there is a need for long-term and large-scale strategies, as these are likely to be more successful and economical than trying to combat individual invaders (Simberloff, 1997). Investigating the role of ecological, genetic and life-history characteristics of invasive organisms has therefore been a central focus of ecological research and experiments in the last five decades. Several studies have illustrated how invasive species can alter the evolutionary pathway of natives in many different ways (e.g. by competitive exclusion, niche displacement, predation and hybridisation) that can ultimately lead to extinction of natives. It is only recently, however, that the role of behaviour during an invasion received attention. Since behavioural adaptations can underpin successful invasion this is a crucial theme for future research. In particular, it is vital to understand how behaviour influences the competitive ability and spread of invasive species (Holway & Suarez, 1999). The formation of large super-colonies by some invasive ants is an example of how behavioural shifts after introduction can increase the invasiveness of species (Hölldobler & Wilson, 1977). In their natural habitat, the Argentine ant (*Linepithema humile*) exhibits strong intra-specific aggression. However, invasive populations in California almost completely lack this form of aggression. This leads to the formation of extremely dense and large colonies that are more competitive than native ant species in this region (Suarez et al., 1999). Behavioural variations can therefore influence the invasive success of a population and shifts in behavioural characteristics following introduction can enhance competitive ability (Holway & Suarez, 1999).

Genetic homogenisation of populations

The term biodiversity does not only address species diversity but also includes the genetic diversity within a species, as defined by the Convention on Biological Diversity (1993). For that reason, the consequences of invasions for native populations that are not fully reproductively isolated from their invaders are of particular interest. Possible results of the invasion of another population could be the coexistence, hybridisation or displacement of the native population. In the latter two cases the invasion would result in a change of the local gene pool and therefore in a loss of biodiversity. Hybridisation is the interbreeding of individuals from genetically distinct species or populations, thereby producing mixed offspring. Introgression on the other hand describes gene flow between two populations that hybridise, when hybrids backcross with one or both parental populations (Lookwood et al., 2007). Hybridisation between the introduced North American mallard (*Anas platyrhynchos*) and the native New Zealand grey duck (*Anas superciliosa*) endangers the survival of the latter as a distinct species (Rhymer et al., 1994). Intraspecific hybridisation can homogenise discrete characteristics of geographically isolated populations (Daehler & Carino, 2001) and as a result affects individual fitness by destroying local adaptations (Storfer, 1999). One important example of introduced individuals endangering native stock of the same species is the escape and subsequent spawning of farmed Atlantic salmon (*Salmo salar*) and other Salmonids into the wild. Farmed salmon has been under artificial growth selection for decades and differs genetically from wild populations. In the wild, where they mix with native populations, their offspring experience high mortality rates which leads to concern for fitness and productivity of native populations due to very large numbers of escaped fish (Hindar et al., 2006). Small populations are especially vulnerable to hybridisation in several respects including infertility of offspring, genetic homogenisation and outbreeding depression (Mooney & Cleland, 2001). The analysis of allozymes and mtDNA in an endemic pupfish species (*Cyprinodon bovinus*) in Texas revealed that all wild populations of this fish contained foreign genetic elements (ranging from 6.1 to 15.1%) from the sheepshead minnow (*Cyprinodon variegatus*), a recently introduced species. The only unmixed individuals left belong to a laboratory population (Echelle & Echelle, 1997).

One of the main aims of my PhD was to investigate how the invasion of individuals influences the existence and survival of a native population belonging to the same species. I asked two main questions: 1) Which traits could help to explain the invasive success of one over the other population when both share the same or at least very similar genetic material. And finally, 2) what are the consequences for the native population that has to share its territory with invaders with whom it is able to mate and produce offspring. The Trinidadian guppy is an ideal model system for addressing questions concerning the effects of invasions of other populations and genetic, life history and behavioural patterns influencing the success of an invading population. There exist several well documented introduction events throughout Trinidad, involving guppies from the same population or fish from different rivers or even drainage systems. Knowing about the origin of introduced fish as well as the time scale and propagule size of the introduction event provides useful background information that might help with interpreting genetic data and mixing patterns. Another reason for choosing the guppy as a model species for this question is the fact that populations belonging to different drainage systems in Trinidad show very high genetic diversity. This diversity is greatly endangered by the introduction and subsequent gene pool mixing of fish belonging to distinct genetic groups.

The guppy as a model species

For many years now, the guppy (*Poecilia reticulata*, Poeciliidae) has been used to shed light on the rapid evolution of behavioural and genetic traits (see Magurran et al., 1992). This small freshwater fish is native to the north-eastern parts of South America including Trinidad and inhabits shallow rivers and ponds. Populations show considerable differences in morphology, behaviour and male colour patterns over relatively short geographical distances. They are also capable of undergoing fast evolutionary adaptations under changing environmental conditions (Reznick et al., 1997). This made the guppy an ideal candidate to investigate natural and sexual selection acting on organisms. A significant part of the variation observed between populations is due to different degrees of predation guppies' face in their natural environment.

General appearance

Guppies exhibit strong sexual dichromatism. Females have a drab, beige colouration and continue to grow throughout their lifetime. They can thereby reach a body length of 3 cm and more.

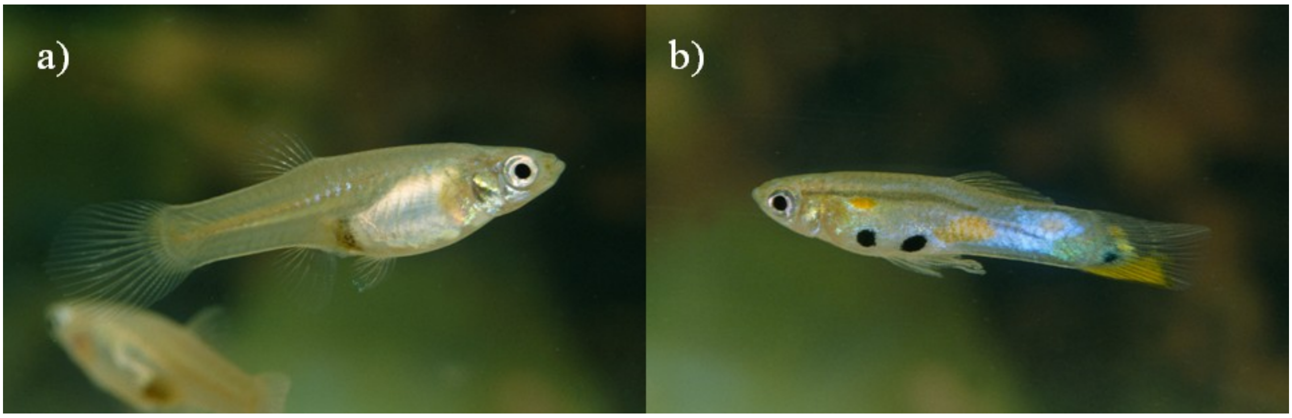


Figure 2: There exist obvious differences both in morphology as well as colouration between female (a) and male (b) guppies (Poecilia reticulata). Females are larger and of a drab beige colouration whereas the often smaller males display various black, orange and yellow colour spots as well as areas of iridescent blue and green.

Males in contrast stop growing after they have reached sexual maturity and normally do not exceed 2.5 cm. They display unique colour patterns, mainly consisting of yellow, orange and black spots which make it possible to individually identify each male (Figure 2).

Predation risk and survival

In general, the existence of different predation regimes is largely responsible for the observed strong differences in guppy behaviour, morphology and male colouration (e.g. Endler, 1980). High predation habitats are normally found in the lower parts of streams and are characterised by the occurrence of the pike cichlid (*Crenicichla alta*), an important guppy predator, and other piscivorous fish species. Up-stream low predation sites on the other hand are mainly occupied by only one other fish species, the killifish *Rivulus hartii*, a smaller omnivorous species that predominantly feeds on guppies (Liley & Seghers, 1975). Males in populations from low predation regimes are more colourful, and females in these populations show strong preferences for brighter male colour patterns. Individuals reach maturity at a later age and females give birth to fewer and larger offspring (Reznick & Endler, 1982). In contrast, male guppies from high predation habitats are less colourful (Endler, 1983). Here guppies reach maturity sooner and females produce more and smaller offspring (Reznick & Bryga, 1987). Female preferences for certain male traits, such as black and orange colour spots, are weaker (Endler & Houde, 1995). At the same time males increase the frequency of sneaky mating attempts and reduce the time they spend courting females, therefore the opportunity for sexual selection to act on certain traits is decreased (Houde & Endler, 1990).

One way to reduce the risk of predation is to form schools with other individuals, a behaviour that functions as a defence against predators in many fish species (Pitcher & Parrish, 1993). The inspection of possible predators by single individuals can also increase the available information the remaining school members gain about the predator and is commonly found in guppies (Kelley & Magurran, 2003a). Female guppies form the core of schools and become familiar with other group members. They spend more time schooling compared to males which trade off the safety of a group with increased movement between schools in search of new mating partners (Griffiths & Magurran, 1998). Guppies occurring in high predation sites spend more time schooling and form larger, more cohesive schools than fish living in low predation habitats (Seghers, 1974).

Reproduction

Guppies are ovoviviparous which means that they have internal fertilisation and females give birth to living offspring. Male guppies have a gonopodium, a modified anal fin that is used as an intromittent organ and transfers spermatozeugmata (sperm bundles) during copulation. At the age of three months guppies are sexually mature and females start to give birth around once every month (Liley, 1966). To achieve a mating, males can employ one of two different mating strategies, and can switch between these according to context (Houde, 1997). They can either court receptive females and display to them, or they can try to circumvent female choice by means of sneaky mating attempts. Males are almost permanently in search of mates and are thereby likely to move considerable distances. Females on the other hand are only receptive as virgins and for a few days after giving birth (Houde, 1997). They then exhibit strong mate choice mainly depending on elements of male colouration and male body size, but otherwise try to escape the continuous male mating attempts. Normally more than one male sires each brood produced by females. Interestingly, the number of fathers per brood is smaller than the number of males that successfully copulated with a given female (Evans & Magurran, 2001). Therefore, Becher and Magurran (2004) concluded that post-copulatory mechanisms exist and females can discriminate between the sperm of different males. At the same time, Evans et al. (2003) argued that sperm competition, by some males producing superior ejaculates, is more important than cryptic female choice.

Russell et al. (2006) demonstrated that at least some sneaky mating attempts are successful and result in the transfer of sperm, and a recent study has shown that males which predominantly use coercive mating tactics are less colourful but produce faster swimming sperm (Evans, 2010) Although the total amount of transferred sperm is generally larger after courtship behaviour, there is much variance and overlapping of the number of transferred spermatozeugmata between both mating tactics (Pilastro & Bisazza, 1999). Therefore, males can sometimes overrule female mate choice with its normally strong preferences for individual male colour patterns and create the basis for sexual conflict.

Sexual conflict and its consequences

An explanation for sexual conflict in guppies is the difference between male and female mating tactics. As long as there are at least a few males around, females are able to find a mating partner and reproduce. Their fecundity depends on size and therefore foraging qualities and survival (Magurran & Seghers, 1994), which is one reason why females spend more time schooling. However, males have to find as many mating partners as possible to maximise their fitness while females are often reluctant to mate. This circumstance leads to sneaky mating behaviour and an increased movement between schools in search of potential mates (Magurran & Seghers, 1994). Strong sexual selection due to female choice seems to reinforce differences between populations caused by natural selection in the first place. These differences can finally lead to reproductive isolation and the establishment of two different species (as reviewed by Magurran, 1996). So far guppy populations inhabiting the same river have not developed any reproductive barriers and are unlikely to do so in the future for several possible reasons. Male guppies can potentially undermine female choice and therefore act against reproductive isolation (Magurran, 1998). A second behaviour also seems to be important for the lack of speciation between populations of the same river: the tendency of male guppies to move between groups.

Movement and gene flow

Since female guppies prefer to mate with unfamiliar males (Hughes et al., 1999) and male guppies can increase their fitness by mating with novel females (Kelley et al., 1999), it can be advantageous for males to move between different schools and populations to gain access to more mating partners. Accordingly, males that move between groups increase the total number of females they come across. These encountered females are also more willing to mate because of their unfamiliarity with the males (Croft et al., 2003).

In contrast to males, female guppies show site fidelity and usually return to their site of origin if moved. Only a small percentage of females (6.9 %, compared to 27.3 % of moving males) left their release pools during an 8-day study with marked fish (Croft et al., 2003). In combination, these two male behaviours, movement between schools and sneaky mating attempts, can lead to strong gene flow across populations and therefore weaken the strength of sexual selection operating in this species (Bisazza, 1993). In 1995, Endler proposed that the absence of speciation in guppies can be explained by the large distance of gene flow compared to the area of a given selection regime. On the one hand, evolution of behavioural and other traits caused by predation-induced selection pressure is fast. On the other hand, continuing gene flow prevents or slows down genetic differentiation between populations and therefore counteracts reproductive isolation and speciation. Male dominance varies between populations (Magurran et al., 1996) and this can further enhance gene flow, if males of a native population are outcompeted by invading males (Magurran & Phillip, 2001).

Speciation and the existence of *Poecilia obscura*

So far scientists are agreed that there exists one context in which Trinidadian guppies exhibit the early stages of reproductive isolation. Two unconnected drainage systems are found in northern Trinidad: the western Caroni- and the eastern Oropuche system, separated only by a short geographic distance at their closest points, and both of them widely inhabited by guppies (Figure 3).

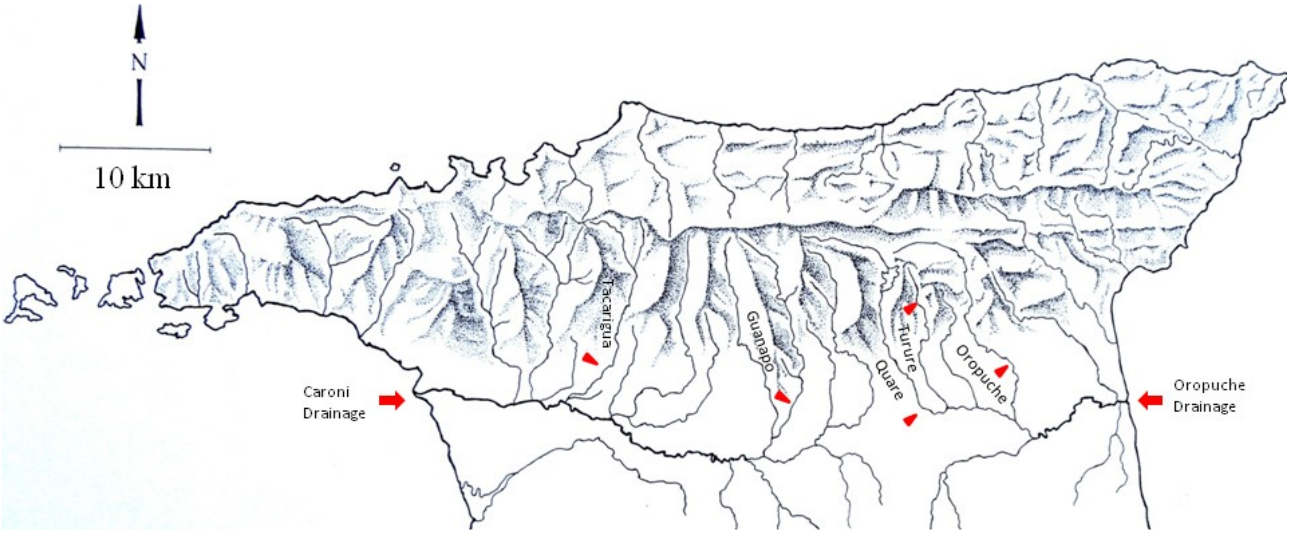


Figure 3: Map of the Northern Mountain Range in Trinidad showing the island's two separate drainage systems flowing to the west (Caroni drainage) and the east (Oropuche drainage). The rivers where experimental fish were collected for this thesis are named and indicated with red arrows.

Not withstanding the fact that mating between individuals from both river systems can produce viable offspring, several studies have revealed that populations from the Caroni and Oropuche drainage belong to genetically distinct groups, separated for up to two million years (Ludlow & Magurran, 2006).

A detailed study of mitochondrial DNA-sequences in several Trinidadian guppy populations across the island by Fajen & Breden (1992) revealed that there is hardly any genetic differentiation between guppy populations within drainages (0.0-1.0% mtDNA variation). The variation between Oropuche guppies and populations native to all other drainage systems lies between 3.9 and 5.6%. An investigation using allozymes came to similar conclusions, showing that gene diversity based on Nei's gene diversity analysis was 66% due to differences between drainage systems, 32% due to within drainage differences and 2% due to within river variation (Carvalho et al., 1991). Recent experiments have detected the existence of some reproductive barriers between Caroni and Oropuche individuals, including hybrid behavioural impairment in the F_1 generation (Russell & Magurran, 2006) and the emergence of gametic isolation (Ludlow & Magurran, 2006). Guppies were thus used to provide novel insight into the speciation process and particularly the importance and order of establishment of different isolating patterns during the separation of populations. More recently, however, Schories et al. (2009) argued that guppies from the Caroni and Oropuche drainage systems do not show the early stages of speciation, but belong to two distinct species, *Poecilia reticulata* and *P. obscura*, that have been separated for several million years. Based on their study of the mitochondrial control (D-loop) region and the cytochrome b gene of several guppy population across Trinidad as well as *Poecilia wingei* individuals from Venezuela, they argued that *P. obscura* forms a cryptic species complex with the other two species. According to this study, separation took place between 2.5 and 5 million years ago based on the cytochrome b sequences and between 0.4 and 4.2 million years ago based on the sequences of the control region. No morphological or behavioural differences could be found to clearly separate both species in the same way as the molecular differences do. Further analysis and discussion is therefore necessary to clarify the species status of the guppy.

During the last few decades, several guppy populations from different Trinidadian rivers were transferred within and between drainage systems for scientific reasons (see Carvalho et al., 1996).

Despite the fact that it was possible to gain new insights into evolutionary processes, some of these experiments also demonstrated how easily the evolution of reproductive isolation can be disrupted.

Haskins's introduction

In 1957, C.P. Haskins introduced 200 guppies (approximately half of them female) from the lower Guanapo (Caroni drainage) to the upper Turure (Oropuche drainage) into a previously guppy-free environment above a barrier waterfall (Shaw et al., 1992). (see Figure 4). This was done in order to investigate the influences of predation regime on guppy life-history and morphology. However, no results were published later on. Because of the ability of female guppies to store the sperm of several males, the number of individuals contributing genetically to the newly founded population might have been larger than 200.

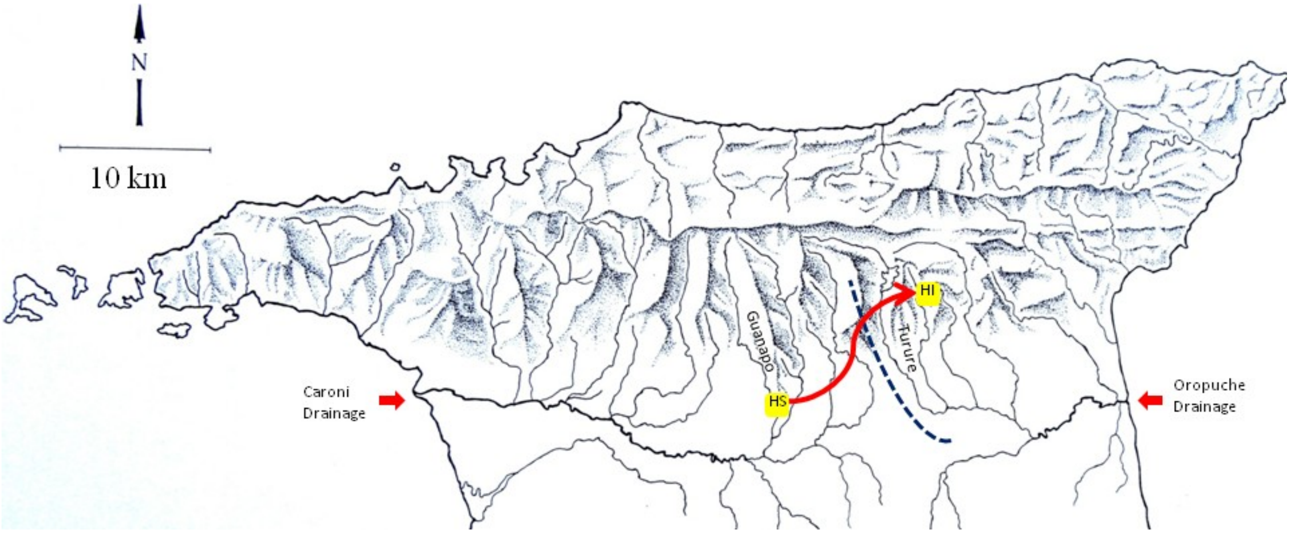


Figure 4: Map of Trinidad showing the origin of the source population (Haskins' source: HS) from the Guanapo and the introduction site (Haskins' introduction: HI) in the Turure used in Haskins's introduction. The red arrow highlights the direction of the transfer. A blue line marks the border between the Caroni and Oropuche drainage system that was crossed during the translocation event.

Before their translocation, Guanapo guppies lived in a high-predation regime where they coexisted with several predators including the pike cichlid, whereas their new habitat was predator free except for *Rivulus hartii*. 37 years later, it became evident through genetic investigations that great parts of the guppy population living in the upper and middle Turure were genetically more closely related to populations from the Caroni system than to populations from the rest of the Oropuche drainage

(Shaw et al., 1992). This means that the introduced fish not only established a viable population in their new environment but also expanded their home range in a downstream direction beyond the barrier waterfall into the middle parts of the river.

In this population, mean heterozygosity was strongly reduced (0.028 compared to 0.049 in the source population) whereas the mean number of alleles in allozymes per locus differed only slightly from the source population (1.2 compared to 1.4). This implies that the founder population was large enough to prevent a narrow bottleneck and population growth after introduction was rapid, so the probability of losing many low frequency alleles was small. Even with reduced mean heterozygosity, there is still a strong potential for fast adaptive evolutionary change in morphology, life-history and behaviour in this and other populations established from few founder individuals (Carvalho et al., 1996). For example, the schooling tendency of the new population above the waterfall was significantly reduced (Magurran et al., 1992) and males had more and larger colour spots, which correlates with the different predation regime the guppy population faces in its new habitat. In their study based on 25 allozymes, Shaw et al. (1992) found that native Turure fish had genetically contributed to the present population below the waterfall, but could not ascertain if the alleles belonging to the original genotype represented a strongly reduced native stock or resulted from inter-breeding. Later studies revealed that only 12% of all genotypes occurring in the parts downstream of the barrier waterfall of the river Turure belong to the original Oropuche matriline (Becher & Magurran, 2000). This study was based on an investigation of the mitochondrial NADH dehydrogenase subunit 2 (NADH2) gene.

The question that now arises is whether the colonisation of the Turure by Guanapo guppies is an ongoing process that will eventually lead to the total replacement of the native genotype or if the displacement reached a stable equilibrium point after some time and therefore allows individuals of the native population to co-exist with their invaders, as was suggested by Becher & Magurran (2000). This would mean that original population still exists, albeit in low numbers. Therefore it is important to determine if and to what extent fish of both populations mix in the wild and how hybrid offspring contributes to further gene flow within the Turure.

Aims of my thesis

What are the reasons that could explain the invasive success of Guanapo individuals over Turure fish? During the course of my PhD I looked at different aspects of guppy biology in search for population differences between fish belonging to the two drainage systems that would help to explain the invasive success of Guanapo fish in the Turure and the vast reduction of the native genotype. Because the original Turure genotype is almost extinct by now, I used a closely related population from the Oropuche instead for my experiments. Because populations within drainage systems share most of their genetic material, I expect fish to show the same or very similar traits.

As mentioned earlier, guppies belonging to different Trinidadian drainage systems show a large amount of genetic variation. Together with their overall ability to evolve quickly and adapt to ecological changes in only a few years, this species provides scientists with an important natural experiment repeated in every single river that is inhabited by guppies across Trinidad, thereby giving insight into processes driving evolution and speciation. Translocations of individuals for scientific reasons and the possible resulting invasion of populations belonging to distant genetic groups endanger this experiment and the diversity found within the system. Therefore it will be advantageous to investigate and better understand the processes that play a role in the invasive success of guppy populations in Trinidad, and develop arguments that hopefully will reduce the ongoing translocations of guppies across river and drainage system borders. Ultimately, two important questions shall be answered. First, how behavioural and genetic interactions between populations can determine the outcome of biological invasions and second, if and how such invasions contribute to the loss of biodiversity.

Chapter content

Chapter 2 gives an overview of Haskins's introduction and explores the genetic consequences for both native and invasive population in the wild. It also investigates if and how far the invasive front has moved downstream since the last time genetic samples were taken (2001, see Russell 2004).

Chapter 3 concentrates on the influence of male mating behaviour and familiarity on the potential invasive success of a guppy population. I asked if males display any mating preferences for females of their own population or do not distinguish between population origin. A further experiment explored potential differences in dominance behaviour between males belonging to different populations and how this could influence the mating success of individuals. The general ability of male guppies to become familiar with their mates was also explored and compared to the ability of females to become familiar with schooling partners.

In Chapter 4 I examined the role female guppies can play during an invasion event and investigated behaviours that could help to increase or decrease the speed with that two populations mix. I looked at the ability of females to distinguish between fish of different populations with varying relatedness to the choosing female and at the overall willingness of females to mate and therefore exert mate choice.

Chapter 5 focuses on life-history patterns of populations and the consequences of population mixing for offspring behaviour. Fish from the Guanapo, Haskins's source population, and Oropuche fish that are closely related to the original Turure population, were crossed and several life-history parameters concerning offspring production examined. Later on, the schooling abilities of mixed newborn fish were tested and compared to those of unmixed fish of several populations across Trinidad.

In Chapter 6 the influence of population mixing on the distribution of alleles was explored in a mesocosm experiment where individuals of two populations from the Guanapo and the Oropuche were mixed in different proportions. This was done to repeat the translocation event from the wild in controlled laboratory settings and to distinguish between effects of population origin and the importance of propagule size for the outcome of an invasive event between Caroni and Oropuche fish.

Chapter 7 finally discusses splitting of the guppy into two different species, *Poecilia reticulata* and *P. obscura* is justified and useful, and therefore combines the results of all relevant experiments of my PhD as well as findings in the literature.

In Chapter 8 I discuss my data in the light of the invasive success of the Guanapo guppies in the Turure and explain how individual behavioural decisions, such as the choice of mating partner, can influence the probability of a successful invasion. I also address general behaviours and traits that could increase or decrease the speed of population mixing after an invasive event even in the absence of obvious behavioural, genetic and life-history differences between the two studied populations.

Chapter 2 Re-sampling and genetic analysis of Turure fish

Abstract

Around 50 years ago, C.P. Haskins introduced guppies from the Guanapo into the Turure, thereby crossing drainage system borders. Here they established a viable population and started mixing with the native Turure fish. Several studies for far have shown that thereby the Guanapo individuals populated vast stretches of the Turure and significantly reduced the proportion of the original Turure genotype. Due to a large amount of genetic differenciation between guppies belonging to the Caroni and Oropuche drainage systems, several authors have suggested that they show the early stages of speciation. This process might now be threatened by the population mixing. One aim of my PhD was to re-sample the Turure to see if both populations have finally reached a stable equilibrium point and coexist next to each other, as was hypothesised before (Becher & Magurran, 2000), or if the invasion of Guanapo guppies is an ongoing process. Here I present data that show that even after this long time interval Guanapo fish are still spreading downstream, thereby leading to a reduction of the original Turure gene pool. These results are in line with the outcome of former studies. In the time that passed between the last investigation before I re-sampled the Turure, the invasive front has moved downstream considerably. Today it has probably passed the Turure's confluence with the Quare many kilometres downstream of the introduction site and is likely to have reached adjoining rivers such as the Quare or even the Oropuche. This means that both populations have not reached a stable equilibrium point where they coexist next to each other, but suggests that the invasion of Guanapo fish and the subsequent reduction of the native Turure population is an ongoing process. I will discuss the consequences of Haskins's introduction for the original Turure population as well as for the overall genetic diversity between different rivers and drainage systems in Trinidad. Furthermore, the impact of gene pool mixing will be considered in the light of biodiversity and the possibly speciation process occurring between Caroni and Oropuche populations.

Introduction

One of the important steps of becoming invasive is based on the ability of translocated individuals to establish a viable population and spread in their new environment. This process is likely to be influenced by the number of individuals released. If the initial population size is small and going through a severe bottleneck, the loss of genetic variability can be very high (Sakai et al., 2001). The loss of many low frequency alleles, mainly by genetic drift, is directly dependant on bottleneck size and can reduce a population's genetic variability to a point that makes it hard to quickly adapt to the new environment this population possibly faces. The amount of reduction in heterozygosity is not only influenced by bottleneck size but also by the speed of population growth after the bottleneck occurred. In the case of a small bottleneck, heterozygosity is likely to stay high if the population increases quickly in size afterwards (Nei et al., 1975). However, a recent analysis of 43 successful aquatic invasions showed evidence that reduced genetic diversity in invasive species is not as widespread as previously thought. High numbers of introduced individuals together with multiple introductions of members of the same source population or species reduced the risk of founder effects (Roman & Darling, 2007).

After a population has successfully adapted to local conditions and has reached a certain density, it can start to spread out across its new habitat. As was reported by Arim et al. (2006), new sites can only be colonised after local population growth and the production of new propagules of invading individuals. Repeatedly investigating the range of a non-native population through time and space to notice any form of range expansion is therefore necessary to draw conclusions about its invasive status. At the same time it is important to examine the impact an alien population has on its new environment in terms of ecology, genetics and behaviour. A negative influence in any of these fields would finally justify calling a population invasive. Beside the threat of predation or competition for resources, an important impact invasive species can have on native populations they encounter, is the possibility of hybridisation between formerly separated groups. If viable hybrid offspring are produced that later backcrosses with one or both parental populations, then introgression may threaten the genetic distinctiveness of populations or species. If, on the other hand, hybridisation leads to the production of sterile offspring, the wasted reproductive effort can endanger population survival, especially if population size is small (Rhymer & Simberloff, 1996).

For my PhD, I mainly concentrated on one particular translocation event, namely Haskins's introduction. More than 50 years ago, in 1957, 200 guppies (approximately half of them female) from a high predation habitat in the lower Guanapo, were transferred by C.P. Haskins to the upper Turure (Shaw et al., 1992). The place of introduction was previously guppy-free and contained only one other fish species, *Rivulus hartii*, a minor guppy predator (Magurran et al., 1992). A barrier waterfall isolated this part of the Turure from the lower parts of the river that were inhabited by a native guppy population (see Figure 5). Nevertheless, individuals of Guanapo ancestry started invading the Turure and thereby mixing with the native Turure population. With this transplant experiment, Haskins not only crossed stream borders but also the border of the two main drainage systems found in the Northern Mountain Range of Trinidad, the western Caroni drainage (Guanapo, Haskins's source) and the eastern Oropuche drainage (Turure, Haskins's introduction HI, see Chapter 1), thereby bringing populations into contact that had been separated for up to two million years (Ludlow & Magurran, 2006). Populations belonging to both drainage systems show a remarkable genetic diversity, that is between 3.9 and 5.6% mtDNA variation (Fajen & Breden, 1992). Carvalho et al. (1991) reported in a study using allozymes, that the genetic diversity based on Nei's gene diversity analysis was 66% due to differences between drainage systems. Guppies from these two drainages might well represent the early stages of the speciation process, as was suggested by Ludlow & Magurran (2006) and Russell & Magurran (2006). Bringing fish belonging to different drainage systems into secondary contact can therefore give interesting insights into the importance and order of the development of separating traits between populations. On the other hand, it can easily reverse the speciation process, and, in the event of population mixing, lead to the loss of distinctiveness between rivers. Should the introduced population turn out to be invasive and drive the native population extinct, the loss of a population, reproductively isolated or not, would also equate to a loss of biodiversity (Convention on Biological Diversity, 1993).

Figure 5: The barrier waterfall that separates the site of Haskins's introduction (directly to the left of the picture) from the rest of the Turure river. Unidirectional movements of guppies down the waterfall are possible while upstream movements are usually not.

Thirty seven years after the introduction took place, genetic investigations revealed that the newly arrived Guanapo fish had managed to establish a viable population and colonised the river parts above the barrier waterfall (Shaw et al., 1992). But they also overcame the natural barrier of their habitat, perhaps with the help of flooding events that are common throughout the wet season, and started to spread below the waterfall, thereby coming in contact with the native Turure population. The spread of Guanapo fish was probably increased by the tendency mostly of male guppies to travel longer distances (Croft et al., 2003). In a study by Shaw et al. (1991) using allozymes, it became apparent that fish from the upper Turure were genetically more closely related to fish from the Caroni drainage than to other populations from the Oropuche drainage, where this river is found. A follow-up study sampling at several different sites at the Turure, Guanapo and Oropuche rivers confirmed that fish from the upper and middle parts of the Turure (Introduction site, Cumaca Bridge, Valencia Road) exhibited nearly exclusively (1st two sites) or a high number (Valencia Road) of Guanapo alleles. Fish from a downstream site of the Turure close to its confluence with the Quare on the other hand only showed allele frequencies very similar to that of other Oropuche rivers (Shaw et al., 1992) which means that at this time the invasive front had not yet reached the end part of the river. In this population, mean heterozygosity (0.028 compared to 0.049 in the source population) was reduced. The only slightly reduced mean number of alleles per locus (1.2 compared to 1.4 in 25 loci) in the founding population is probably explained by fast population growth after the introduction which prevented the loss of many low frequency alleles (Carvalho et al., 1996). That this newly established population still contained enough genetic diversity for fast adaptive evolution to work on morphology, life-history and behaviour was demonstrated by Magurran et al. (1992). The authors of this study showed that the schooling tendency of fish was significantly reduced above the waterfall but not in the middle stretches of the Turure. This means that the individuals of this population adjusted their behaviour towards the low predation pressure they were facing after the introduction above the barrier waterfall but increased the time they spent schooling with the once more raising predation level in the middle parts of the river. Males living above the waterfall also displayed more and larger colour spots than Guanapo males from the (high predation) source population. Again, this correlates with the different predation regime the fish face in their new environment (Magurran et al., 1992).

In their study using 25 allozymes, Shaw et al. (1992) found that native Turure fish had genetically contributed to the present population below the waterfall but could not ascertain if the alleles belonging to the original genotype represented a strongly reduced native stock or resulted from inter-breeding. Later studies revealed that only 12% of all genotypes occurring in the parts downstream of the barrier waterfall of the river Turure belonged to the original Oropuche matriline (Becher & Magurran, 2000). This examination was based on an investigation of the mitochondrial NADH dehydrogenase subunit 2 (NADH2) gene. One year later, Russell (2004) sampled several sites along the Turure, including the confluence with the Quare, again using the NADH2 gene for analysis. 60% of all fish expressed the original Turure haplotype at this site while 40% of examined haplotypes were of Guanapo origin. Another study conducted by Suk & Neff (2009), this time using microsatellites, could again show that significant gene flow must have taken place from the introduced population in the upper Turure to the lower parts of the river. In 2010, Willing et al. looked at the population history of guppies using SNPs and affirmed that fish from the middle stretches of the Turure clustered with populations from the Caroni drainage rather than the Oropuche drainage. However, this study only investigated one site along the river and therefore does not give a more detailed insight into the current extent of the invasion.

So what is the situation in the Turure almost 20 years after the invasion's discovery? Is the colonisation of this river by fish of Guanapo origin an ongoing process that will eventually end in the complete replacement of the native population, or did the observed process reach a stable equilibrium point that allows individuals of both populations to co-exist without threatening each others' survival? This latter possibility was suggested by Becher & Magurran (2000). To examine the current state of the Turure population and the geographic extent of the invasion of Guanapo fish in the Turure was one main aspect of my PhD. These results were then set in context with the outcomes of former studies, firstly to follow the invasion of Guanapo fish in the Turure through time to investigate the speed of spread and geographic distances overcome by this population, and secondly, to focus on the threats for genetic variability between drainage systems and the loss of biodiversity in the Turure. Because populations belonging to the Caroni and Oropuche drainage system are thought to show the early stages of speciation, the observed mixing of gene pools can reverse this development, another circumstance that makes a repeated investigation of the Turure and adjacent rivers important.

For this reason I re-sampled the Guanapo at the site of Haskins's source, several sites along the Turure including the introduction site and the confluence with the Quare, and two sites at the Oropuche river. I then analysed my genetic data in cooperation with the Max Planck Institute for Developmental Biology in Tuebingen, Germany, where I tested the hypothesis that Guanapo fish had continued to spread downstream since the last investigation and further reduced the natural Turure genotype. For this purpose, I used SNP markers specific for Guanapo and Oropuche (Turure) fish to examine the current genetic composure of populations from different sites along the Turure. SNPs, or single-nucleotide polymorphisms, are a variation in the genetic sequence of two members of the same species/population or a chromosome pair within an individual, where a single nucleotide differs at a particular locus. In most cases, SNPs only have two alleles. An advantage for genetic mapping and sequencing is the high abundance with which SNPs occur throughout the entire genome (Berger et al., 2001). In contrast to microsatellites that can have multiple alleles, it will be easy to calculate the proportions of alleles from each parental population based on the two alleles per SNP. They are therefore very useful to analyse closely related populations/species and can be used to answer the question if Guanapo guppies are still spreading down the Turure and to what extend the original population still exists. I also hypothesise to find a gradual rise in the amount of SNPs belonging to the native Turure population between different sites of the Turure while moving downstream and further away from the introduction site.

Methods

Fish used for the genetic analysis were caught at several sites across the Northern Mountain Range, Trinidad, using a seine net and hand nets. Ten adult fish per site consisting of males and females were killed with an overdose of Tricaine Methanesulfonate immediately after catching, transferred into 96% Ethanol and stored in a freezer. Only three adults could be caught at the Toco Main Road site of the Oropuche.

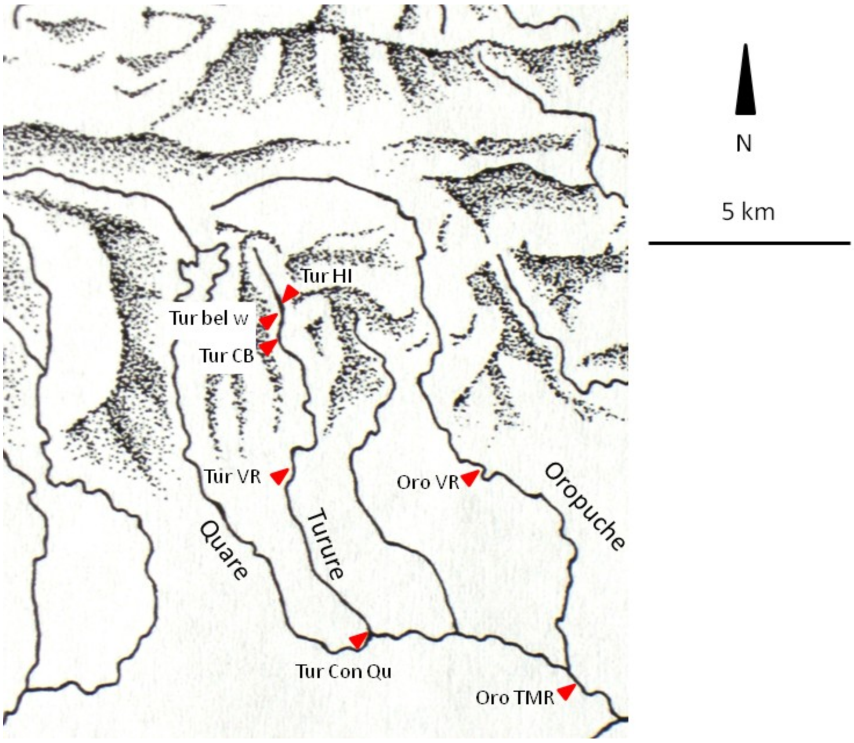


Figure 6: Detail of the Northern Mountain Range map showing the Turure and Oropuche. Red arrows indicate the sites where fish collection took place. HI: Haskins's introduction, bel w: below waterfall at introduction site, CB: Cumaca Bridge, VR: Valencia Road, Con Qu: Confluence with Quare, TMR: Toco Main Road. Not shown: Haskins's source, Guanapo.

Sampling took place in June 2009 except for the Introduction site and directly below the waterfall (both Turure) where no fish were present at that time. Fish from these two sites were collected in June 2010 (see Figure 6 for a map showing all collection sites). The grid references of all sites are listed in Table 1.

Table 1: Grid references of sites used to catch fish for the re-sampling of the Turure. The drainage system each river belongs to is indicated if relevant.

Population	Grid reference number	Site name	Drainage system
Guanapo	PS 913 765	Eastern Main Road (Haskins's source)	Caroni
Oropuche	QS 042 788	Valencia Road	Oropuche
Oropuche	QS 078 719	Toco Main Road	Oropuche
Turure	PS 997 817	Haskins's introduction	
Turure	PS 997 817	Below waterfall	
Turure	QS 003 809	Cumaca Bridge	
Turure	QS 002 784	Valencia Road	
Turure	QS 023 738	Confluence with Quare	

In June and July 2010, all fish samples were taken to the Max Planck Institute for Developmental Biology in Tuebingen, Germany, for genetic analysis where population specific markers already existed due to a genome-wide SNP study of guppy population history in Trinidad and Venezuela (Willing et al., 2010).

DNA was isolated using the DNeasy Blood & Tissue Kit from Qiagen. 4.5 mg of the tail muscle tissue per fish were cut into small pieces and transferred into a 1.5 ml microcentrifuge tube. The tissue of five adult fish was pooled in one tube, leading to two tubes per site, each containing 22.5 mg of tissue. 180 µl ATL buffer was added per tube, followed by 20 µl proteinase K. All samples were vortexed and incubated overnight at 56°C on a rocking platform. The next morning, tubes were vortexed for 15 seconds before 200 µl AL buffer was added. The samples were vortexed again, 200 µl 99% ethanol was added and the samples thoroughly mixed. This mixture was then pipetted into a DNeasy Mini spin column in a 2 ml collection tube and centrifuged at 8000 rpm for 1 min. Both flow-through and collection tube were discarded and the spin column was placed in a new 2 ml collection tube. 500 µl AW1 buffer was added and the samples centrifuged at 8000 rpm. Flow-through and collection tube were discarded again, a new collection tube used and 500 µl of AW2 buffer added. The samples were then centrifuged at 14000 rpm for 3 min. and flow-through and tubes discarded. The spin columns were transferred to new 1.5 ml microcentrifuge tubes, 200 µl AE buffer was added for elution and samples incubated for 15 min. at room temperature. Afterwards the tubes were centrifuged at 8000 rpm for one min. This final step was repeated once, this time using only 100 µl elution buffer and fresh microcentrifuge tubes to increase the DNA yield.

After isolation, the DNA concentration of all samples was measured and the two samples per site combined. Water was used to dilute each DNA sample to an end-concentration of 25 ng/µl, each containing the DNA of 10 animals. All samples were stored at 4°C.

For DNA amplification the following PCR protocol was used:

Template	2.5 µl [25ng/ µl]
H_2O	4.4 µl
PCR buffer	1 µl [10x Thermobuffer (NEB B9004S)]
dNTPs	1 µl [2mM]
Taq polymerase	0.1 µl [5000 U/m (NEB M0267L)]
Fw-primer	0.5 µl [10 pmol/ µl]
Rev-primer	0.5 µl [10 pmol/ µl]

All PCR amplifications run in Peltier Thermal Cyclers pTC-200 from Bio-Rad under the following conditions with a reaction volumes of 10 µl for each PCR:

Initial denaturation	96°C for 3 min.	
40 cycles of		
	Denaturation	96°C for 30 sec.
	Annealing	56°C for 30 sec.
	Extension	68°C for 30 sec.
Final extension		68°C for 5 min.

The markers used in this experiment were developed by Tripathi et al. (2009) for a complete linkage map of the guppy. Before genetic analysis, 28 markers were selected that were likely to distinguish between Guanapo and Oropuche populations, based on the study on guppy population history by Willing et al. (2010). Out of these, only 4 produced clear results in pure Guanapo and Oropuche fish (see Chapter 6 for further details), therefore only these four markers were used in this experiment.

Table 2: Sequences of forward and reverse primer for each marker that produced clear results in pure Guanapo and Oropuche fish. The linkage group of all markers is shown as well. (Sequences from: Tripathi et al. (2009), Data supplement no 2 & 3).

Marker	Forward primer	Reverse primer	Linkage group	GenBank accession no
M 654	TTTACATCCCACACCTTCAATC	TGTGAATGCTCAACCAAACTC	LG02	FH890962
M 978	GGCCCATCTGGATAGAGTG	TTAACATCTTGTGGAGTTATGCTG	LG23	FH893187
M 1033	AATCAGTCAGTTTACAAAGTCTGGTC	TGGAGACGCAATCAGTGG	LG10	FH893635
M 1042	ACAACATTCTATGGGTGAAAGAAG	GCTCATTGTAAGGGTAGTGTGC	LG09	FI903158

Following the PCR, the product was electrophorised on a 1.5% Agarose/ethidiumbromid gel and 4% TAE buffer. Two µl PCR product were therefore mixed with 5 µl Blue juice (0.2% Bromphenol blue, 25% Glycerol, in H_2O) and loaded on the gel. One run lasted approximately 20 to 30 min. at 120 Volts. The amplified DNA was stored at - 20°C.

Subsequently, excessive primers were removed from the product using a mixture of 1.2 µl Exo I E.coli and 1.2 µl FastAP, a thermosensitive Alkaline Phosphatase. Following digestion which took approximately 30 min., the mix was prepared for sequencing:

Template	1 µl [25ng/µl]
H_2O	6 µl
Sequencing buffer	2 µl [5x Sequencing buffer (AB 4305603)
Fw-primer	0.5 µl [10 pmol/µl]
BDT	0.5 µl [Big Dye Terminator v3.1 from Applied Biosystems Cat. no. 4336921 (undiluted)]

The following sequencing program was used for amplification:

Initial denaturation	96°C for 30 sec.
40 cycles of	
Denaturation	96°C for 20 sec.
Annealing	50°C for 10 sec.

Extension 60°C for 4 min.

The amplified product was then sequenced, which took place using a sequencer from Applied Biosystems ABI 3730 XL. The final DNA sequences for each marker were analysed with the help of the pregap4 and gap4 software from the Staden-package (GAP v 4.10) to investigate all SNPs that were informative between the Guanapo and Oropuche (Turure) populations.

Results

The four markers used in the genetic analysis of the re-sampled Turure fish resulted in 15 meaningful SNPs that could be analysed. These SNPs clearly distinguished between Guanapo and Turure (Oropuche) origin (see Table 2).

Table 3: Meaningful SNPs within the sequences produced by 4 different markers. The base changes within each locus are indicated, starting with the nucleotide found in Oropuche fish.

Marker	SNP	SNP	SNP
654	110 (T/A)	111 (T/C)	161 (G/C)
	356 (T/C)	370 (A/G)	515 (A/G)
	547 (G/A)		
978	152 (A/C)	194 (A*/GT)	220 (A/C)
	267 (A/T)	380 (C/T)	
1033	215 (T/C)	217 (T/G)	
1042	173 (C/T)		

The percentage of the original Turure allele compared to the introduced Guanapo genotype was determined for each SNP by estimating the proportion of the area of both peaks in percent in case two alleles were present (see Appendix I for an alignment example for a SNP at the marker 978). The mean results for all SNPs per site were then used to investigate the genetic change that took place in the Turure after the introduction of Guanapo fish and the following invasion of the middle and lower parts of the river. As can be see in Figure 7, SNPs from fish collected at Haskins's source at the Guanapo differ remarkably from the ones typically found in the Oropuche and are nearly homozygous for the 15 chosen loci (Mean amount of SNPs identical with the nucleotide typically found in Oropuche fish:3.57% ± 4.97 S.D.). The number of SNPs displaying the original Oropuche (Turure) nucleotide is slightly higher at the introduction site in the Turure and directly below the barrier waterfall (both sites: 8.57% ± 9.49 S.D.). One kilometre further downstream, at Cumaca Bridge, the percentage of SNPs displaying the native state has doubled (15.00% ± 15.57 S.D.) and nearly is the same in Turure fish caught at Valencia Road (12.14% ± 11.88 S.D.). At the confluence of Turure and Quare, nearly 40% of tested SNPs belong to the original genotype (38.18% ± 9.82 S.D.). In comparison, 98% of Oropuche fish collected at Valencia Road displayed a Oropuche typical nucleotide at each SNP (97.78% ± 6.67 S.D.). This value was slightly lower at Toco Main Road (91.25%, ± 16.42 S.D.), but only three fish could be used for genetic analysis at this site.

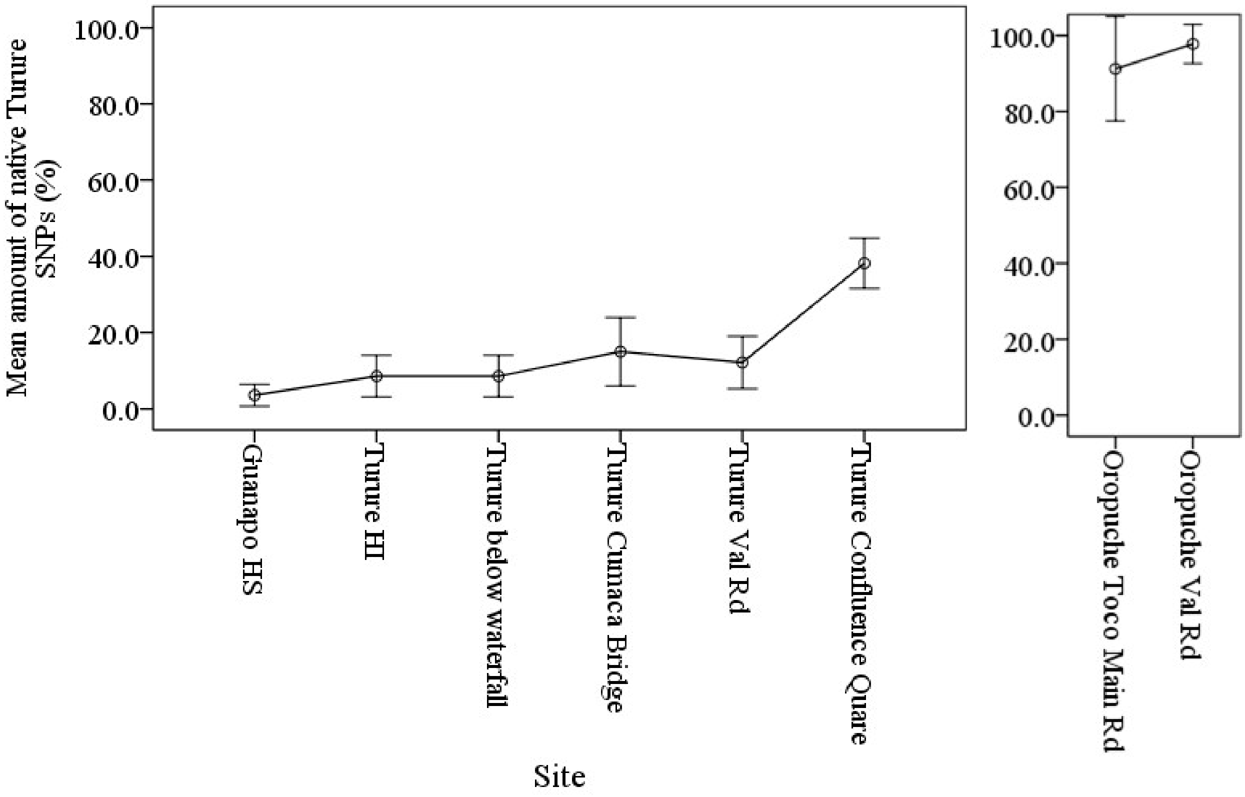


Figure 7: The amount of native Turure and Oropuche genetic sequences compared to the invading Guanapo genotype. The percentage of the Oropuche state of all SNPs per site was taken in order to illustrate the change of genetic composition across the river Turure. Two sites at the Oropuche were sampled to compare the Turure results to a likely original genotype. N = 10 fish per site, except Oropuche Toco Main Road: n = 3. HS: Haskins's source; HI: Haskins's introduction. Error bars represent 95% CI.

Discussion

More than 50 years after their first introduction by C.P. Haskins, the invasion of Guanapo fish in the Turure is an ongoing process. The genetic analysis of fish sampled at several sites along the Turure river supported the results of earlier studies (e.g. Shaw et al., 1991; Shaw et al., 1992) that found the original Turure genotype below the introduction site and in the middle stretches of the river vastly reduced and replaced by the invasive Guanapo population. However, my results showed that the invasive genotype has spread even further in the meantime, thereby affirming my hypothesis. By now the invasive front has reached and probably passed the Turure's confluence with the Quare, many kilometres downstream of the introduction site. Only 40% of analysed SNPs at this place still resembled the original genotype whereas the other 60% came from fish with Guanapo ancestry. The fact that fish from both populations were not entirely homozygous for each SNP is likely to have introduced some variation to my data, but the general trend was still clearly observable.

So far, several different methods of analysis [allozymes (Shaw et al. (1992), mtDNA: NADH2 gene (Becher & Magurran, 2000) and microsatellites (Suk & Neff, 2009)] have been used to examine the genetic composition of fish found in the Turure. All of them showed similar results with regard to the existence of population mixing within this river. There was, however, a difference in the distance from the introduction site where the invading genotype could still be found. Therefore it is possible to follow the spread of Guanapo genes in the Turure population throughout the years. If comparing my own results from fish collected in 2009 and 2010, with a former study done by Stephen Russell in 2001 (Russell, 2004), it becomes obvious that in this relatively short amount of time the Guanapo genotype still spread downstream and displaced the original Turure genotype even further. 60% of fish collected at the Turure's confluence with the Quare still expressed a native Turure genotype in 2001, while 8 years later only 40% of all analysed SNPs belonged to this original population (see Figure 8 for a comparison of different studies). However, in contrast to my own study, Russell used mtDNA for genetic analysis, so results might not be completely comparable. Still, the general trend is obvious. This not only means that in the past 20 years after the first discovery of the introduction Guanapo fish spread further downstream the Turure and displaced the native population, but also that it is very likely that by today introduced fish entered the Quare and possibly even the lower reaches of the Oropuche, a river that is joined by the Quare some kilometres further downstream. It also implies that both populations have not reached a stable equilibrium point as was suggested by Becher & Magurran (2000), but that an introduction experiment carried out to better understand the ability of guppies to quickly adapt to new environments may ultimately lead to the disappearance of an entire population.

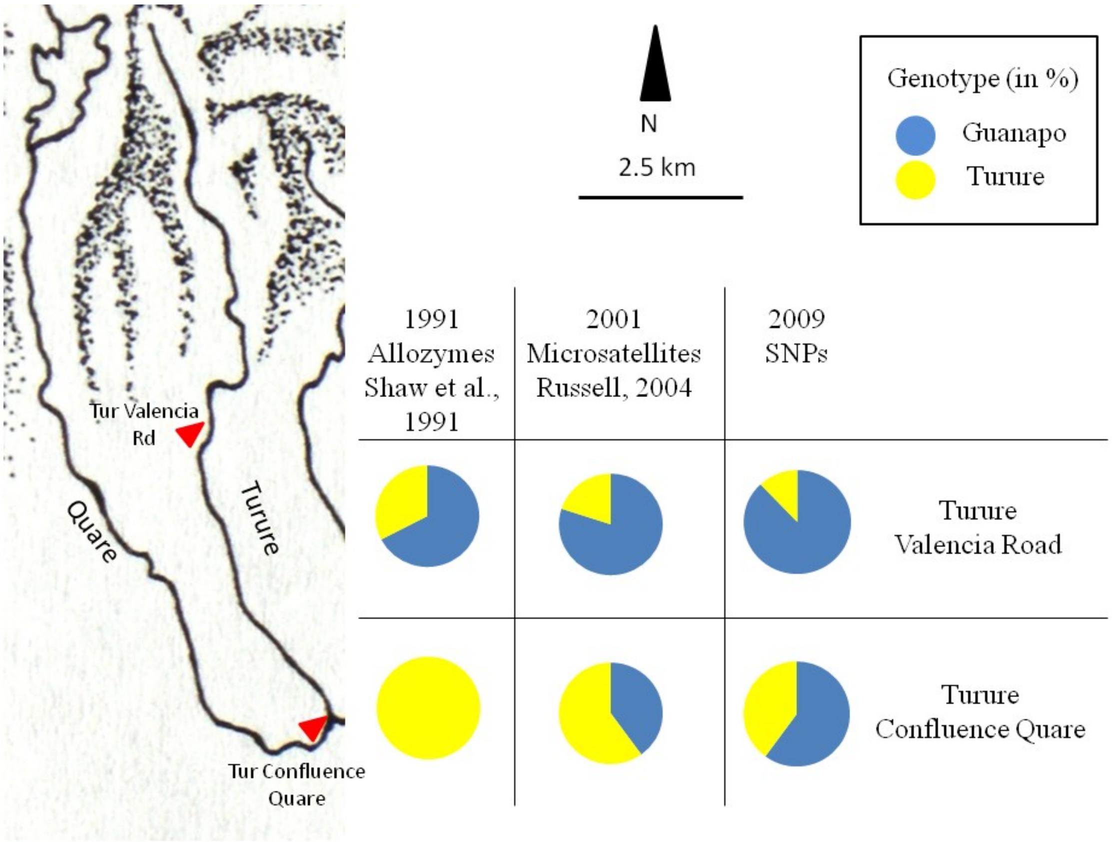


Figure 8: Comparison of the continuing spread of Guanapo fish and the decline of the original Turure genotype through time. Different methods were used for analysing the genetic composure of fish in the studies shown here, and the results might therefore hard to compare. However, the general trend of the increase in Guanapo alleles within the Turure is obvious.

This development not only endangers the survival of the native Turure population which is now nearly extinct, but also the genetic diversity displayed by populations belonging to the Caroni and Oropuche drainage system. Guppy populations that face the same ecological parameters, especially predation regime, show similar adaptations in behaviour, life-history traits and morphology across drainage systems. However, no genetic exchange took place between these systems for up to two million years and guppies inhabiting them belong to genetically distinct groups (Ludlow & Magurran, 2006) and display a large amount of between-system genetic variation (e.g. Fajen & Breden, 1992). This has led several authors to suggest that the guppy might be on its way to speciation (Ludlow & Magurran, 2006; Russell & Magurran, 2006). Having a set of populations that display the early stages of reproductive isolation can be very useful for examining the development and order of isolating traits that arise and finally lead to complete separation. This potential speciation event is now influenced by population mixing as well as the continuing spread of Guanapo alleles in the Turure and possibly also in adjacent rivers. The reduction of distinctiveness between drainage systems resulted in the loss of genetic differences between emerging separate genotypes and could reduce the likelihood that *Poecilia reticulata* will eventually split into two species. Because of mostly uni-directional movement downstream and the existence of barrier waterfalls it is unlikely that the spread of Guanapo genes will affect every river and tributary in the Oropuche drainage system. However, the reduction of differing genetic raw material for selection or drift to work on could set back or at least slow down the process of speciation.

In the long run, the extirpation and possible extinction of the native population as well as the threat of an ongoing mixing of the invaders with populations living in rivers close to the Turure will lead to another example of biotic homogenisation. In the case of Haskins's introduction, this does not so much mean the replacement of a local specialist by an cosmopolitan generalist but more the loss of a diverse range of different genotypes while a single population becomes more abundant. It is unlikely that this homogenisation of gene pools will endanger the survival of guppies as a species, but some of the diversity guppies became famous for in the first place will be lost because of a scientific interest to understand exactly this diversity. Deliberate introduction experiments in Trinidad and elsewhere in the world are ongoing. Therefore scientists should ask themselves whether translocation experiments are really necessary to answer a particular question, given the possible implications for the study species, ecology and biodiversity itself. With the knowledge of possible consequences of this sort of experiments, some of them shown in this study, great care should be taken with any kind of species translocation. In case of any remaining doubts concerning possible future consequences, scientists should consider alternative solutions such as lab experiments and modelling before risking a species'/populations' existence.

To examine exactly where the invasive front of Guanapo fish lies today, and if any rivers other than the Turure are affected by the mixing of gene pools, sites upstream and downstream of the Quare, that are close to its confluence with the Turure, should be sampled and genetically analysed. Equally, sites further downstream where the Quare joins the Oropuche might already be influenced by the invasive population and therefore would be interesting to observe. Furthermore, it would be helpful to identify more markers and SNPs that clearly distinguish between Guanapo and Oropuche (or Quare) fish, to get a more detailed result in places where population mixing is an ongoing process at the moment. Analysing individuals instead of groups of 10 might help to increase the resolution in locations that are currently reached by the invasive front.

Chapter 3 Male mate choice based on familiarity and genetic distance to females

Abstract

Male mate choice and general mating behaviour will have an impact on the mixing of two populations in an invasive event. In case of the guppy, *Poecilia reticulata*, male mating behaviour is particularly important due to the ability of males to forcefully overrule female choice. In this chapter, I asked how familiarity and genetic distance between male guppies and their mates influence their mating decisions and what consequences this can have for population mixing after an invasion. I tested the general ability of male guppies to become familiar with females in a mating context, a behaviour that would enable the selection of new mates a male has not already mated with. Contrary to my expectations, male guppies did not prefer unfamiliar females as mating partners, even after spending up to 18 days with them in the same tank. I will discuss the differences between male and female ecology and the consequences this has on the importance and development of short term familiarity in a mating context. Further experiments looked at the ability or the interest of males to distinguish between females of varying relatedness/genetic distance. A preference of males for females of their own population would prevent fast population mixing in an invasive event, but males did not seem to distinguish between population origins of females. Next to mate choice, male dominance behaviour and an increased ability to secure matings could influence a population's invasive success. Again, contrary to expectation, the presence of male guppies from the Guanapo did not change the mating behaviour of Oropuche fish, and aggression between males was hardly detectable. Dominance behaviour of Guanapo males over the native Turure males is therefore unlikely to be a reason for increased success of the Guanapo genotype in the Turure. I discuss how the absence of population preferences in male mate choice could have contributed to the population mixing in the Turure by overruling female choice with forced copulations despite any behavioural superiority of Guanapo males.

Introduction

Male guppies express many morphological and behavioural traits that set them apart from females of the same species. A male almost stops growing after he reaches sexual maturity and therefore stays significantly smaller than an average female (Reznick & Miles, 1989). In contrast to the cryptic coloured females, male guppies often express a broad range of colouration, mainly consisting of black, orange and yellow spots and sometimes areas of green, white and iridescent blue (Houde, 1997). With the help of these specific colour patterns most males can be individually distinguished. There exists a great amount of colour variation between fish of high and low predation populations within the same river, with males from low predation sites generally being more colourful and conspicuous than their high predation counterparts (Endler, 1980). Differences in the amount of colouration also exist between populations belonging to different rivers across Trinidad (Houde, 1988). Unlike females, male guppies only spend a limited amount of time per day foraging and are mostly engaged in courting females and finding mating partners (Magurran & Seghers, 1994). To maximise their reproductive output, male guppies have to copulate with as many females as possible and are therefore constantly in search of new mates (Kelley et al., 1999). They trade off the relative safety of staying in a school with familiar fish, against switching between groups, thereby encountering more females (Griffiths & Magurran, 1998). Male guppies are likely to move considerable distances (Croft et al., 2003), a behaviour that could contribute to a mixing of populations after an invasive event. They exhibit less pronounced anti-predator behaviours than females (Magurran & Seghers, 1994). Together with their conspicuous colouration this increases the predation risk to male guppies compared to females (Rodd & Reznick, 1997).

The predation regime experienced by a population not only influences the amount of male colouration but also the mating tactic used by males. Male guppies employ two different mating strategies and can switch between them according to context. They can court receptive females using sigmoid displays. During a display, a male bends his body in a S-shape and quivers his fins. This type of courtship behaviour can last several seconds and allows female choice. The other mating tactic frequently used is a sneaky mating attempt where a male tries to force an unreceptive female to copulate by thrusting his gonopodium at her genital pore (Houde, 1997). Evans et al. (2003) showed that these forced copulations can lead to sperm transfer. After mating successfully with a female, male guppies display several jerky swimming motions, a behaviour that normally indicates the successful transfer of sperm (Liley, 1966). Males facing high predation regimes are more likely to employ sneaky mating tactics and females living in these places can receive up to one sneaky mating attempt per minute (Magurran & Seghers, 1994). After a mating, several postcopulatory effects can influence the amount of offspring a male fathers. More ornamented males seem to be better sperm competitors and produce superior ejaculates (Pilastro et al., 2002). However, a recent study revealed that males that favoured sneaky mating attempts over sigmoid displays were generally less colourful but had faster swimming sperm than males that mostly display towards females (Evans, 2010). The quality of a male can have several direct consequences for offspring fitness. For example, the offspring of more colourful males are better at evading capture (Evans et al., 2004). Male size also plays a role in offspring quality. Size has a father-son heritability, large males produce offspring with higher growth rates and the daughters of large fathers produce more offspring (Reynolds & Gross, 1992).

Choosing the right mating partner can therefore be essential for reproductive success, and mate choice is observed in both sexes of *P. reticulata*. One aspect of behaviour found in guppies that could influence their choice of mating partner is their ability to become familiar with conspecifics. Familiarity is a form of social recognition found in many animal species and it has repeatedly been shown that fishes have the cognitive capacity to distinguish between familiar and unfamiliar individuals. For example, Brown & Colgan (1986) discovered that one year old bluegill sunfish (*Lepomis macrochirus*) spent significantly more time with familiar rather than with unfamiliar conspecific individuals. These choices can be subtle - three-spined sticklebacks (*Gasterosteus aculeatus*) discriminate between conspecifics from their own habitat and fish of different habitats within the same stream or lake with a resolution of less than 200 m using self-referent chemical cue matching (Ward et al., 2007). Being able to recognise individual identities or certain common characteristics like chemical cues associated with habitat members is advantageous in a range of contexts including schooling or foraging. Chivers et al. (1995) discovered that groups of familiar fathead minnows (*Pimephales promelas)* form more cohesive schools and engage in more anti-predator behaviour than unfamiliar groups; both behaviours are likely to increase the survival rate of fish in the presence of predators. Groups of familiar three-spined sticklebacks are better at exploiting a food resource than unfamiliar groups are (Ward & Hart, 2005). However, these effects are not universal. In a recent study Kydd & Brown (2009) showed that captive reared rainbowfish (*Melanotaenia duboulayi*) exhibited a strong tendency to school with conspecifics, but in contrast to wild populations did not display any preference for familiar fish. The authors suggested that this result could be explained by a loss of the ability to recognize individual fish, or the lack of benefits associated with becoming familiar in the absence of predators under captive conditions.

The guppy has frequently been used to examine familiarity and its development. A study by Magurran et al. (1994) found that female guppies prefer to school with other familiar females, and it takes around 12 days for these females to become familiar with each other (Griffiths & Magurran, 1997a). Croft et al. (2004) similarly found that it takes 12 days for familiarity to develop between male guppies in a schooling context. Guppies also choose to associate with the more cooperative of two fish after predator inspection – an outcome that depends on their ability to discriminate individuals. In this context visual cues alone appear to be sufficient (Dugatkin & Alfieri, 1991). Reproduction is another domain in which familiarity is important. Both male and female guppies prefer to mate with unfamiliar mates. Females seem to favour males with novel colour patterns over males with patterns they are familiar with, a behaviour that helps explain the extreme phenotypic variation found in natural guppy populations (Hughes et al., 1999). Males also associate with unfamiliar females over familiar ones after spending six weeks in the same tank (Kelley et al., 1999).

However, although there is now compelling evidence that familiarity recognition plays an important role in mediating social interactions amongst fish, the time needed to establish familiarity and benefits of becoming familiar with other individuals are almost certainly based on the behaviour and ecology of a species or a sex (see Ward & Hart, 2003 for a review). To date it is assumed that in guppies learned recognition of familiar individuals is uniform across contexts (e.g. Griffiths & Magurran, 1997b; Ward et al., 2003) meaning that no sex differences exist and the two sexes respond in the same way.

In the guppy, females tend to form cohesive schools for predator avoidance (Seghers, 1974). Males on the other hand trade off the safety of schools with increased movement between schools in order to mate with as many different females as possible (Griffiths & Magurran, 1998). They also show less site fidelity and may move considerable distances; males are therefore likely to encounter many different schools of females during their lifetime. In 2003, Croft et al. showed that 27.3% of males had left their pool of release after an eight day period, compared to 6.9% of emigrated females.

Unless they are trapped in a pool with the same set of fish for several weeks - a likely scenario in the upper parts of rivers during the dry season - males will not encounter the same females for extended periods of time and therefore may not have either the need or the opportunity to develop any familiarity with them. Because the ecology and behaviour of male and female guppies differ significantly, I predicted that there will be differences in the development and importance of familiarity between the sexes. The speed with which discrimination is achieved is likely to be linked to the benefits that recognition of familiar individuals delivers. This suggests that the rate at which familiarity learning may be acquired is context dependent.

In the first part of this chapter I tested if familiarity between male guppies and potential mates is established at the same rate as learned recognition of school males, that is in 12 days. To do this I examined the same focal males repeatedly every 3 days for 18 days. The development of familiarity on a finer scale was also investigated, testing every day for 6 days to see if males remember and discriminate against females they have recently mated with. Finally, the original experiment by Griffiths & Magurran (1997a) was repeated, to confirm that familiarity recognition amongst females develops as expected and that there has been no shift in behaviour linked to captivity.

The second part of this chapter concentrates on the importance of different degrees of relatedness on the mate choice behaviour of male guppies. Female preferences and the traits underlying female choice are the subject of Chapter 4. Because females are only receptive as virgins and during a short period after giving birth approximately once every month, males should be choosy as well. Ojanguren and Magurran (2004) found that male guppies are able to discriminate between receptive and non-receptive females and direct a larger amount of courtship behaviour towards the former. They also prefer the larger of two females if the size difference exceeds 2 mm (Dosen & Montgomerie, 2004). Males as well as females prefer to mate with unfamiliar partners (Kelley et al., 1999; Hughes et al., 1999), a behaviour that could be important for inbreeding avoidance. At the same time, Endler & Houde (1995) discovered a slight but significant female mating preference for males of her own population. But do males equally differentiate between population origin of female guppies and prefer to mate with fish from their own population or are they more likely to court and try to mate with every female they encounter?

Frequent sneaky mating attempts by males overrule female mate choice with its normally strong preferences for individual male colour patterns and create the basis for sexual conflict. In principle, therefore, invading Guanapo males could mate with Turure females, even if these females prefer males of their own population, and in doing so contribute to a spread of Guanapo genotypes in the invaded places. A second behaviour that could be responsible for the success of Guanapo fish in the Turure River is dominance behaviour or the exclusion of inferior rivals from access to females by dominant males. It is possible that the dominant behaviour of male Guanapo guppies relative to Turure fish is contributing to the observed replacement by the monopolization of mating opportunities and the exclusion of inferior Turure males. Male guppies rarely engage in direct combat, but can try to monopolise females or jockey for the right mating position in the presence of a rival (Houde, 1988). In an early study, Magurran & Seghers (1991) showed that there exists significant variation in the levels of male aggression between different populations of *P. reticulata*. In spite of strong female preferences for colourful males, unattractive but dominant males can gain mating success by excluding other males from mating (Kodric-Brown, 1992). This was the case even if the female did not prefer the dominant male. Population density as well as operational sex ratio influences the amount of male-male competition. High operational sex ratios led to a reduction of male courtship displays and an increase in antagonistic behaviour between males (Jirotkul, 1999a), the same was true for higher population densities (Jirotkul, 1999b). Familiarity between males does also play a role in the observed amount of competition and dominance behaviour. Males familiar with each other showed significantly less antagonistic behaviour whereas unfamiliar males engaged in more aggressive interactions, possibly to establish a dominance relationship (Price & Rodd, 2006). The risk of predation will also decrease male-male competition, but not male courtship displays and mating attempts (Kelly & Godin, 2001).

Here I tested the mating behaviour of male guppies from the Guanapo and Oropuche. In a choice experiment males were allowed to choose between females from their own and from the other population. I expected to either find no difference in choice of mating partner between populations or a preference in Oropuche males for females from their own population, whereas at the same time Guanapo males do not discriminate between population origin. The first outcome could be interpreted as part of a general basis for explaining gene pool mixing after an invasive event whereas the second result would also give a possible explanation for the success of invading Guanapo fish over the native population. In a second experiment, the mating behaviour of males was tested, this time allowing the fish to interact freely. A focal Oropuche male that had access to a

Guanapo and a Oropuche female was either tested with two other Oropuche males or two Guanapo males. Due to possible dominant behaviour of Guanapo males I predicted that in the presence of these males the focal Oropuche male would reduce the amount of mating behaviour directed towards females compared to the presence of males of his own population.

Methods

Male familiarity with females in a mating context

This study was carried out between November 2008 and October 2009 in St Andrews using descendants of guppies from the Lower Tacarigua, Trinidad, that had been kept in the laboratory for several years. Twenty four adult males were individually placed in small tanks (31 x 20 x 21 cm). All tanks were layered with coarse gravel and contained a filter and a plastic plant for cover. Each male was then paired with a virgin but mature female aged between four and six months and was free to court and mate with her. Virgin females are likely to mate indiscriminately with the first male they encounter (Houde, 1988) and male courtship behaviour was frequently observed in the holding tanks. Fish within the same tank will hence forth be called familiar, fish from different tanks, unfamiliar. Tanks were separated with sheets of paper to prevent the animals from seeing each other. Fish were kept on a 12 L: 12 D cycle using 18W fluorescent daylight bulbs. The tank temperature was maintained at 23.5 ± 0.5°C. The water in newly set-up tanks was treated with STRESS COAT® to further eliminate chloride ions and help fish to rebuild their slime coat after handling. All fish were fed with TetraMin flake food once daily in the morning, and on days when experiments were conducted, about half an hour before the first trial. Newborns were fed with TetraMin Baby powder food once a day. In order to raise virgin females, newborn guppies were isolated shortly after birth, transferred into small tanks (31 x 20 x 21 cm) and raised individually. All tanks contained a filter and were layered with gravel. At the age of approximately 3 months, fish were sexed and all females transferred into a tank containing virgin females only, where they were kept until use in experiments. The total body length (TL) of each fish was taken by measuring from the tip of the snout to the end of the caudal fin. Therefore adult fish were either directly measured using a caliper, or were photographed in a small petri dish using a Nikon Coolpix P5100 digital camera and graph paper. Their length was then measured on the computer using ImageJ, a public domain, image processing program. The length of newborn and fish was always measured from photographs.

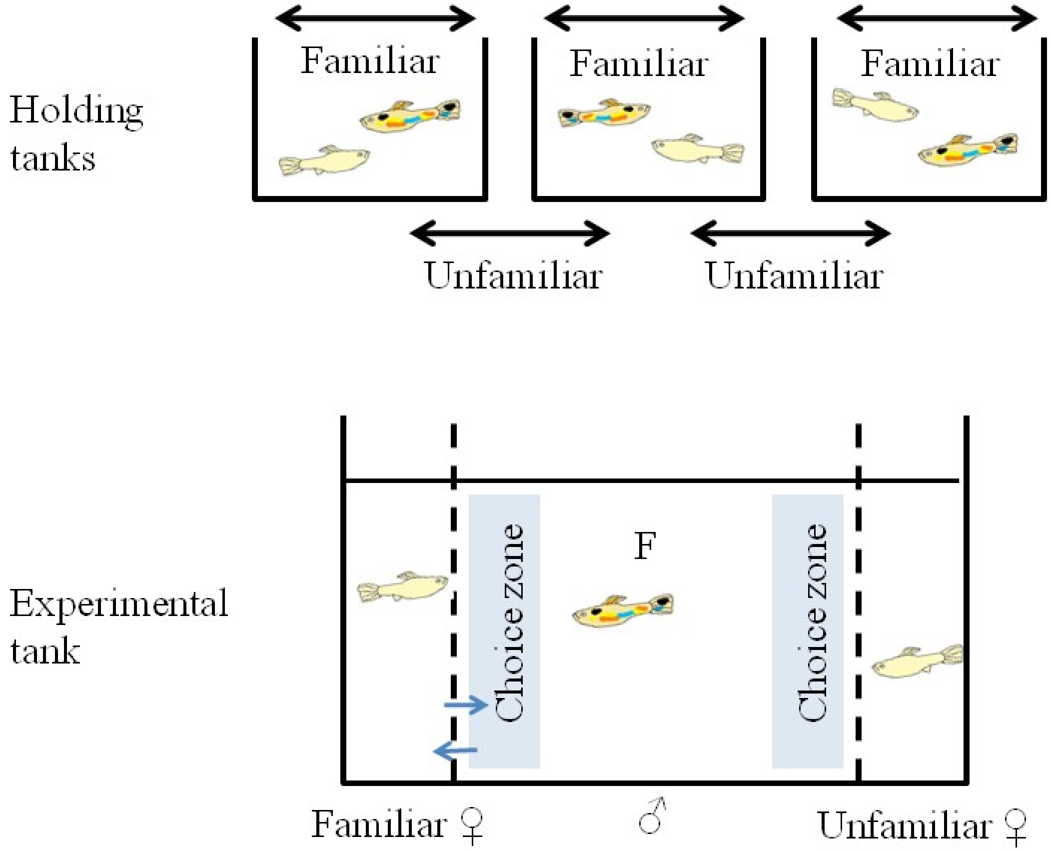


Figure 9: Set-up of holding tanks and experimental tank used to test for the development of familiarity in male guppies. Fish within the same holding tank are called familiar, fish from different holding tanks are unfamiliar with each other. The choice zones in front of the Plexiglas dividers are highlighted in blue. The focal male is marked with an F.

All trials took place between 0900 hours and 1500 hours using a choice tank (46 x 31 x 30 cm) with a gravel floor. The tank was divided into three compartments by clear, perforated Plexiglas. Both side compartments measured 5.5 cm, the middle compartment measured 35 cm. Thin black lines 6 cm apart from the dividers marked two choice zones, this distance equals approximately three body lengths in male guppies. The tank was filled 20 cm deep with water. Three sides of the tank were covered with black plastic to provide a uniform background. Before each trial, the familiar female from the focal male's home tank and a size-matched unfamiliar female from a different experimental holding tank were randomly placed inside the two end compartments of the tank (Figure 9). Within each trial females differed no more than 5% in total body length and the male had never seen the unfamiliar female before. The male was then transferred to a clear plastic bottle in the middle of the tank from where he could see both females and given 10 min. to acclimatise. Afterwards the bottle was carefully lifted and the male was able to swim freely inside the tank. Next, the time he spent in each of the two choice zones either facing the females or displaying to them was measured for another 10 min. After the end of each trial all fish were transferred back to their original tanks and the water in the experimental tank was changed.

All males were repeatedly observed on days 3, 6, 9, 12, 15 and 18 using the same familiar and a different unfamiliar female each time. After the last trial the fish were transferred into large stock tanks and played no further part in this study. The same experiment was repeated using a novel set of fish and testing on days 1, 2, 3, 4, 5 and 6. A control for any effects of repeatedly measuring the same fish was carried out where fish were only tested once after six days. To test for a general loss of the ability to become familiar with other conspecifics after spending several years in captivity, I repeated the experiment originally designed by Griffiths & Magurran (1997a), using 30 females of similar size that were housed in three separate holding tanks in groups of 10 during the course of the experiment. The females were chosen from 10 different stock tanks to avoid using fish that are already familiar with each other. Out of each holding tank 7 individuals (= 21 females in total) were randomly picked and tested every 3 days over 21 days, using a choice tank containing 2 stimulus shoals with 3 fish each, one shoal from the same tank (= familiar) and one from a different holding tank (= unfamiliar). All females were size matched before the trials. The further set-up of this experiment was the same as in the previous male-female trials.

Male mate choice and male mating behaviour based on genetic distance to females

The following experiments were carried out in May 2008 in Trinidad, using wild caught guppies from the Guanapo and Oropuche River. Fish were transferred into six stock tanks (45 x 31 x 30 cm) containing a filter and some stones for cover in a laboratory at the University of West Indies. Black plastic visually separated tanks. The fish were given five days to acclimatise to laboratory conditions before trials started. Fish were kept on a 12 L: 12 D cycle using 18W fluorescent daylight bulbs. The tank temperature was maintained at 26 ± 0.5°C. Water used to fill the tanks was taken from the tab and left to age for at least 48 hours. The water in newly set-up tanks was treated with STRESS COAT®. Fish were fed with TetraMin flake food in the morning about half an hour before the first trial and freshly hatched Artemia larvae in the evening. The total body length (TL) of each experimental fish was taken by measuring from the tip of the snout to the end of the caudal fin using a caliper.

Male mate choice trials took place between 0800 hours and 1600 hours in a choice tank (60 x 30 x 20 cm deep) layered with coarse gravel. The tank was divided into three compartments using clear perforated Plexiglas that allowed water circulation. Both side compartments measured 9 cm in length, the middle compartment measured 42 cm. Two 6 cm long choice zones were marked with thin black lines in front of the side compartments; a distance that equals approximately three body lengths in adult male guppies. The tank was filled 20 cm deep with water and three sides of it were covered with brown paper to create a neutral background.

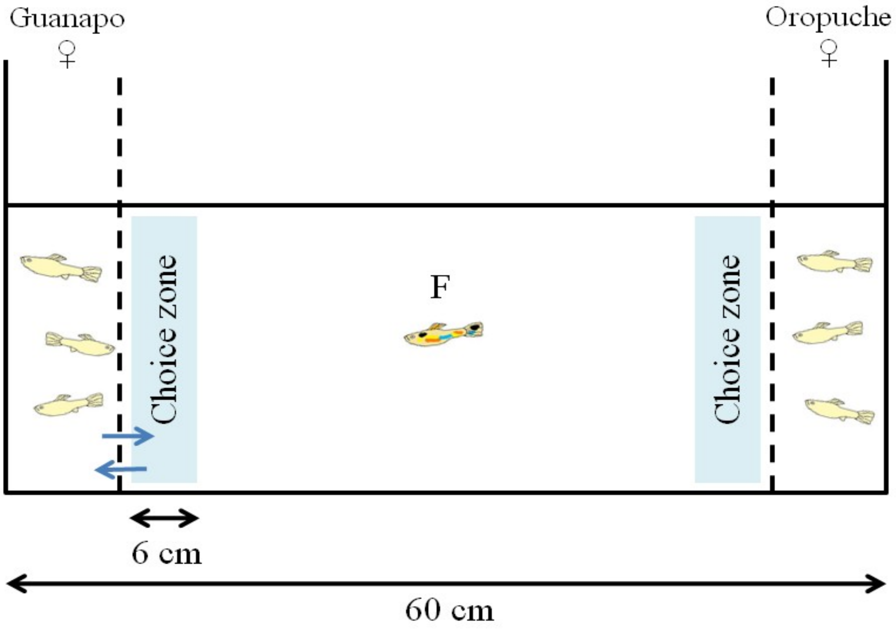


Figure 10: Experimental set-up used to test for population preferences in mate choice in male guppies belonging to either the Guanapo or Oropuche population. The choice zones are highlighted in blue. The focal male is marked with an F.

Twenty-four size-matched females, 12 from the Guanapo and the Oropuche, respectively, were used throughout the experiment and divided into four groups of three females per population. The two end compartments of the observation tank were stocked with either three Oropuche (= one group) or three Guanapo females, respectively. Fish were allowed at least one hour to settle down. Before the trial, a focal male was gently placed into a clear plastic bottle in the middle compartment of the observation tank and allowed 5 min. to settle before the bottle was carefully removed (Figure 10). The time the focal male spent in each choice zone was then measured and the number of sigmoid displays towards females was recorded for 15 min. At the end of each trial, the size of the male was measured. Then he was transferred back into one of the population stock tanks and played no further part in this experiment. A total of 46 males (23 per population) were tested. After every three trials the position of the females inside the observation tank was changed to control for any side preferences in males. After 6 trials a new set of females was used. No combination of female groups was used more than once during the experiment. A complete water change took place every evening after the last trial.

For the male mating behaviour experiments, the bottoms of five replicate observation tanks (45 x 22 x 20 cm) were layered with coarse gravel and three sides of each tank were covered with brown paper. All tanks contained an air-stone. A screen of brown paper containing small holes in front of each tank shielded the observer from the aquaria. Two females, one Guanapo and one Oropuche fish, were transferred into each of the observation tanks and allowed two days to settle. In order to distinguish between females, they differed slightly in size. Because male guppies prefer the larger of two females as mating partners if the size difference exceeds 2 mm (Dosen & Montgomerie, 2004), the larger fish belonged to the Oropuche population in three tanks and to the Guanapo population in two tanks. Before the trial, a focal male (Oropuche population) and two other males either from his own (Oropuche) or the Guanapo population were transferred into one of the observation tanks and given 15 min. to settle (Figure 11). All males could be identified by individually recognisable colour patterns. Each trial lasted for 10 min. and the following behaviours of the focal male towards both females were recorded:

1. Number of sigmoid displays
2. Number of sneaky mating attempts
3. Number of times jockeying for position together with one or both other males

4. Time spent following females

The term 'jockeying for position' describes a behaviour when two or more males that follow a female at high speed, try to bring themselves into the right mating position while at the same time they attempt to deny the same to rivals.

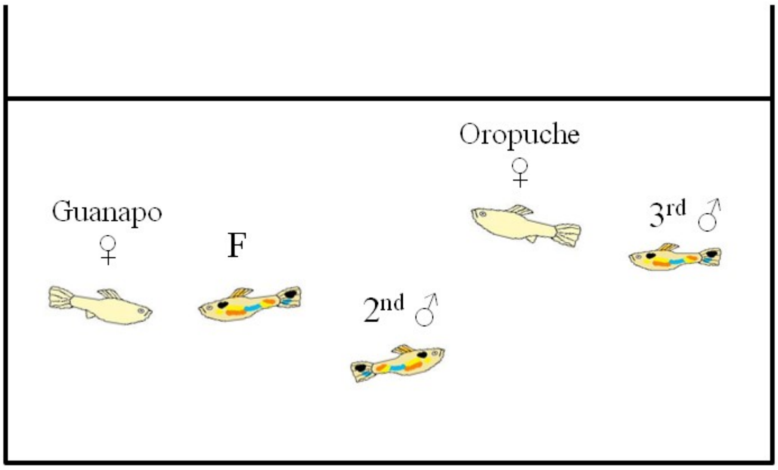


Figure 11: Set-up of male mating behaviour experiments to test for the influence of population origin of rival males on a focal Oropuche male guppy. The focal male is marked with an F. The 2nd and 3rd male either belonged to the focal male's own population (Oropuche) or to the Guanapo population. The tank also contained one Oropuche and one Guanapo female.

At the end of each trial, the size of all males was measured and they were transferred back into their stock tanks. No male was used more than once as a focal male, but it is possible that some of the 2nd and 3rd males were used more than once, though never on the same day. A total of 50 focal Oropuche males were tested, half of them together with two Oropuche males, the other half together with two Guanapo males.

Results

Male familiarity with females in a mating context

All data were arcsine transformed before analysis in order to achieve normal distribution. After spending 18 days in the same tank, male guppies showed no significant preferences for unfamiliar over familiar females (Repeated measures ANOVA, $F_{1,46} = 0.898$, $P = 0.348$) and patterns of male choice varied substantially during the course of the experiment (Figure 12c). Measurements of the mating behaviour of male guppies on six successive days also showed that male guppies do not distinguish between females they have recently mated with and unfamiliar females (Repeated measures ANOVA, $F_{1,48} = 0.492$, $P = 0.486$). As before, preferences for familiar and unfamiliar females changed markedly from day to day and no clear pattern could be seen. The overall changes in male choice behaviour were accompanied by considerable variation between individual focal males (Figure 12a).

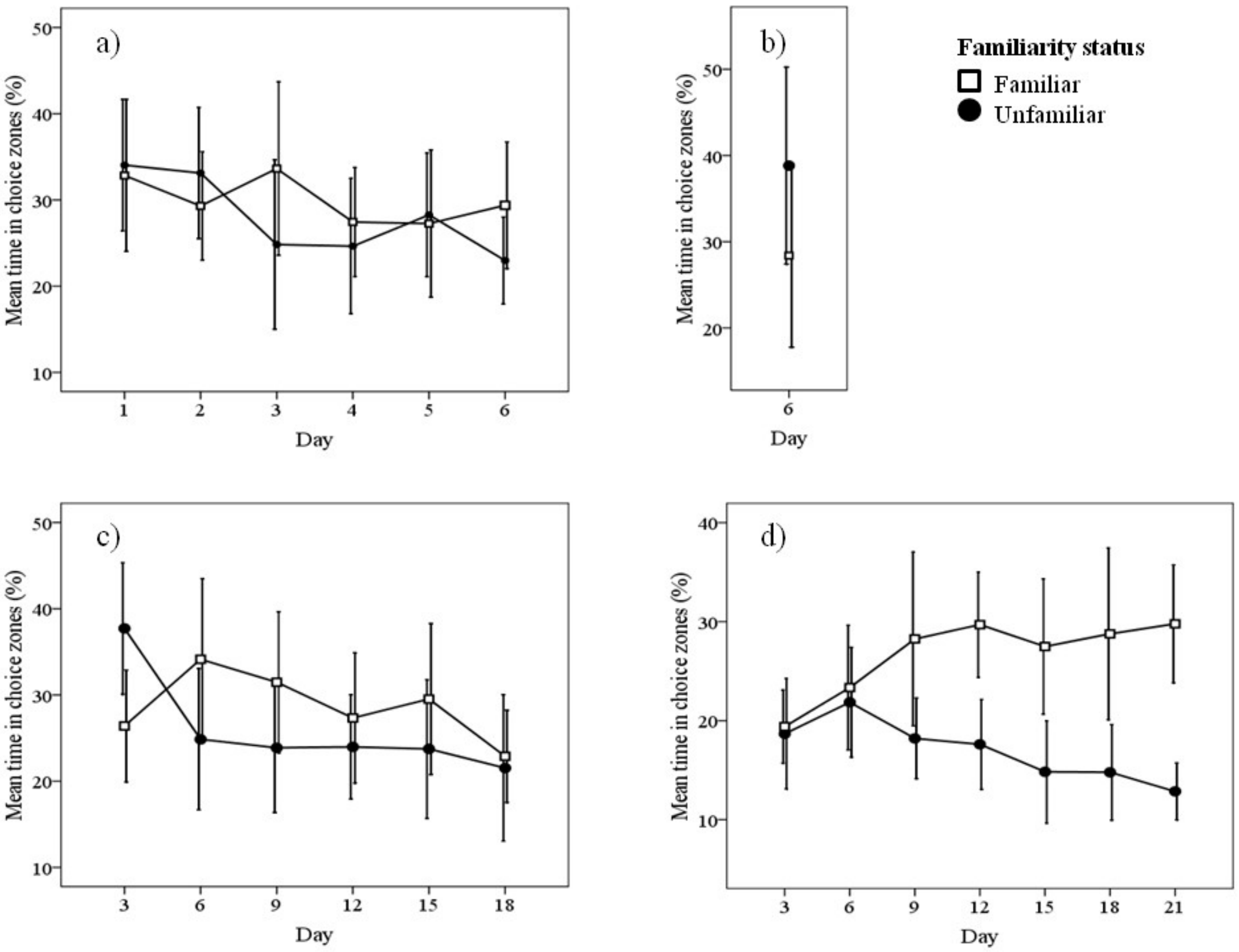


Figure 12: The percentage of time a male guppy spent in the choice zone with the familiar (□) *or unfamiliar* (●) *female: (a) on six successive days; (b) measured once after 6 days during which the focal fish were not exposed to trial conditions; (c) measured every 3rd day for 18 days. (d) The percentage of time a female guppy spent in the choice zone with a set of 3 familiar* (□) *or 3 unfamiliar* (●) *females, measured every 3rd day for 21 days. Error bars represent 95% CI.*

A control was run to test for any effects of repeated measurements on male behaviour where fish were only tested once after six days. Again, males did not distinguish between females (One-way ANOVA, $F_{1,44} = 1.887$, $P = 0.176$; Figure 12b). Female guppies on the other hand became familiar with each other and preferred to school with familiar fish after 12 days, as previously found by Griffiths & Magurran (1997a), and exhibited this preference until the end of the experiment at day 21 (Repeated measures ANOVA, $F_{1,21} = 20.12$, $P < 0.001$; Figure 12d).

Females used as companion fish with males in the 18 day treatment were significantly smaller than females used in the other three treatments (One-way ANOVA, $F_{3,111} = 11.83$, $P < 0.001$; Post Hoc

Test: LSD). Because familiar and unfamiliar females within each trial never differed more than 5% or 2 mm in total body length, a size difference between treatments does not seem to be of great importance. There existed no size difference between males used in different treatments (Kruskal-Wallis test, $\chi^2 = 4.52$, d.f. = 2, P = 0.105), the mean size of males being 1.95 cm ± 0.14 S.D.

Male mate choice and male mating behaviour based on genetic distance to females

No size difference existed between males (Mann-Whitney U test, Z = - 1.829, $n_{Gua} = 23$, $n_{Oro} = 23$, P = 0.067) or females (Mann-Whitney U test, Z = - 0.895, $n_{Gua} = 12$, $n_{Oro} = 12$, P = 0.371) from the Guanapo and Oropuche used for the male mate choice trials. Guanapo males had a mean size of 2.01 ± 0.13 cm, while Oropuche males were on average 2.09 ± 0.13 cm. Guanapo females had a mean size of 2.33 ± 0.21 cm, and Oropuche females had an average size of 2.47 ± 0.35 cm. I then asked if males behaved differently towards different sets of females, but neither the total time males spent in the choice zones (Kruskal-Wallis test, $\chi^2 = 4.85$, d.f. = 7, P = 0.678) nor the total number of sigmoid displays directed towards females (Kruskal-Wallis test, $\chi^2 = 4.09$, d.f. = 7, P = 0.770) differed between female groups. All data were therefore pooled.

No difference was found between the total time males from the Guanapo or Oropuche spent in the two choice zones (Mann-Whitney U test, Z = - 0.692, $n_{Gua} = 23$, $n_{Oro} = 23$, P = 0.489). Guanapo males did not spend significantly more time in the Guanapo choice zone than did Oropuche fish (Mann-Whitney U test, Z = - 1.066, $n_{Gua} = 23$, $n_{Oro} = 23$, P = 0.286). Likewise, Oropuche males did not spend more time in the Oropuche choice zone with females from their own population than did Guanapo males (Mann-Whitney U test, Z = - 0.781, $n_{Gua} = 23$, $n_{Oro} = 23$, P = 0.435) (Figure 13).

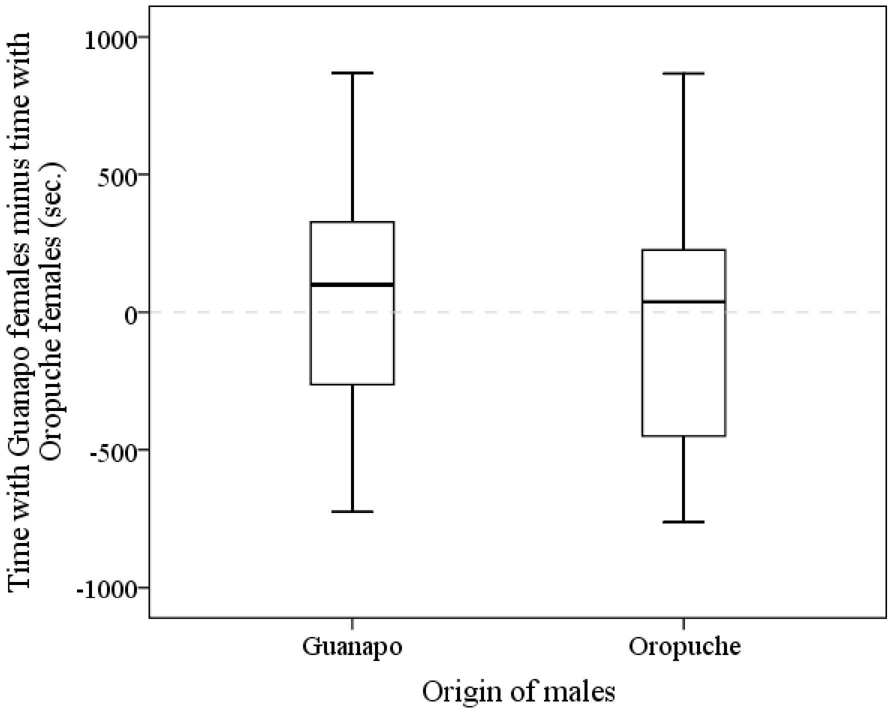


Figure 13: The time Guanapo and Oropuche males spent close to Oropuche females was subtracted from the time they spent with Guanapo females. A value of 0 would indicate that males did not prefer females of a certain population. No differences were found between Guanapo and Oropuche males. Medians and interquartile ranges are shown.

The total number of sigmoid displays directed towards females did not differ between Guanapo and Oropuche males (Mann-Whitney U test, Z = - 1.304, n_{Gua} = 23, n_{Oro} = 23, P = 0.192). Guanapo males did not display more often towards Guanapo females than did Oropuche males (Mann-Whitney U test, Z = - 0.087, n_{Gua} = 23, n_{Oro} = 23, P = 0.930).

Similarly, Oropuche males did not direct more sigmoid displays towards Oropuche females than did Guanapo males (Mann-Whitney U test, Z = - 0.969, n_{Gua} = 23, n_{Oro} = 23, P = 0.332) (Figure 14).

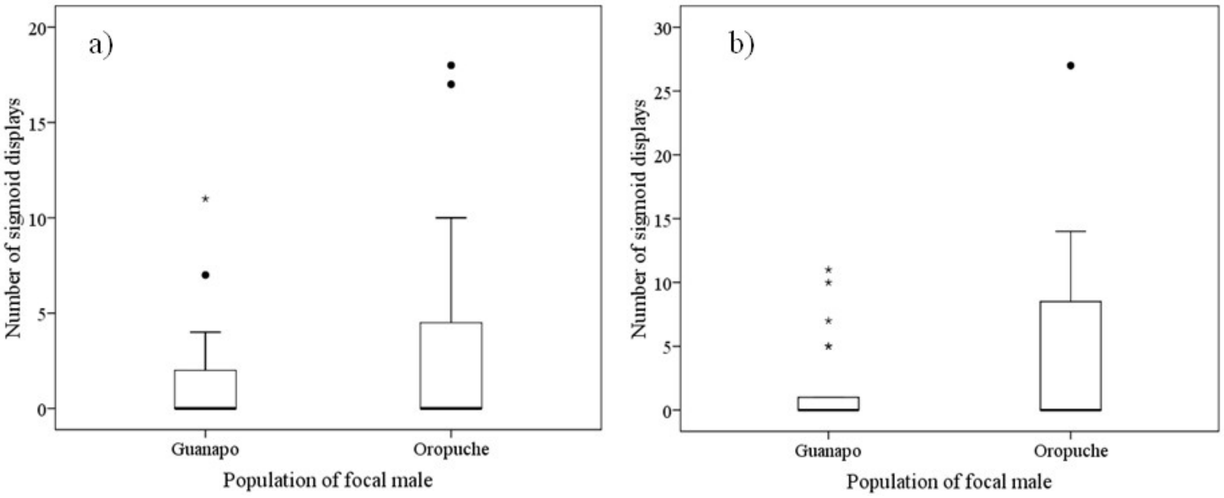


Figure 14: The number of sigmoid displays directed towards Guanapo (a) or Oropuche (b) females by the focal male in a mate choice trial. No difference existed in the choice behaviour based on population origin of the focal male. Medians,interquartile ranges and outliers are shown.

Male size did not differ between treatments (a focal Oropuche male tested with either two Oropuche or two Guanapo males) in the male mating behaviour trials (Mann-Whitney U test, $Z = -0.058$, $n_{Gua} = 23$, $n_{Oro} = 23$, $P = 0.954$). Neither did females from the Oropuche and Guanapo differ in body length between tanks (Mann-Whitney U test, $Z = -0.568$, $n_{Gua} = 7$, $n_{Oro} = 5$, $P = 0.570$). No difference in the recorded behaviours existed between the five experimental tanks (Sigmoid displays: Kruskal-Wallis test, $\chi^2 = 2.10$, d.f. = 4, $P = 0.718$; Sneaky mating attempts: Kruskal-Wallis test, $\chi^2 = 0.941$, d.f. = 4, $P = 0.919$; Time following females: Kruskal-Wallis test, $\chi^2 = 1.733$, d.f. = 4, $P = 0.785$; Jockeying for position: Kruskal-Wallis test, $\chi^2 = 1.941$, d.f. = 4, $P = 0.747$). The data of all tanks were therefore pooled.

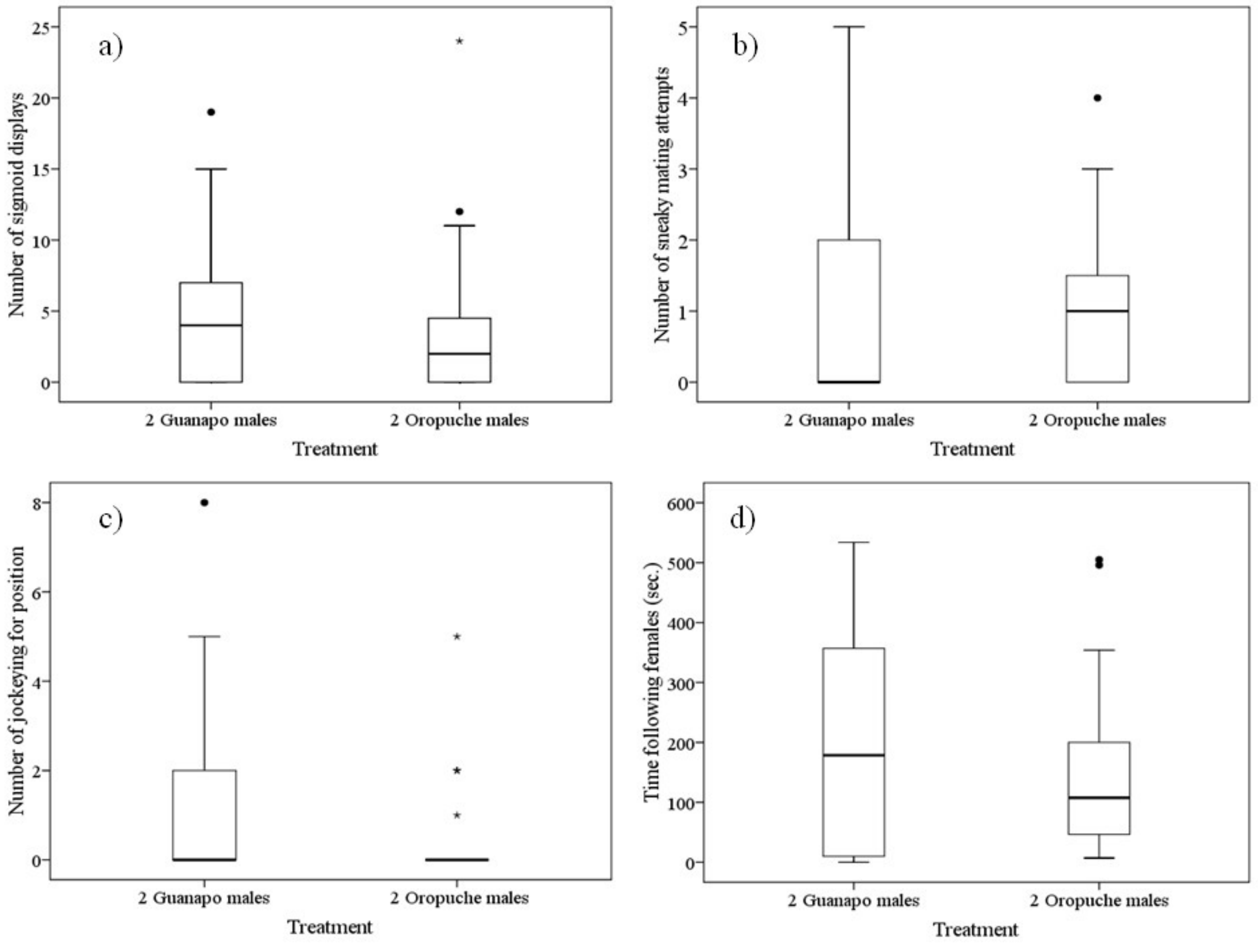


Figure 15: The number of sigmoid displays, sneaky mating attempts and jockeying for the right mating position as well as the mean time focal Oropuche males spent following females in the presence of either two Guanapo or two Oropuche males: No significant difference existed between any of the tested traits. Medians, interquartile ranges and outliers are shown.

Oropuche males did not behave differently towards females in the presence of either Oropuche or Guanapo males, and no significant differences existed between the number of sigmoid displays towards females (Mann-Whitney U test, $Z = - 1.071$, $n_{Gua} = 26$, $n_{Oro} = 24$, $P = 0.284$), the number of sneaky matings (Mann-Whitney U test, $Z = - 0.533$, $n_{Gua} = 26$, $n_{Oro} = 24$, $P = 0.594$), the time the spent following females (Mann-Whitney U test, $Z = - 0.272$, $n_{Gua} = 26$, $n_{Oro} = 24$, $P = 0.786$) and the number of times they jockeyed for the right mating position with one or both other males in both treatments (Mann-Whitney U test, $Z = - 1.832$, $n_{Gua} = 26$, $n_{Oro} = 24$, $P = 0.067$) (Figure 15). Females from the Oropuche and the Guanapo received the same number of sigmoid displays (Mann-Whitney U test, $Z = - 1.056$, $n_{Gua} = 7$, $n_{Oro} = 5$, $P = 0.291$) and were followed for the same amount of time by the focal male (Mann-Whitney U test, $Z = - 1.218$, $n_{Gua} = 7$, $n_{Oro} = 5$, $P = 0.223$). There were however significantly more sneaky mating attempts on Oropuche than Guanapo females (Mann-

Whitney U test, Z = - 2.15, $n_{Gua} = 7$, $n_{Oro} = 5$, P = 0.032) (6.20 ± 3.35 sneaky mating received by Oropuche females vs. 2.14 ± 1.57 sneaky mating attempts received by Guanapo females).

Discussion

Neither familiarity with females nor genetic distance between mating partners seemed to influence the mating decisions of male guppies in a series of choice experiments. In contrast to female guppies, who develop familiarity with their schooling partners within 12 days, male guppies did not become familiar with females in the same short amount of time. Females on the other hand were still able to develop familiarity with other females even after spending several years in captivity, so that a general loss of the ability to become familiar with other conspecifics due to laboratory conditions can be ruled out as an explanation for my findings. Male guppies did also not prefer courting females from their own population over females from a different population in a choice experiment and might not even be able to distinguish between female origin. The presence of rival males from the invading Guanapo population neither altered nor reduced Oropuche male mating behaviour compared to the presence of rivals from the focal male's own population.

Male familiarity with females in a mating context

Male guppies did not discriminate between familiar and unfamiliar females in a choice experiment, despite spending 18 days in a tank with the same female. Overall patterns of male mate choice varied markedly and were not predictable from one day to the next. These results are strengthened by similar findings by Zajitschek et al. (2006) where male guppies did not prefer unfamiliar over familiar females and did not allocate more sperm to the former in a series of experiments. The authors assumed that experimental males behaved in that way because they only had access to a single female during the mating trial. Another possible reason mentioned was the fact that these males had no opportunity to mate for several days or even weeks before they were tested which could have increased their general willingness to mate independently of their mate's familiarity status. In my experiment, males always had the opportunity to mate while in their holding tanks, and they had the choice between a familiar and an unfamiliar female during the trial but still did not discriminated between females. However, it was shown by Griffiths & Magurran (1997a) and confirmed by my own experiment that female guppies need 12 days to become familiar with other females in a schooling context. Therefore, it is possible to assume that, in contrast to captive reared rainbowfish (Kydd & Brown, 2009), the absence of familiarity between male guppies and their mates is unlikely to be due to inbreeding effects or the result of spending several years in captivity.

In order to maximise their lifetime reproductive success, females must avoid predation and exhibit behaviours that reduce the risk of predator attacks. Associating with familiar fish makes schools more cohesive and school members less exposed to predators – females thus benefit by learning to recognise their schoolmates (Magurran, 2005). Male guppies on the other hand achieve a greater reproductive output if they copulate with as many females as possible and are therefore constantly in search of new mates (Kelley et al., 1999). They trade off the relative safety of staying in a school with familiar fish against switches between schools, and in this way encounter more females (Griffiths & Magurran, 1998). Due to this constant movement it seems unlikely that a male will meet with the same female very often during his life, and there seems to be little need for male guppies to invest time into short term familiarity with females. However, it is known that males have the general ability to become familiar in a schooling context (Croft et al., 2004). My results are further supported by a study on wild guppies by Griffiths & Magurran (1997b), which demonstrated that the ability of females to recognize other individual females decreased as the number of females present increased. This suggests that female guppies are not capable of becoming familiar with very large numbers of other females.

If males were more likely to encounter the same females on a regular basis, it would be advantageous for a male to remember a female he has recently mated with in order to direct more time to other potential mates. Guevara-Fiore et al. (2009) found that guppy males are able to distinguish between virgin and recently mated females and vary their mating behaviour according to the situation. If guppy males are also able to individually distinguish between females they themselves have mated with and females mated by other males, familiarity even after a very short amount of time (= hours to days) would be expected, but as my results indicate, males do not seem to be able to remember the female of a recent mating.

The situation changes if a male is trapped with the same females over a long period of time, a scenario likely to happen in the upper stretches of rivers during parts of the dry season when unconnected pools in otherwise dry rivers prevent fish from moving around. In a recent study, Ward et al. (2009) confirmed that female guppies were able to recognize individual conspecifics, whereas three-spined sticklebacks were not. The authors argued that populations or species restricted to small habitats, like isolated pools, or where the advantage of moving between groups is low, would be more likely to individually recognize familiar conspecifics than populations or species inhabiting open areas or encountering large numbers of individuals. Again, only females were tested, but the results of this study support my findings when the differences in lifestyle between the sexes of guppies are considered. After spending six weeks with the same females, male guppies preferred to court unfamiliar females in an experiment carried out by Kelley et al. (1999). However, the cues that male guppies use to distinguish between familiar and unfamiliar females have not yet been investigated. Dosen & Montgomerie (2004) found that male guppies choose the larger of two females if the size difference exceeds 2 mm, so size could be used to distinguish between conspecifics. Here I used size matched females that never differed by more than 5% of their total length. These differences also never exceeded 2 mm, so that size probably could not be employed as a cue to distinguish between mates. Because female guppies have uniform drab colouration, no obvious visible differences exist between fish, at least not for the human observer. It is possible that males are able to recognise individual females but it could also be that they simply match habitat-specific chemical cues like smell to identify familiars. This mechanism of self-referent chemical cue matching is found in three-spined sticklebacks (Ward et al., 2005; Ward et al., 2009).

To address this question further, experiments testing for any mechanisms that lead to familiarity in male guppies and their mates are required. An experiment similar to the ones described here, but this time repeatedly testing the same males around once per week for six weeks could finally answer the question how long it takes for male guppies to become familiar with their mates. Another interesting question is whether males from the upper sections of rivers, where the restriction to unconnected pools during parts of the dry season is likely, and males from downstream sections that are constantly flooded show differences in the development of familiarity in a mating context. The result that male guppies are not able to distinguish between familiar and unfamiliar fish after a relatively short amount of time emphasises how important it is to include ecological and behavioural differences between species or even sexes before extrapolating data on a broader scale.

Male mate choice and male mating behaviour based on genetic distance to females

No significant differences could be found in any tested behaviour in the second set of experiments looking at the importance of genetic distance between mating partners for male mate choice. Unlike females, who were reported to slightly prefer mating with males from their own population (Endler & Houde, 1995), males guppies did not discriminate between female origin but spent the same amount of time with females of both populations in a choice experiment. This result was the same for males from two different populations, the Guanapo (belonging to the Caroni drainage) and the Oropuche (Oropuche drainage). A difference in choice behaviour between these populations that could lead to selective mating is therefore unlikely to be one of the reasons for the invasive success of Guanapo fish after their introduction in the Turure. The absence of a preference for females of their own population could either be the inability of males to distinguish between female origin or a general lack of male interest in the source of mating partners. Male guppies have been reported to prefer virgin females over recently mated ones (Guevara-Fiore et al., 2009) but did not distinguish between familiar and unfamiliar females after spending up to three weeks in the same tank (see familiarity experiment in this chapter). It might therefore be that they generally lack the ability to differentiate between females, at least in the short term.

Magurran & Seghers (1991) found that the level of male aggression significantly differs between populations, and a year later Kodric-Brown (1992) showed that unattractive but dominant males can gain significant mating success by monopolizing females even if their mating partner did not prefer them. However, the occurrence of dominance behaviour like jockeying for the right mating position in the presence of rival males was low in all male mating behaviour treatments conducted here. Furthermore, an absence of behavioural differences between treatments make it unlikely that this type of behaviour played an important role during the colonisation of the Turure by Guanapo fish and a subsequent mixing of populations. Oropuche males did not reduce the amount of mating behaviour directed towards females in the presence of two males from the invading Guanapo population compared to the presence of two males from their own population. As in the choice experiment, they also did not distinguish between female origin and equally courted females from both populations.

But the observed inability or unwillingness of males to differentiate between female origin can still influence the outcome of an invasive event and the speed of gene-pool mixing, even in the absence of obvious differences between the mating behaviour of male guppies from the Guanapo and the Oropuche. Because male guppies can overrule female choice with forced copulations, the importance of female preference to mate with males from her own population is weakened. Therefore it is possible for populations to mix quickly after an invasive event without the invading males necessarily showing superior or more dominant mating behaviour than males belonging to the native population.

To further investigate potential differences in male behaviour, male mobility and the likelihood of males moving long distances could be compared between populations. Croft et al. (2003) showed that 27.3 % of males left their pool of release during an 8-day study. A higher travel distance compared to Turure fish could give an advantage to Guanapo males and could increase the speed with which they encounter new, unmixed parts of the native population further downstream of the Turure.

Chapter 4 Female receptiveness to male courtship and population differences in female schooling behaviour

Abstract

Several types of female behaviour could influence the mixing of two populations and therefore two gene pools after an invasive event. Female guppies show a slight but significant preference to mate with males of their own population, a behaviour that might slow down the process of population mixing if females mate mostly according to their choice. Here I asked how willing females are to mate in general and found a very low tendency to respond to male courtship behaviour except when virgins, even after having been separated from males for up to 6 months. This reluctance to mate while having a choice could increase the importance of sperm transfer during forced copulations. Because male guppies do not seem to distinguish between population origin of their mating partners, this could enhance the speed of population mixing after an invasion. A further question asked was if female guppies prefer more closely related schooling partners (from their own population) over distantly related females (from other populations or another species). Female guppies do not seem to be able to discriminate between populations of different origin and are therefore likely to form mixed schools in the wild when fish of more than one population coexist in the same place. The ability of female guppies to distinguish between their own species and the swamp guppy, *Poecilia picta*, was only found in two of the five tested populations, one in each drainage system. I will discuss the consequences of the observed reluctance to mate and the females' inability to differentiate between their own and other populations of the same species with regard to the ecological implications they have for population mixing during an invasion.

Introduction

Female guppies differ in many morphological and behavioural respects from males of the same species. They grow throughout their lifetime and reach a significantly larger size than males (Reznick & Miles, 1989). Females only express a drab beige colouration compared to the colourful red, orange and black spots displayed by many males and are therefore less conspicuous (Houde, 1997). Because of their larger size female guppies need to spend more time foraging, an activity that is dedicated a large amount of time every day (Magurran & Seghers, 1994). Females school more than males (Griffiths & Magurran, 1998) and often stay together with the same group of individuals with whom they become familiar in less than two weeks (Griffiths & Magurran, 1997a). Other predator avoidance behaviours such as anti-predator responses are also more pronounced in females than in males (Magurran & Seghers, 1994) and females tend to inspect predators at a greater distance (Magurran et al., 1992). These differences in colouration and behaviour lead to a generally decreased risk of predation in female guppies (Rodd & Reznick, 1997).

Female guppies are only receptive as virgins after reaching sexual maturity at the age of approximately three months, and for a few days after giving birth to a brood of living offspring about once a month (Liley, 1966). During these periods they display strong mate choice depending on various male traits such as colouration and size, but pronounced differences in preferred traits and the strength of female choice exist between populations (Endler & Houde, 1995). Virgin females on the other hand do not seem to discriminate much between males until they have mated at least once (Endler & Houde, 1995). Unreceptive females normally try to avoid the continuous male courtship displays and mating attempts. They can store sperm for some time and are able to produce one brood per months for several months after their last mating (Winge, 1922). These broods become smaller over time as sperm stores are becoming depleted (Magurran, 2005). Having been isolated from males for some time should therefore increase female receptiveness and their willingness to mate. Female guppies have a degree of control over the duration of matings and the number of transferred sperm during a copulation. Solicited matings with preferred males last longer and more sperm is transferred (Pilastro et al., 2007), but some sperm can also be received during sneaky mating. Evans et al. (2003) detected sperm from forced copulations in 44.5% of non-receptive females. After mating with a male, females are less responsive if presented with a second mate, but responsiveness increases if this male is more ornamented than the one they previously mated with (Pitcher et al., 2003). Multiple mating leads to a shorter gestation time in females, and broods with more than one father are commonly found in the wild (Evans & Magurran, 2000). The same authors also found further advantages of multiple mating such as the production of larger broods with better schooling abilities and escape responses. Therefore females would be expected to mate with preferred males on a regular basis and should be especially keen after being isolated from males for several weeks or months.

This chapter explores the existence of differences in schooling behaviour of females from several rivers belonging to the Caroni and the Oropuche drainage system in Trinidad. Furthermore their ability to distinguish between members of their own and other populations was tested, a trait that, beside other behavioural patterns, could help to explain the invasive success of Guanapo fish

(Caroni drainage) in the Turure (Oropuche drainage) from a female perspective. In the first part of this chapter the general female willingness to mate was examined to get a better understanding of to what extent female decisions and, in particular, their choice of mating partners influence reproduction and the speed at which the two populations mix. Here I tested the responsiveness of females towards male courtship behaviour using females of varying receptive status (virgins and females isolated from males for 1 month, 3 months and 6 months, respectively). I predicted significant differences in female responsiveness to male courtship behaviour between these groups with virgin females being most receptive, while the general eagerness of already-mated females would increase with the amount of time they had been isolated from males. I expected to find females isolated for 6 months showing a similar responsiveness towards male courtship as virgin females. The second part of the study focused on the ability of female guppies to distinguish between their own population and other guppy populations of varying relatedness in a laboratory experiment.

Marked differences in the aforementioned female behaviours as well as several life-history traits exist between populations from low and high predation sites. Females that only face minor predation risk mature later and produce fewer, larger offspring (Reznick & Endler, 1982). Their preference for brightly coloured males is more pronounced (Breden & Stoner, 1987) and males spend more time courting females than trying to overrule female choice with forced copulations (Luyten & Liley, 1985). Sexual selection is therefore stronger and can quickly produce highly coloured males and female preference for them (Houde & Endler, 1990). In high predation sites, on the other hand, males are often less colourful (Endler, 1983) and are more likely to seek access to females via sneaking (Luyten & Liley, 1985). Here females can receive up to one sneaky mating attempt per minute (Magurran & Seghers, 1994). In addition, they are less choosy which means that sexual selection in high predation sites is less articulate (Houde & Endler, 1990). Fish also tend to school more and form more cohesive schools than fish in low predation habitats (Seghers, 1974)

But how do populations that face similar predation regimes in different Trinidadian drainage systems differ from each other? Despite their genetic diversity they are experiencing very similar living conditions, a fact that seems to produce larger behavioural and life-history differences within fish living in low or high predation regimes in the same river than between populations from two different rivers that experience the same level of predation (Endler, 1995). However, Endler & Houde (1995) found differences in female preferences for male colouration and the amount of colour expressed in males between populations across several rivers in both drainage systems. The question now is whether these various differences go alongside genetic differences between the two drainage systems and indicate the existence of two subgroups, or even two different species as was recently suggested by Schories et al. (2009), or if population differences are not strongly correlated with the geographic isolation of both river systems but appear at random. The schooling behaviour of females belonging to several populations of both systems was investigated to explore differences within and between drainage systems.

Schooling together with other individuals is a common anti-predator strategy in fishes (Pitcher & Parrish, 1993) and can also enhance foraging quality (Pitcher et al., 1982). Commonly, schools are sorted by size and dominated by one species (reviewed by Krause et al., 2000) which increases anti-predator benefits for each individual (Theodorakis, 1989). In the guppy, schooling is an important anti-predator strategy. Guppies that occur in high predation sites where they coexist with the pike cichlid (*Crenicichla alta*) and other major guppy predators, spend more time schooling and form larger, more cohesive schools than guppies living in low predation sites and only experience minor predation (Seghers, 1974). Female guppies form the core of schools and become familiar with other group members. They then prefer to school with familiar over unfamiliar fish. Females spend more time schooling compared to males which trade off the safety of a group with increased movement between schools in search of new mates (Griffiths & Magurran, 1998). Schools are commonly sorted by size (Croft et al., 2003b). Mixed schools with the swamp guppy (*P. picta*) can occasionally occur in places where their distributions overlap (Magurran, 2005). Females of these closely related species look very similar and are hard to tell apart from a distance, at least for the human observer. In laboratory studies naïve male guppies were reported to initially prefer courting the often larger *P. picta* females. Only after several days did they learn to distinguish between *P. picta* and females of their own species and ceased courting heterospecifics (Magurran & Ramnarine, 2004). A recent study has shown that female guppies are able to individually recognize other conspecifics. They can also distinguish between different groups of fish on the basis of habitat cues such as smell (Ward et al., 2009). But do females equally distinguish between conspecifics of other populations and *P. picta* females and prefer to school with their own kind? Magurran et al. (1994) demonstrated that guppies could not distinguish between their own and a different population by visual cues alone.

In this experiment female guppies were allowed to choose between schooling partners of their own population, of the same drainage system, of a different drainage system and from a different but closely related species (*P. picta*) while having access to visual as well as odour cues. I observed the general schooling behaviour of females belonging to several populations across drainage systems to investigate if existing differences followed the genetic relatedness of populations or occurred at random. The hypothesis tested was that females guppies should be able to differentiate between guppies and fish from another species, preferentially schooling with the former, but might not be able or interested in differentiating between schooling partners from their own and another population. The absence of a genetic correlation does of course not exclude another non-random but unknown factor to explain any differences between populations and drainage systems, therefore further experiments looking at population differences might me useful. I also examined the ability of females to distinguish between members from their own population and females from other populations or species of varying relatedness. The ability to differentiate between their own and another population could help to decrease the speed at which two or more populations mix during an invasion.

Methods

Schooling behaviour and population recognition

The experiments for this study were carried out between April and May 2009 and in February 2010 at the University of the West Indies, Trinidad, using wild caught guppies from high predation sites at the Guanapo, Lower Tacarigua (both belonging to the Caroni drainage), Quare, Turure and Lower Oropuche (Oropuche drainage) as well as *Poecilia picta* females. See Table 4 for grid references.

Table 4: Grid references of populations used during the female schooling. The drainage system each river belongs to is indicated if important.

Population	Grid reference number	Nearest road	Drainage system
Lower Tacarigua	PS 774 763	off Churchill Roosevelt Highway	Caroni
Guanapo	PS 913 765	Eastern Main Road	Caroni
Quare	PS 979 779	Valencia Road	Oropuche
Oropuche	QS 042 788	Valencia Road	Oropuche
Turure	QS 002 784	Valencia Road	

In 2009, experimental females from the Oropuche and Guanapo were separated from stock fish directly after catching and kept individually in small plastic tubs filled with 1 litre of water for the course of the experiment. This was done to prevent females from becoming familiar with each other. No female spent more then 9 days in her plastic tub. A water change took place every 3 – 4 days. Stock fish were kept in several big tanks (45 x 31 x 30 cm), containing a filter and a plastic plant and visually separated from each other by black plastic. In 2010, experimental females from the Oropuche, Guanapo, Quare, Lower Tacarigua and Turure were split into three groups per population and transferred into the same number of tanks (45 x 31 x 30 cm), again, to prevent them from becoming familiar with other experimental fish. All tanks contained a filter, a plastic plant and some *Elodea canadensis* for cover. Black plastic visually separated the tanks.

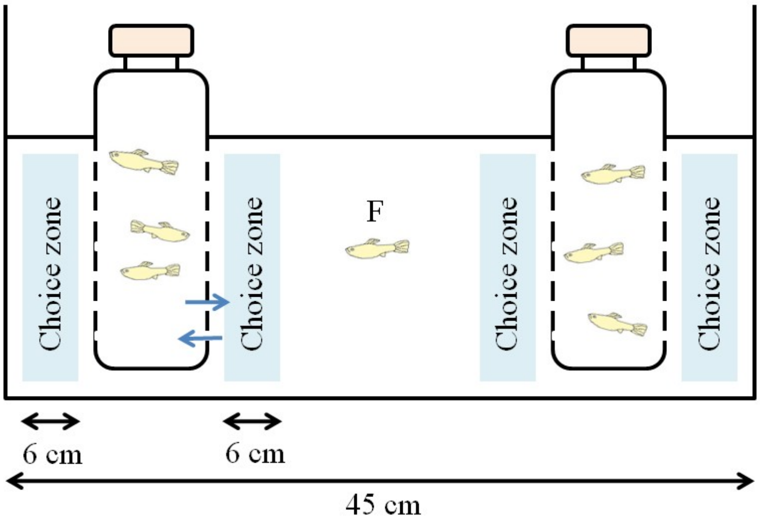


Figure 16: Experimental set-up of the choice tank used to test for population preferences in schooling female guppies. The choice zones around the Plexiglas bottles that contain the companion fish are highlighted in blue. The focal female is marked with an F.

All fish were kept on a 12 L : 12 D cycle, the water temperature was maintained at 26°C. Fish were fed with TetraMin flake food in the morning about half an hour before the first trial and freshly hatched *Artemia* larvae in the evening. The trials took place between 0800 hours and 1700 hours using two identical choice tanks (45 x 31 x 30 cm) layered with coarse gravel and filled with water to 20 cm. Three sides of each tank were covered in black plastic to provide a uniform background. Two clear 600 ml Plexiglas bottles containing several small holes for water exchange were placed at either end of the tank. Each bottle was stocked with 3 size matched females unfamiliar with the focal fish from a stock tank of the focal female's own population and 3 size matched females of a different population or species, respectively (Figure 16). The following treatments were tested:

- 2009 (Oropuche and Guanapo females)
    1. Fish of the focal female's own population vs. females of her own population (control)
    2. Fish of the focal female's own population vs. females from the same drainage system
    3. Fish of the focal female's own population vs. females from the other drainage system
    4. Fish of the focal female's own population vs. females of a different but closely related species (*P. picta*) (Figure 17)

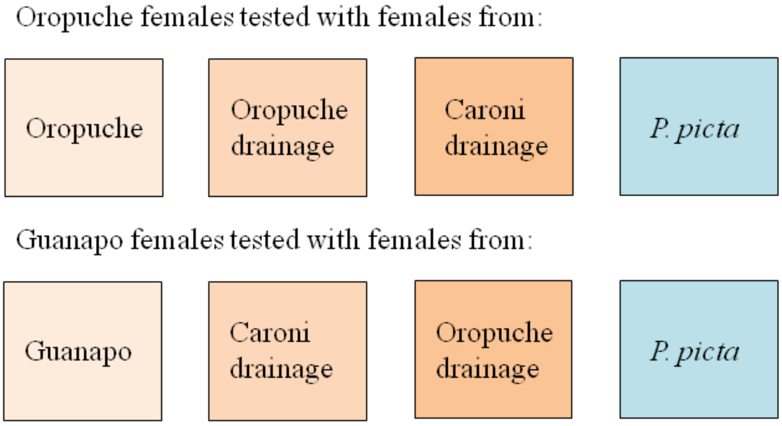


Figure 17: Treatments used to test for schooling preferences of focal females with fish of varying relatedness. Females could choose between fish from their own population, fish from the same drainage system, fish from a different drainage system and P. picta.

- 2010 (Oropuche, Guanapo, Quare, Lower Tacarigua and Turure females)
  1. Fish of the focal female's own population vs. *P. picta* females

One set of companion females was used for several trials and afterwards transferred back into their stock tank. Before each trial the bottles containing the fish were randomly placed at both ends of the experimental tank, where the fish quickly settled and the focal female was carefully transferred into a small starter box in the middle of the back of the tank. The trial started as soon as this fish left the box and swam freely around the tank. For 10 min. the time the female spent within 6 cm (approximately 2 body length) of both bottles was recorded. In 2009, 26 Guanapo females and 28 fish from the Oropuche were each tested four times, once in each condition, with a day of rest between each trial. The order of the different treatments was randomised for each female and their total length was measured after the first trial. In 2010, females were only tested once and their total length was measured before they were transferred back into their home tank.

Female responsiveness to male courtship

All trials for this experiment were carried out in St Andrews between September and December 2009 using descendants from the Lower Tacarigua that had spent several generations in captivity. Before the experiment, 30 size-matched females per treatment were transferred into ten small tanks (31 x 20 x 21 cm) layered with gravel and containing a filter and a plastic plant for cover. Each tank was stocked with three individuals, sheets of paper between the tanks prevented the fish from seeing each other. Fish were kept on a 12 L: 12 D cycle using 18W fluorescent daylight bulbs. The tank temperature was maintained at 23.5 ± 0.2°C. All fish were fed with TetraMin flake food once daily about half an hour before the first trial. The following treatments were tested during the course of this experiment:

1. Virgin females (n = 24)
2. Isolated females (1 month) (n = 24)
3. Isolated females (3 month) (n = 21)
4. Isolated females (6 month) (n = 21)

To isolate females, stock fish were separated from males for 1, 3 or 6 months, respectively. In order to raise virgin females, newborn guppies were isolated shortly after birth, transferred into small tanks (31 x 20 x 21 cm) and raised individually. At the age of 3 months fish were sexed and all females transferred into a tank containing virgin females only. Virgins used in this experiment were approximately between 8 and 10 months old. Because females of the other treatments used in this experiment were roughly matched for size, they are likely to belong to an age class similar to that of the virgin females.

Four observation tanks (31 x 20 x 21 cm) were set up, layered with gravel and supplied with a filter and a plastic plant. Three sides of each tank were covered with black plastic to provide a uniform background. All observation tanks were stocked with one adult female and two juveniles acting as companion fish. In the evening before the trial, four focal females were randomly chosen and their total length measured. They were then transferred into the four observation tanks to settle overnight. In order to be able to distinguish between the focal female and companion fish, the two females within one tank differed slightly in size. For the focal fish this size difference was classified as either larger or smaller than the companion female. Half of the focal females were larger than their companion fish, the other half were smaller. The following morning two individually recognisable males were chosen from two of three holding tanks containing six males and four females each and carefully transferred into one of the experimental tanks. The trial started as soon as both males displayed normal swimming behaviour and courted the females. Four trials were run per day. Each lasted for 1 hour and the following behaviours of or towards the focal female were measured:

1. Number of sigmoid displays
2. Number of sneaky mating attempts
3. Number of female responses (orientate and possibly swim towards the male)
4. Number of matings
5. Time until first and second mating

After the end of the trials all focal fish were transferred back into their stock tanks and played no further part in this experiment. Males were moved back into one of the holding tanks but were never used more than once per day. The companion fish stayed in the observation tanks throughout the experiment.

Results

Schooling behaviour and population recognition

Guanapo and Oropuche females used in the 2009 schooling experiment were of equal size (t-test, t = - 0.98, d.f. = 53, P = 0.332). The total time fish spent schooling did not differ during days for Guanapo females (One-way ANOVA, F = 0.32, d.f. = 3,100, P = 0.808) and data for all days were combined. Total schooling time did however differ for Oropuche females (One-way ANOVA, F = 3.64, d.f. = 3,112, P = 0.015). A LSD Post Hoc Test found that Oropuche females spent significantly more time schooling on day 1 than during the other days (Day 1 and 3: P = 0.014, Day 1 and 5: P = 0.007, Day 1 and 7: P = 0.006). The total schooling time during the other days did not differ, even after re-analysing days 3 to 7 without day 1 (One-way ANOVA, F = 0.52, d.f. = 2,84, P = 0.949). Because schooling time did not change after day 1 and no general trend was obvious, data for all days were pooled.

A significant difference between the total time females of both populations spent schooling was found (One-way ANOVA, F = 7.22, d.f. = 1,218, P = 0.008). Guanapo females on average schooled for 363.3 sec ± 116.1 S.D. out of 600 sec whereas Oropuche fish only spent a mean time of 327.4 sec ± 80.9 S.D. close to their companion fish. To test if a female spent more time with her own population than with the other population, the time she spent schooling with the other population was subtracted from the time she spent with her own population. I then tested these values for a significant difference from 0. Guanapo fish neither preferred their own population over females from the same drainage system (One-sample t test, t = - 1.16, d.f. = 25, P = 0.257), nor did they distinguish between schooling partners from their own population and fish from a different drainage system (One-sample t test, t = - 1.79, d.f. = 25, P = 0.086). Guanapo females also did not prefer fish from their own population over *P. picta* (One-sample t test, t = - 1.40, d.f. = 25, P = 0.174). As expected, no difference was found when females had to choose between two sets of fish both belonging to their own population (One-sample t test, t = - 0.87, d.f. = 25, P = 0.392). Oropuche females showed similar results when tested with fish from their own population (One-sample t test, t = - 2.02, d.f. = 28, P = 0.053), fish from their own drainage system (One-sample t test, t = - 1.52, d.f. = 28, P = 0.139) or fish from a different drainage system (One-sample t test, t = 0.53, d.f. = 28, P = 0.597). However, Oropuche females significantly preferred to school with their own population over schooling with *P. picta* (One-sample t test, t = 3.24, d.f. = 28, P = 0.003) (Figure 18). This difference persisted after adjusting the significance level to 0.0125 using the sequential Bonferroni procedure (Rice, 1989) to correct for multiple testing.

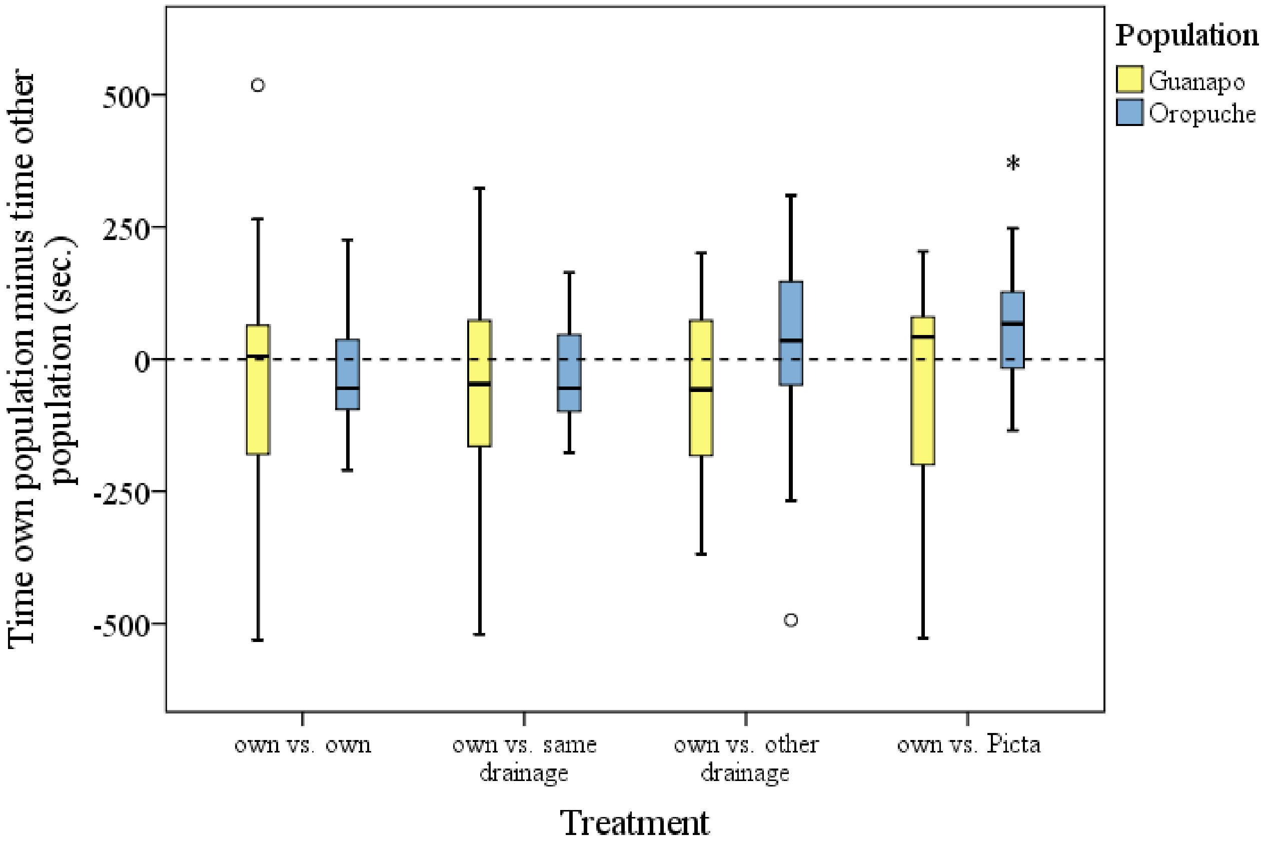


Figure 18: The time Guanapo and Oropuche females spent schooling with fish from a different population was subtracted from the time they spent with their own population. A value of 0 would indicate that no difference between these two measurements exists. Only Oropuche females did significantly prefer to school with fish from their own population over schooling with P. picta. No other differences existed within treatments. Medians, interquartile ranges and outliers are shown.

In 2010, at least one population of fish differed from the rest in total body length (Kruskal-Wallis test, $\chi^2 = 31.63$, d.f. = 4, $P < 0.001$). However, populations still differed significantly in their schooling time after size was accounted for (ANCOVA, $F_{4,120} = 3.53$, $P = 0.009$). Females from the Oropuche (One-sample t test, $t = 3.81$, d.f. = 24, $P = 0.001$, $\alpha_1 = 0.01$) and from the Lower Tacarigua (One-sample t test, $t = 2.97$, d.f. = 24, $P = 0.007$, $\alpha_2 = 0.0125$) spent significantly more time with their own population than with *P. picta* (Figure 19). No difference was found between the time spent with their own population and the time spent with *P. picta* in Guanapo fish (One-sample t test, $t = -0.93$, d.f. = 20, $P = 0.363$), Turure fish (One-sample t test, $t = -0.83$, d.f. = 24, $P = 0.415$) and Quare fish (One-sample t test, $t = 0.54$, d.f. = 24, $P = 0.594$).

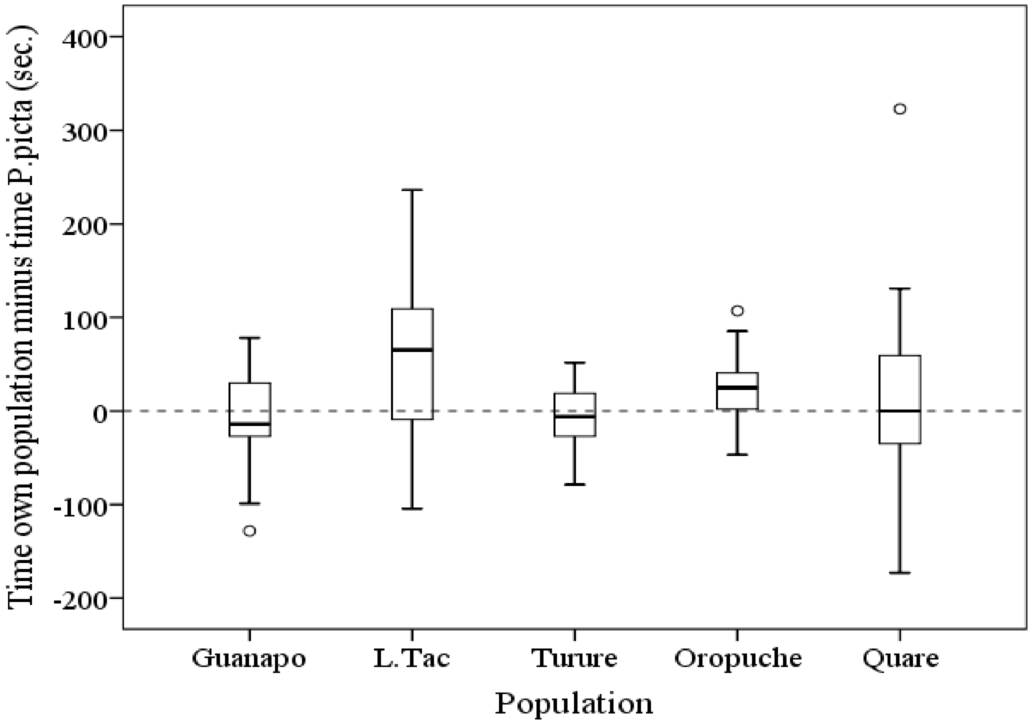


Figure 19: The time guppy females from several populations spent schooling with P. picta was subtracted from the time they spent with their own population. A value of 0 would indicate that no difference between these two measurements exists. Females from the Oropuche and the Tacarigua preferred schooling with their own population over schooling with P. picta whereas females from the Guanapo, Turure and Quare did not distinguish between species origin. Medians, interquartile ranges and outliers are shown.

Female responsiveness to male courtship

There was a significant size difference between the females of different treatments in this experiment (Kruskal-Wallis test, $\chi^2 = 9.76$, d.f. = 3, P = 0.021). Again, treatments differed significantly in the number of sneaky matings females received and the number of responses to male courtship displays after size was accounted for (Sneaky mating: ANCOVA, $F_{3,89} = 4.34$, P = 0.001; Female responses: ANCOVA, $F_{3,89} = 3.18$, P = 0.028). Because the number of sigmoid displays directed towards females did not differ between treatments (see below), no further test was carried out. The number of female responses was combined with the number of matings received by the focal male as an indicator of a female's receptiveness towards male courtship behaviour. No difference was found between trials run in tanks 1 to 4 for the number of sneaky mating attempts directed towards the focal female (Kruskal-Wallis test, $\chi^2 = 1.51$, d.f. = 3, P = 0.681), the number of sigmoid displays (Kruskal-Wallis test, $\chi^2 = 2.37$, d.f. = 3, P = 0.500) nor the number of female responses or matings (Kruskal-Wallis test, $\chi^2 = 0.67$, d.f. = 3, P = 0.881). The data of all tanks were therefore pooled. Further tests investigated if the size difference between the focal female and the companion fish influenced the number of sneaky mating attempts (Mann-Whitney-U test, Z = - 0.29, $n_{larger} = 46$, $n_{smaller} = 44$, P = 0.773), the number of sigmoid displays (Mann-Whitney-U test, Z = - 1.00, $n_l = 46$, $n_s = 44$, P = 0.317) or the number of female responses and matings (Mann-Whitney-U test, Z = 0.00, $n_l = 46$, $n_s = 44$, P = 1.000), but no effect was found.

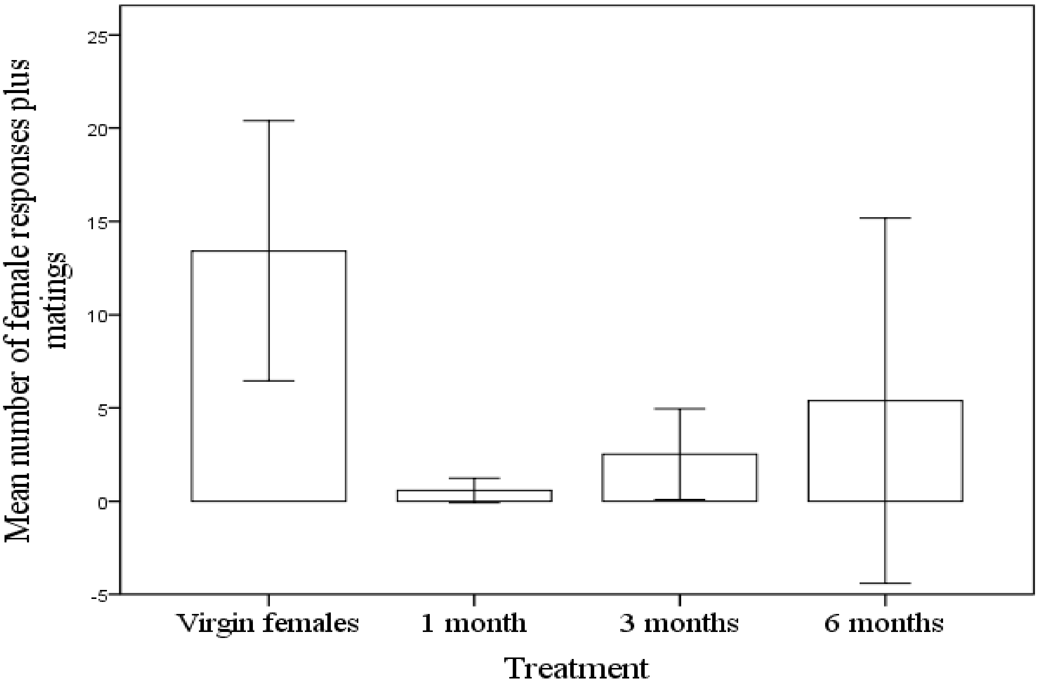


Figure 20: The number of female responses to male courtship behaviour combined with the number of matings the females engaged in for all tested treatments. Error bars represent 95% CI.

87.5% of virgin females responded to male courtship behaviour and/or mated, compared to 20.8% of 1 month females and 28.6% of 3 months and 6 months females, respectively. This corresponded with a mean of 13.83 responses and matings per virgin female, 0.58 responses per 1 month female, 2.52 responses per 3 months female and 5.3 responses per 6 months female, respectively (Figure 20). Kruskal-Wallis tests were used to control for differences in any of the aforementioned behaviours between the four treatments. There was a significant difference between treatments in the number of sneaky mating attempts directed to the focal female (Kruskal-Wallis test, $\chi^2 = 16.08$, d.f. = 3, $P = 0.001$). The number of female responses and matings also differed significantly between treatments (Kruskal-Wallis test, $\chi^2 = 32.03$, d.f. = 3, $P < 0.001$). No difference was found in the number of sigmoid displays directed to the focal female (Kruskal-Wallis test, $\chi^2 = 5.79$, d.f. = 3, $P = 0.122$) (Figure 21). Pairwise Mann-Whitney U tests were carried out to determine which groups differed in each treatment for significant Kruskal-Wallis test results when this seemed reasonable.

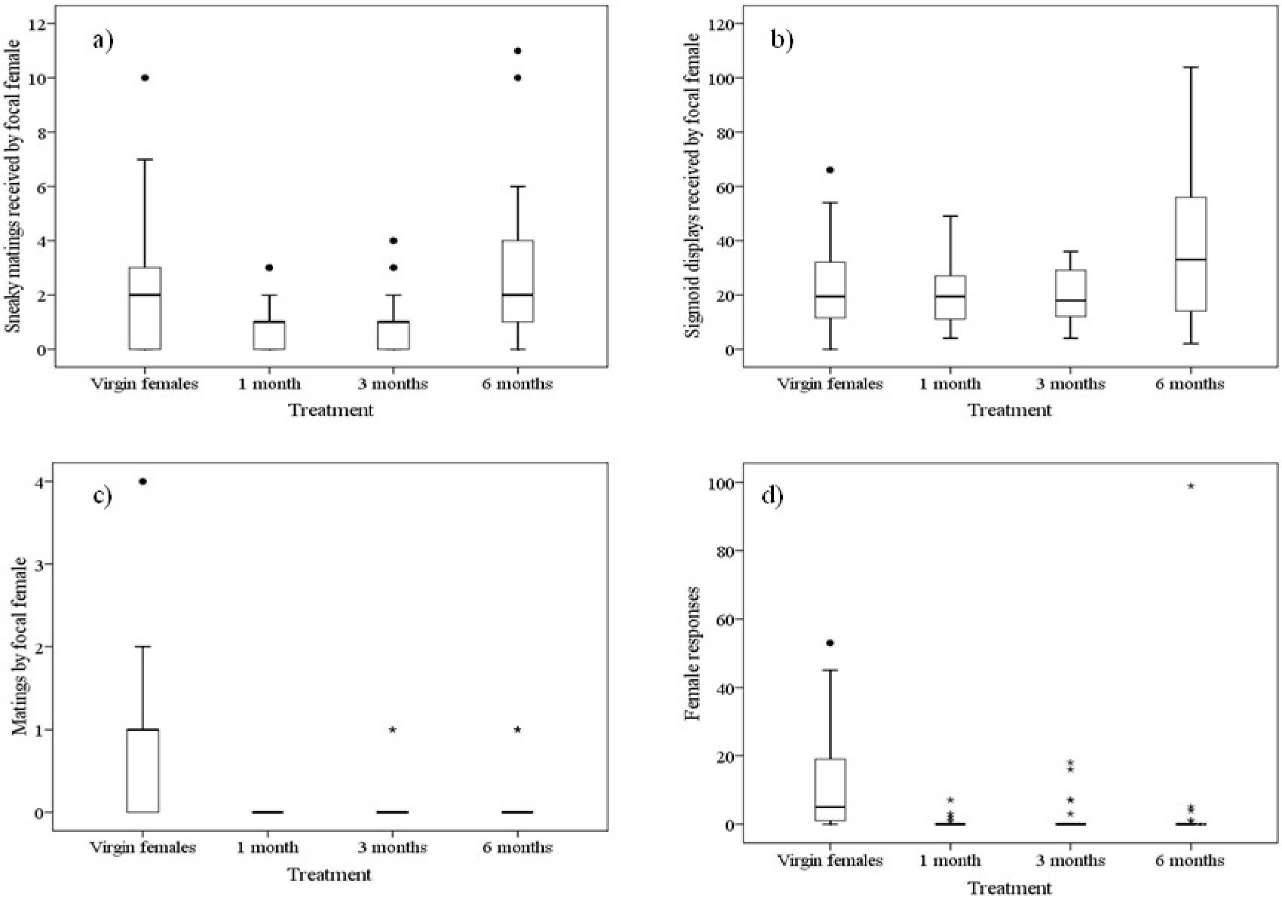


Figure 21: The number of sneaky mating attempts (a), sigmoid displays (b) and matings directed to or received by the focal female (c) as well as the number of female responses to male courtship behaviour (d) for the four treatments of this experiment. Medians, interquartile ranges and outliers are shown.

In order to correct for multiple testing, the significance level was adjusted using the sequential Bonferroni procedure. Virgin females showed a significantly higher number of responses to male courtship behaviour and matings than females from all other treatments. Females from the 6 months treatment received significantly more sneaky mating attempts than 1 month and 3 months females. So did virgin females, but the results were not significant after adjusting α_6 to 0.017. See Table 5 for all P-values and α-statistics.

Table 5: Results of pairwise Mann-Whitney U test conducted for mating behaviours with significant Kruskal-Wallis test results. The significance level was adjusted using the sequential Bonferroni procedure.

Behaviour	Treatment	Z	P-value	α-statistics
Sneaky mating attempts	Virgin – 1 month	-2.19	0.028	$\alpha_6 = 0.017$
	Virgin – 3 months	-2.04	0.042	-
	Virgin – 6 months	-1.08	0.282	-
	1 month – 6 months	-3.43	0.001	$\alpha_4 = 0.010$
	3 months – 6 months	-3.19	0.001	$\alpha_5 = 0.013$
Female responses + matings	Virgin – 1 month	-4.85	< 0.001	$\alpha_1 = 0.006$
	Virgin – 3 months	-3.67	< 0.001	$\alpha_3 = 0.008$
	Virgin – 6 months	-4.04	< 0.001	$\alpha_2 = 0.007$

Discussion

Female guppies did not seem to be able to distinguish between females from their own and other populations of varying relatedness. Fish from some populations, but not others, preferred to school with their own kind over schooling with *P. picta*. These preferences were not restricted to populations from only one drainage system, but seemed to appear randomly in both systems. Females generally showed a low willingness to mate except when virgins, even after having been separated from males for up to 6 months. Their responsiveness towards male courtship behaviour slightly increased with increased time of separation, but not as much as originally expected.

Schooling behaviour and population recognition

Magurran et al. (1994) found that females from two populations preferred to school with familiar over unfamiliar fish from their own population, but did not discriminate between unfamiliar fish of their own and a different population. In their experiments females could only use visual cues to choose their schooling partners, but had no access to odour cues. The availability of odour cues is important for example in differentiating between members of other groups or populations in the stickleback (*Gaterosteus aculeatus*), where fish were able to discriminate between their own group and members of a different group that were geographically separated by just 200 metres. This choice was based on the existence of habitat cues such as the differences in smell produced by feeding on different food sources (Ward et al., 2007). In a later experiment, Ward et al. (2009) found that guppies were able to distinguish between their own tank members and members from another tank based on the smell produced by different food and water sources. In the experiments described in this chapter, fish had access to visual as well as odour cues when choosing between schooling partners. The inability to differentiate between their own and other populations could be explained by the same living and feeding conditions these fish experienced before and during the experiment, a situation similar to an invasive event. There seem to be no population specific differences that are not based on habitat cues that could help female guppies to distinguish between female origin. In an invasive event, as happened in the Turure, different populations would experience the same living conditions and exploit the same food resources, so that they are very likely to produce similar olfactory cues. This would have made it impossible for individual fish to distinguish between their own and the other population, and it is therefore likely that mixed schools were commonplace after the invasion took place. When combined with the finding of Chapter 3, which showed that male guppies did not seem to discriminate between females of different origin, but court any female they encounter, these results indicate the possibility of an increased speed of population and gene pool mixing after an invasion.

It was previously noted that female guppies from two different populations, the Lower Aripo and the Lower Turure, preferred to school with their own kind over schooling with *P. vivipara*, a closely related and very similar looking species (Magurran et al., 1994). It was therefore concluded that guppies generally prefer to school with members of their own species. However, here I tested the ability to discriminate between species in five different populations and found a preference for the focal female's own population over *P. picta* in only two of them, thereby only partly proving the original hypothesis that guppy females should prefer to school with their own species over schooling with *P. picta*. Accordingly, it seems to be likely that this preference is not found in all guppy populations but is restricted to some of them. The preference of female guppies from some populations but not others to school with fish from their own species, was not restricted to one drainage system but occurred in two populations (from the Oropuche and the Tacarigua) belonging to opposite systems. Further experiments are needed to verify the occurrence and distribution of this trait. The fact that the two populations that distinguished between their own kind and *P. picta* belonged to two different drainage systems excludes this trait from being of use when trying to find behavioural or genetic differences that would justify the split of *Poecilia reticulata* into two separate species as suggested by Schories et al. (2009).

Female responsiveness to male courtship

The finding that female willingness to mate was very low except when virgins, and only slightly increased after 6 months of separation from males was unexpected. Because female guppies can store sperm they are able to produce broods for several months after their last mating. But these sperm storages become depleted with time (Magurran, 2005), so females responsiveness towards male courtship behaviour should increase with prolonged time of isolation. The fact that only 6 out of 21 females (28.6%) in the 6 months trial responded to courtship behaviour and/or mated in an one hour long experiment seems to indicate that female guppies have a very low tendency to mate on a voluntary basis during later stages of their life. It is of course possible that these results will differ between different populations or lab bred and wild fish, so more experiments have to be carried out in order to search for general traits, but I frequently noticed the reluctance of females to mate in several populations of wild guppies (personal observation). This low responsiveness would decrease the possibility for pronounced female choice and at the same time increase the importance of forced copulations for reproduction in guppies. There existed a slight but significant preference of female guppies for males from their own population over males from alien populations (Endler & Houde, 1995). In a context where an invasive population enters the territory of a native population, this could lead to a slower mixing rate of gene pools. But if female guppies are reluctant to mate while they can choose, the importance for offspring production via forced copulations increases. And because male guppies do not seem to distinguish between population origin of females, there might be another explanation for an increased mixing of gene pools.

In summary, neither female schooling behaviour and their lack of within-population preference for schooling partners nor a general reluctance of females to mate regularly and thereby perform strong choice for within-population mating partners would stop or slow down the mixing of populations. Instead, in an invasive event this would be likely to encourage and speed up gene pool mixing.

Chapter 5 Life-history traits during a population crossing experiment and schooling behaviour of mixed and pure bred offspring

Abstract

The contact of partly reproductively isolated populations after an invasion, as happened during Haskins's introduction, can give rise to the production of hybrid offspring. These genetically mixed individuals can display behaviours and life-history traits that differ from those of their parents. If some of these traits influence the hybrids' chances of survival, this in turn can affect further population mixing and the direction the invasive process takes. In case of differing influences of parental population origin, offspring reproduction could become skewed between populations. In this chapter I asked if the inter-breeding of fish belonging to populations from the Guanapo and Oropuche has an effect on reproductive traits. No differences such as days until birth and number of offspring could be detected between populations. Newborn fish were then tested in pairs in a schooling experiment to see if and how population origin and especially mixed ancestry influences schooling behaviour. All newborns schooled but significant differences existed between populations. Offspring from the Guanapo and Oropuche significantly differed in their schooling time while newborns from the population crossing experiment displayed a lot of variation. This was comparable to the results obtained from Turure fish that are likely to be genotypically mixed as well. The schooling time of mixed offspring was significantly different between F_1 individuals depending on the origin of the father and the mother of the tested fish. There seems to be a slight effect of population origin in mixed fish on schooling behaviour, an interesting result in the face of the ongoing population mixing in the Turure. Because non-optimal schooling behaviour can reduce an individual's chances of survival, a change of schooling ability due to mixed parentage could channel the direction and speed of further population mixing after an invasion took place by selective survival of individuals with certain genotypes.

Introduction

Several studies have shown that guppy populations from the Caroni and Oropuche drainage system in Trinidad belong to genetically distinct groups that have been separated from each other for up to 2 million years (Ludlow & Magurran, 2006). When Fajen & Breden (1992) analysed mitochondrial DNA-sequences of several Trinidadian populations across the Northern Mountain Range, they found that the variation between Oropuche and Guanapo guppies lay between 3.9 and 5.6% while they hardly detected any genetic differentiation between guppy populations within drainages (0.0 – 1.0% mtDNA variation). A study investigating allozymes came to similar conclusions, showing that the gene diversity based on Nei's gene diversity analysis was 66% due to differences between drainage systems, 32% due to within drainage differences and 2% due to within river variation (Carvalho et al., 1991). More recent investigations detected the existence of some reproductive barriers between Caroni (Tacarigua river) and Oropuche (Oropuche) populations that included hybrid behavioural impairment in the F_1 generation (Russell & Magurran, 2006). Compared to parental controls, male F_1 mating behaviour was decreased. The authors also showed the emergence of hybrid breakdown in the form of smaller brood size and lower sperm counts in the F_2 generation. No reductions were found in female fecundity, implying that guppies obey Haldane's rule for sterility (Laurie, 1997) which states that when in the offspring of two different animal races one sex is absent, rare, or sterile, it is the heterogametic sex. In the same year, Ludlow & Magurran (2006) reported the existence of partial gametic isolation between guppies belonging to different drainage systems. In a competitive context the sperm of native males was more successful in fertilising eggs than the sperm of males belonging to the other drainage system. This effect was symmetrical for both drainages. In a non-competitive situation no differences in sperm performance could be found. The genetic divergence between drainage systems together with arising reproductive barriers is a sign of emerging post-copulatory isolation and indicates that guppies from the Caroni and Oropuche drainage systems are on the verge of splitting into two separate species. Schories et al. (2009) on the other hand argued that populations from both drainage systems belong to two distinct species that already separated between 2.5 and 5 million years and 0.4 and 4.2 million years ago, depending on the genetic regions analysed in their study (cytochrome b and mitochondrial control region).

Any form of reduced reproductive performance due to not completely compatible genomes might influence the outcome and direction of population mixing after an invasive event or help to keep not fully reproductively isolated, sympatric species separate. Vamosi & Schluter (1999) showed that male F_1 hybrids between sympatric benthic and limnetic sticklebacks (*Gasterosteus aculeatus* complex) had reduced mating success when competing with limnetic males in the wild. Sneaking behaviour in Atlantic salmon (*Salmo salar*) leads to the fertilisation of a high proportion of brown trout (*Salmo trutta*) eggs, but hybrids show low survival rates and are therefore not threatening interspecific barriers (Garcia-Vazquez et al., 2002). But the appearance of non-adaptive behaviours outside a mating context, such as schooling behaviour, could have an impact on the performance of genetically mixed offspring as well.

Because the propagule size in Haskins's introduction was large enough to prevent a large loss of genetic diversity, the mean allele frequency of the introduced population is only slightly lower than the frequency found in the source population (1.2 vs. 1.4) (Carvalho et al., 1996). The newly established population in the Turure still contained enough genetic diversity for fast adaptive evolution to work on behaviour. For example, the schooling tendency of fish above the waterfall was significantly reduced, while it was well developed in the middle stretches of the Turure (Magurran et al., 1992). This means that the individuals of this population adjusted their behaviour towards the low predation pressure they were facing after the introduction, but increased the time they spent schooling with the once more raising predation level in the middle parts of the river. The optimal amount of schooling behaviour, based on the presence or absence of certain predators, is essential to decrease the risk of predation without wasting feeding or mating opportunities. Magurran & Seghers (1991) detected a trade-off between the amount of anti-predator behaviour and resource defence in guppies. Individuals belonging to low predation populations that exhibit a decreased amount of schooling behaviour were more aggressive while defending a food patch than their high predation counterparts. The authors argued that the shorter time-lag before the onset of aggressive behaviour in low predation habitats might be advantageous for individuals to quickly defend resources. In high predation habitats on the other hand, an increase in safety due to many schooling partners is of higher importance.

Chapter 4 investigated the schooling behaviour of adult females belonging to several different populations across the Northern Mountain Range. In the present chapter, the schooling tendency of newborn fish was tested and compared to that of adult fish from the same population. In another study done by Magurran & Seghers (1990), it was shown that newborn guppies school from birth, but again that differences between populations exist. The amount of time spent schooling did not necessarily match the pattern found in adult fish. These discrepancies were explained by different predation pressures acting upon newborns and fully grown fish. While adults in low predation habitats are relatively safe from the only predator present, *Rivulus hartii*, guppy offspring are an important component of its prey and should therefore show well-developed schooling behaviour. In high predation habitats on the other hand, the main predation threat is imposed on grown-up fish. Nevertheless, because cannibalism of newborns by adult guppies can occur independently of predation regime, schooling abilities should be developed in any case (Magurran & Seghers, 1990).

But what happens to a behaviour as important for survival as schooling together with other fish in the case of gene pool mixing? Differences in a population's tendency to school are controlled by genetic factors and correlated with the predation pressure this population faces (Seghers, 1974). Despite coming both from high predation populations, however, female guppies belonging to the Guanapo and Oropuche spent different amounts of time schooling, as was shown in Chapter 4. Here I investigated whether similar differences existed in the schooling behaviour of newborn fish. Behavioural differences in schooling time could influence survival rates of individuals belonging to different populations living in the same habitat, e.g. after an invasive event. More important than behavioural population differences in newborn guppies per se is the influence of mixed parentage on offspring schooling ability. How does parental origin influence schooling behaviour in hybrids? Variation from the optimal schooling tactic due to genetic constraints could decrease the chances of survival for mixed offspring and could therefore influence the amount and direction of population mixing in the Turure.

Other behavioural and life-history traits vary between low and high predation habitats. Females experiencing high predation mature earlier and produce more and smaller offspring per brood (Reznick & Bryga, 1987) than female guppies from low predation habitats. However, no large variations in reproductive traits are expected to exist between Guanapo and Oropuche fish as both of them belong to high predation populations (Willing et al., 2010). Decreasing rates of offspring production that occur after a population mixing event could therefore indicate the existence of reproductive barriers.

In this study, I investigated the influence of mixed parentage on several reproductive traits including days until birth, number of offspring and offspring size. I crossed virgin Guanapo and Oropuche females with males belonging to the opposite population. I then explored the schooling behaviour of F_1 fish and compared this to the time pure bred newborns from several different populations across the Northern Mountain Range spent schooling. Possible influences of the origin of each parent on the schooling tendency of mixed fish were also taken into account. I tested for differences in schooling behaviour of offspring with an Oropuche mother and Guanapo father vs. offspring with a Guanapo mother and Oropuche father and predicted to find that population origin of the parents has an effect on the schooling behaviour of mixed offspring. Differences here might help to understand the inheritance patterns of schooling behaviour in future experiments.

Methods

During March to June 2009, the lab-born offspring of wild caught Guanapo and Oropuche females were raised in groups of four in small plastic tubs containing 1 litre of water. From the age of approximately 6 weeks, juveniles were checked every four to six days for the development of a gonopodium, the modified anal fin of males. As soon as fish could be sexed, virgin females were transferred into one of three stock tanks (45 x 31 x 30 cm) per population. All tanks contained a filter and a plastic plant. Females remained in these tanks until January 2010, when they were used for the population crossing experiment.

All experiments were carried out between January and March 2010 at the University of the West Indies, Trinidad, using wild caught fish or their direct descendants. Fish were kept on a 12 L: 12 D cycle using 18W fluorescent daylight bulbs. The tank temperature was maintained at 26 ± 0.5°C. Water used to fill the tanks was taken from the tab and left to age for at least 48 hours. The water in newly set-up tanks was treated with STRESS COAT®. Fish were fed with TetraMin flake food in the morning about half an hour before the first trial and freshly hatched *Artemia* larvae in the evening. Juveniles were fed with TetraMin Baby powder food in the morning and freshly hatched *Artemia* larvae in the evening.

Population crossing

In January 2010, 27 virgin females from the Oropuche and 18 females from the Guanapo were transferred into individual white plastic tanks (25 x 14 x 14 cm), filled with 2.5 litres of water. The tanks contained some *Elodea canadensis* for cover and the water was changed every four days. Each female was then combined with one wild-caught male of the opposite population (Oropuche female x Guanapo male; Guanapo female x Oropuche male) and left to interact freely. Tanks were checked for newborn offspring on a daily basis, starting three weeks after the introduction of the male. Once a clutch of juveniles was born, the number of offspring and the date of birth were noted. A picture was taken of each newborn and their mother for length measurements. The offspring were then transferred into one of two holding tanks (45 x 22 x 20 cm; sorted by treatment), where they were kept for 1-3 days until enough fish were born to run several trials. This was done in order to minimise the chance of testing siblings together in the same trial. The holding tanks contained a filter and some *Elodea* for cover; the bottom was layered with coarse gravel. Three sides of the tanks were covered with black plastic to provide a uniform background.

Differences in schooling behaviour between pure and hybrid offspring

Juvenile schooling trials took place using a white metal tray (34 cm in diameter), filled with 2 cm of water. Two randomly chosen newborns were carefully transferred into the tray and given 5 min. to settle. The time fish spent schooling together within 3 cm (= 3.5 body lengths) of each other was then recorded for another 5 min. (see Evans & Magurran, 2000). After the trial, a picture was taken of both fish for length measurement and they were transferred into a new holding tank. No fish was used more than once during the course of this experiment. In trials where fish of other populations were needed, these came from wild-caught females used for other experiments. See Table 6 for the grid references of all populations.

Table 6: Grid references of populations used during the crossing experiment and subsequent offspring schooling trials. The drainage system each river belongs to is indicated where relevant.

Population	Grid reference number	Nearest road	Drainage system
Lower Tacarigua	PS 774 763	off Churchill Roosevelt Highway	Caroni
Guanapo	PS 913 765	Eastern Main Road	Caroni
Quare	PS 979 779	Valencia Road	Oropuche
Oropuche	QS 042 788	Valencia Road	Oropuche
Turure	QS 002 784	Valencia Road	

Newborns of several different populations, as well as hybrid offspring, were tested in a series of trials, always using the standard protocol described above. For a complete list of treatments, including the numbers of observed pairs, see Table 7.

Table 7: The population origin of both experimental juveniles and number of tested pairs per treatment in the schooling behaviour time trials.

Population	Tested with	N
Lower Tacarigua	Lower Tacarigua	25
Guanapo	Guanapo	25
Quare	Quare	25
Oropuche	Oropuche	25
Oropuche	Guanapo	25
Turure	Turure	25
[Guanapo mother x Oropuche father]	Guanapo	20
[Oropuche mother x Guanapo father]	Oropuche	20
[Guanapo mother x Oropuche father]	[Guanapo mother x Oropuche father]	20
[Oropuche mother x Guanapo father]	[Oropuche mother x Guanapo father]	20

To compare juvenile schooling behaviour to that of adult females, 25 Guanapo and Oropuche females, respectively, were tested for their general schooling tendency in a choice tank (see Chapter 4 for methods). Instead of two sets of females offered as companion fish, one bottle inside the tank remained empty. The time females spent within two body length of the bottle containing size matched females from their own population was measured for 10 min. (Figure 22).

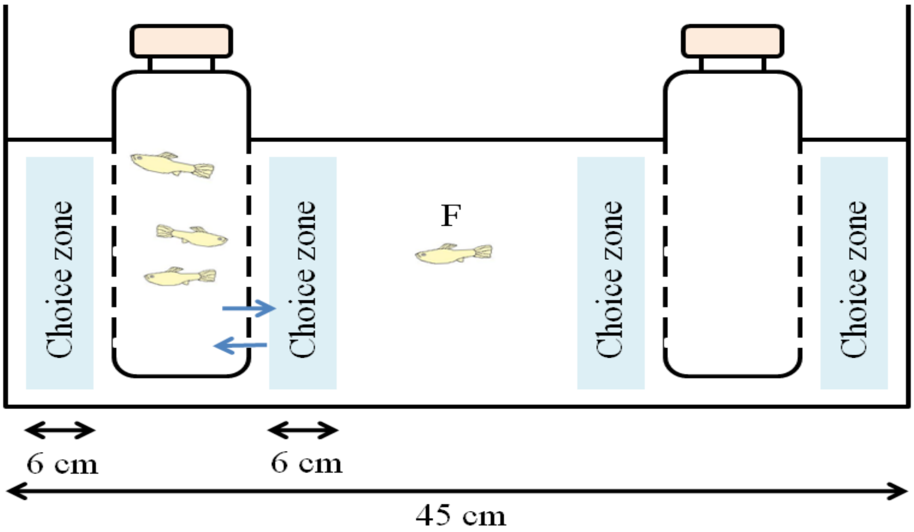


Figure 22: Experimental set-up of the choice tank used to test for the general schooling tendency of adult females. The choice zones around the Plexiglas bottles that either contained a set of companion fish or stayed empty, are highlighted in blue. The focal female is marked with an F.

Results

Population crossing

Of the 27 Oropuche females paired with a Guanapo male, 20 females produced a total of 130 offspring within 55 days. Two females died during the course of the experiment, and five females did not give birth within 55 days. Of the 18 Guanapo females paired with an Oropuche male, 15 females produced a total of 114 offspring within 55 days. Two females died before producing any offspring, and one female did not give birth until the end of the experiment. Guanapo females had a mean size of 2.74 cm ± 0.10 S.D. and needed an average of 40.27 days ± 7.36 S.D. to give birth to a mean number of 8.79 offspring ± 2.91 S.D. Oropuche females reached a mean size of 2.75 cm ± 0.15 S.D. and took an average of 41.55 days ± 4.73 S.D. to produce a mean clutch size of 7.49 ± 2.03 S.D. There was no difference in the mean size of females (Mann-Whitney-U test, $Z = -0.276$, $n_{Gua} = 15$, $n_{Oro} = 20$, $P = 0.782$) or in the average time needed to produce offspring (Mann-Whitney-U test, $Z = -0.501$, $n_{Gua} = 15$, $n_{Oro} = 20$, $P = 0.616$). To compare results from the population crossing experiment with unmixed fish, wild type Guanapo and Oropuche females were included in the analysis of brood size and offspring size. No difference was found in the mean brood size of females belonging to different treatments (Kruskal-Wallis test, $\chi^2 = 1.199$, d.f. = 3, $P = 0.753$). However, there was a difference in the mean offspring size between treatments (One-way ANOVA, $F_{8,261} = 10.05$, $P = 0.004$). A LSD Post Hoc test found that offspring produced by Oropuche mothers and Guanapo fathers were significantly smaller than newborns produced by females in the other three treatments (Gua ♀ Oro ♂: $P < 0.001$, Guanapo: $P < 0.001$, Oropuche: $P = 0.001$) (Figure 23).

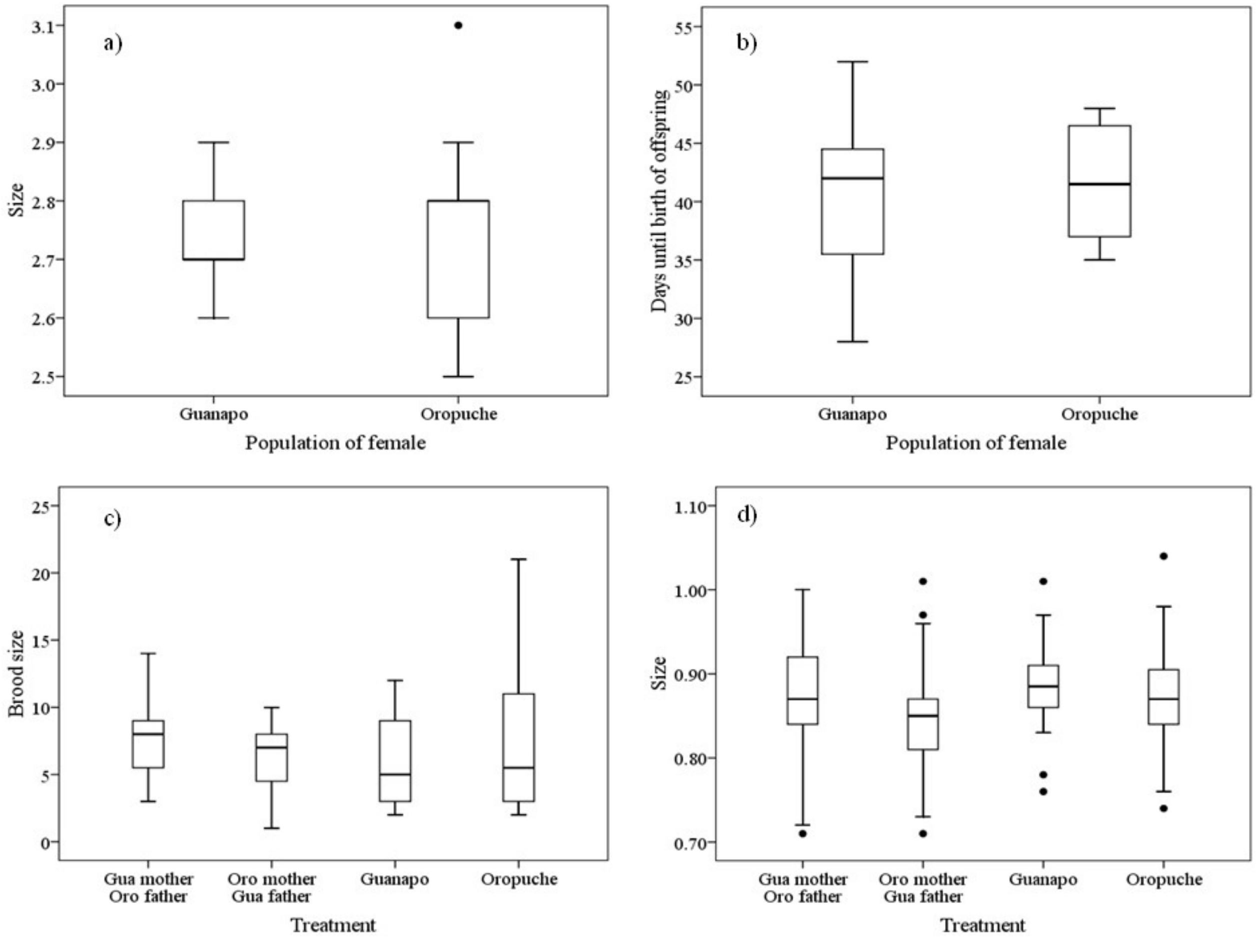


Figure 23: The size (a), the number of days until the birth of offspring (b) and brood size (c) of experimental females as well as offspring size per treatment (d). Data of wild type Guanapo and Oropuche females and offspring are included for better comparison (c+d). No differences existed between any female traits, but offspring from the Oropuche mother - Guanapo father cross were significantly smaller than newborns produced in the other three treatments. Medians, interquartile ranges and outliers are shown.

Differences in schooling behaviour between pure and hybrid offspring

The results for the time newborn fish spent schooling in different treatments were arcsine transformed in order to achieve normal distribution. There was a significant size difference between the treatments in the newborn schooling trials (Kruskal-Wallis test, $\chi^2 = 16.28$, d.f. = 8, P = 0.039) (Figure 24), therefore size was included as a covariate while testing for the effect of treatments on the time newborns spent schooling. However, offspring tested in different treatments differed significantly from each other in their schooling activity after the effect of body size was accounted for (ANCOVA, $F_{8,269} = 15.66$, $P < 0.001$).

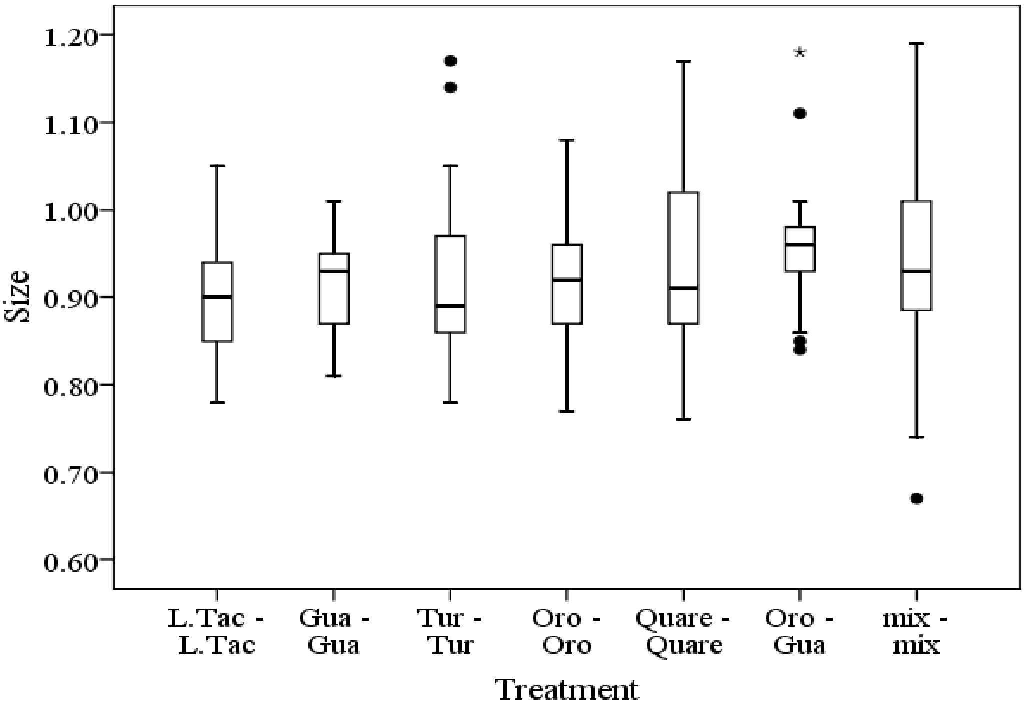


Figure 24: Total body length of offspring belonging to different treatments of the schooling experiment. A significant size difference existed between at least one of the treatments and the rest. Medians, interquartile ranges and outliers are shown.

A significant difference existed between treatments in the amount of time fish spent schooling (One-way ANOVA, $F_{8,269} = 15.69$, $P < 0.001$). Newborns in the Guanapo treatment spent significantly less time schooling than fish from all other treatments. There was also a difference between offspring from the Oropuche – Guanapo and the mixed – mixed treatment. Guanapo newborns spent a mean time of 47.3 sec ± 25.2 S.D. out of 300 sec schooling, while Oropuche newborns on average stayed close to their companion fish for 117.5 sec ± 49.0 S.D. When offspring from both populations were tested together, the mean time they spent schooling (102.8 sec ± 38.7 S.D) was intermediate to the results from single population treatments (Figure 25a). Compared to the other treatments, mixed fish, as well as Turure offspring that are likely to be of mixed parentage, showed a large amount of variation in the time they spent schooling (Mixed – mixed: 142.2 sec ± 74.2 S.D.; Turure – Turure: 127.2 sec ± 75.5 S.D.). (Figure 25b).

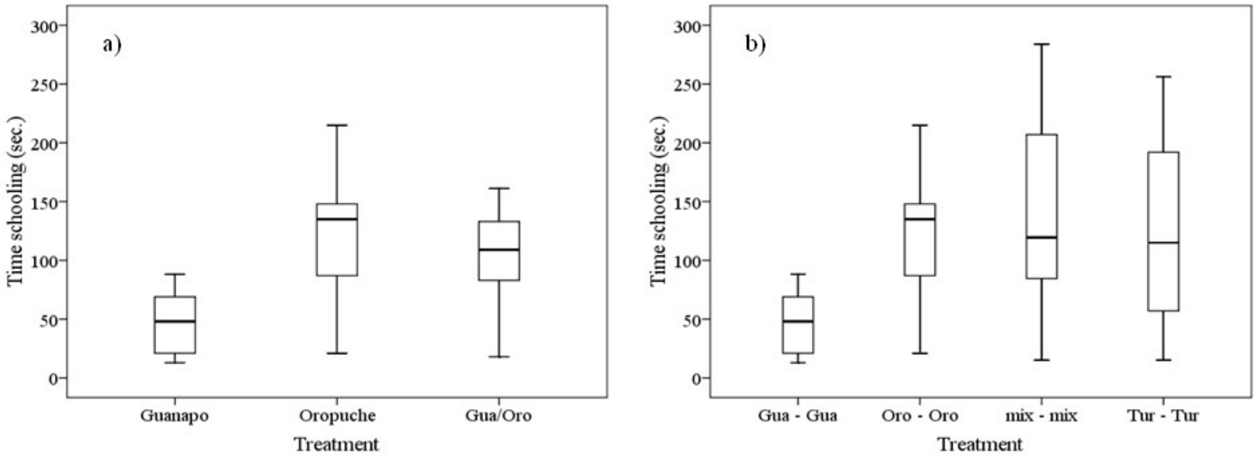


Figure 25: The time newborn fish from different populations or mixed parentage spent schooling. Guanapo offspring schooled significantly less than Oropuche newborns while the time spent schooling was intermediate when a Guanapo and a Oropuche fish were tested together (a). Mixed offspring showed a lot of variation in the amount of time it spent schooling, a result that resembled the schooling behaviour of Turure newborns (b). Medians and interquartile ranges are shown.

The relevant results of the LSD Post Hoc test and α-statistics are listed below (Table 8). In order to correct for multiple testing, the significance level was adjusted using the sequential Bonferroni procedure.

Table 8: Relevant results of the LSD Post Hoc tests carried out after an One-way ANOVA showed a significant difference between treatments in the time newborn fish spent schooling. The significance level was adjusted using the Bonferroni procedure.

Compared treatments	P-value	α
[Gua-Gua] – [Oro-Oro]	< 0.0001	$\alpha_1 = 0.00089$
[Gua-Gua] – [Oro-Gua]	< 0.0001	$\alpha_2 = 0.00091$
[Gua-Gua] – [Mix-Mix]	< 0.0001	$\alpha_3 = 0.00093$
[Gua-Gua] – [Tur-Tur]	< 0.0001	$\alpha_4 = 0.00094$
[Oro-Gua] – [Mix-Mix]	0.007	-
[Oro♀Gua♂-Oro♀Gua♂] - [Gua♀Oro♂-Gua♀Oro♂]	< 0.0001	$\alpha_5 = 0.00096$

To investigate if and how parental origin in a cross influences their offspring's behaviour, fish from mixed treatments were further divided into two groups depending on their parents' population:

1. Guanapo mother x Oropuche father
2. Oropuche mother x Guanapo father

As can be seen in Figure 26, fish differed in the amount of time they spent schooling depending on their parents' origin when two fish of mixed parentage were tested together (see Table 8 for P-values). Treatments testing one fish of mixed ancestry and either a pure bred Oropuche or Guanapo newborn did not differ from each other.

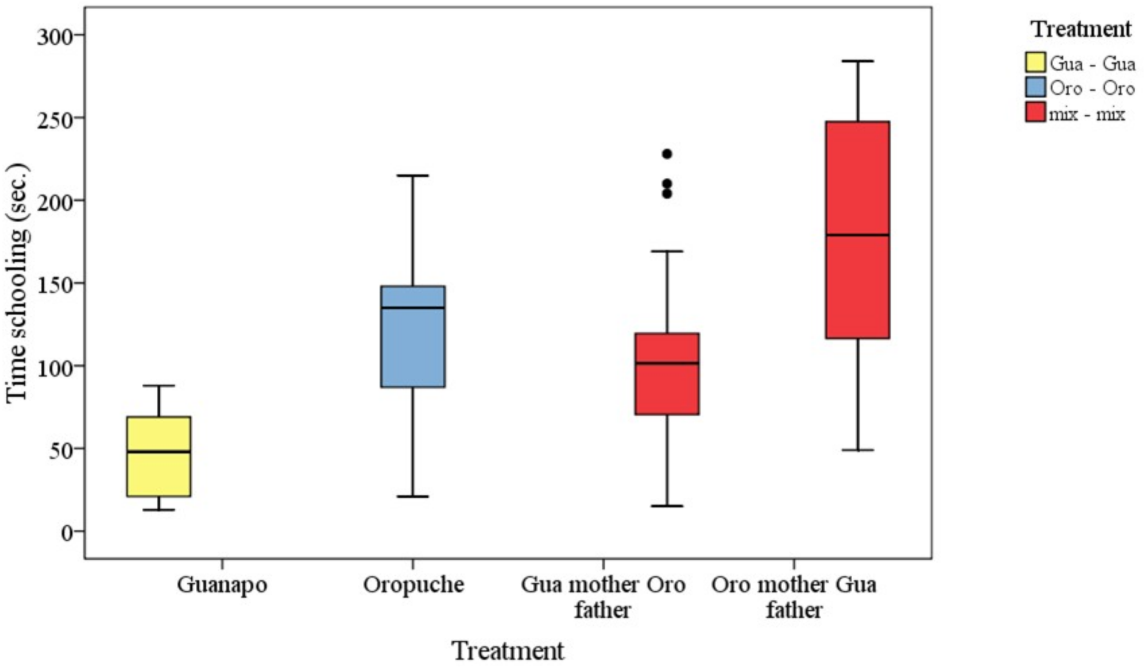


Figure 26: The results of the mixed treatments were split into two groups based on the population origin of both parents. A significant difference existed between (Gua mother – Oro father) and (Oro mother – Gua father) treatments. Medians, interquartile ranges and outliers are shown.

Furthermore, the time individuals spent schooling was compared between females and offspring of each population. Visible differences existed between each tested group of females and offspring belonging to the same population (Figure 27).

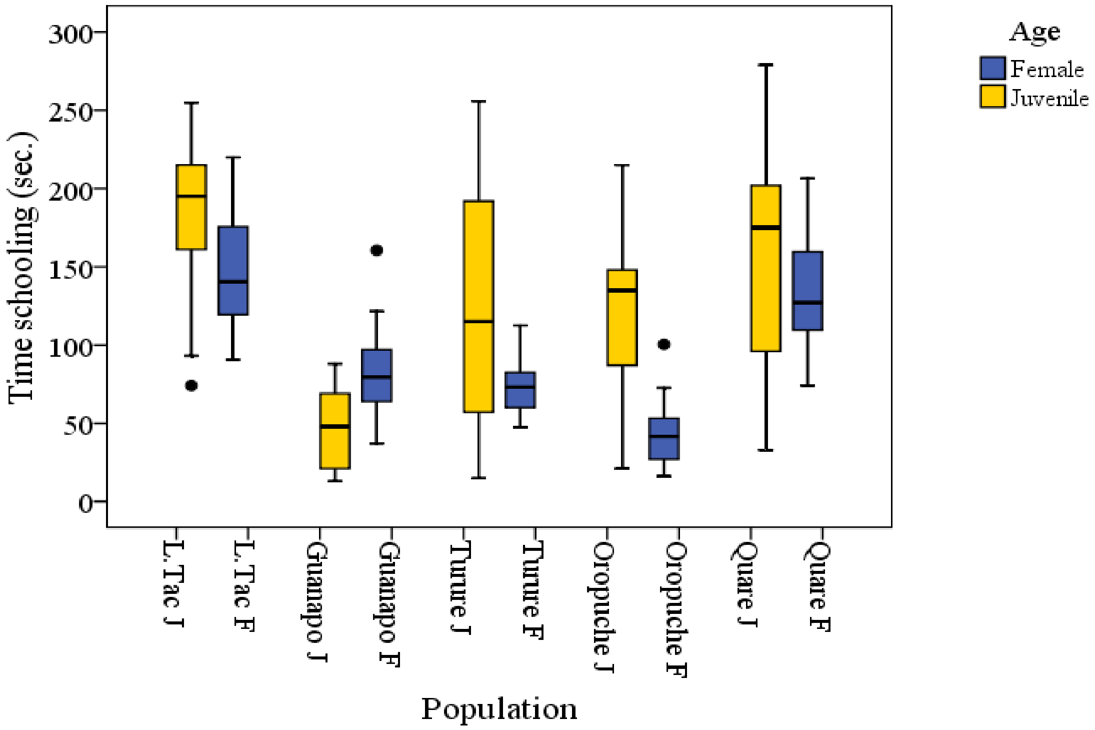


Figure 27: The time fish from all tested populations spent schooling is compared to the time of adult females of the same origin. A difference between females and offspring was detected in all populations. Medians, interquartile ranges and are shown.

In order to better be able to compare the results of females and s, the schooling time of females was divided by 2 to account for the longer time each trial lasted (10 min. vs. 5 min. for newborns). However, because experimental procedures between newborn sand females differed quite remarkably, only the actual schooling time of adults and newborns was plotted in a graph, without using statistics. In case of the Tacarigua (♀: 147.2 sec, newborns: 188.3 sec), Turure (♀: 72.8 sec, newborns: 127.2 sec), Oropuche (♀: 43.8 sec, newborns: 117.5 sec) and Quare (♀: 133.3 sec, newborns: 158.6 sec), adult females spent less time schooling than newborns, while Guanapo females schooled more than newborn fish of the same population (♀: 82.3 sec, newborns: 47.3 sec). Adult females generally exhibited less variation in the amount of time they spent schooling together compared to newborn fish.

Discussion

Female reproductive traits such as number of days until birth, body size and brood size did not differ between treatments depending on population origin and crossing scheme. However, newborn fish from the Oropuche mother – Guanapo father treatment were significantly smaller than newborns produced in the other treatments. When compared to their mothers, all tested offspring showed a different schooling time that was longer in newborns than in adults in all but one population. Guanapo newborns schooled significantly less than Oropuche fish and when offspring of both populations was tested together, the time spent together lay between the values for single population treatments. Mixed offspring tested together displayed a high degree of variation in schooling behaviour which resembled the results in Turure offspring. When separated by population origin of their parents, mixed newborn fish with a Guanapo mother and Oropuche father spent significantly less time schooling than offspring with a Oropuche mother and a Guanapo father, which means the hypothesis that population origin of the parents has an influence on newborn schooling behaviour can be accepted.

Population crossing

The similarities between female reproductive traits in both crosses, such as days until birth and brood size, indicate that both populations face similar predation pressure in the wild and therefore display comparable morphological and life-history traits, as was discovered by Reznick & Bryga (1987) in an introduction experiment. No difference existed between brood size of females belonging to mixed crosses and pure bred control females. These results are in agreement with those of Ludlow & Magurran (2006), who reported the existence of partial genetic isolation between guppies belonging to different drainage systems in a competitive context but not in single male trials. In my population crossing experiment, a single male was paired with each virgin female, therefore sperm competition could not have influenced the result. However, due to the relatively small sample size it would be hard to detect minor differences in these traits. For the same reason it is also difficult to interpret the smaller body size in newborn fish from the Oropuche mother – Guanapo father treatments compared to the other cross and pure bred offspring from both populations. This might be an artefact that would disappear with a larger sample size. Nevertheless, it might also show that it makes a difference for mixed offspring as to which population their mother and their father belong. If that is the case, a non-symmetrical way of inheritance would be expected between the sexes, with either the mother or the father contributing more to a particular trait. However, it might also be that an increase in sample size would show a smaller body size in hybrids of both crosses. In a study looking at the consequences of lineage mixing in the North

American bison (*Bos bison*), it has been found that hybrid F_1 newborns had slower growth rates and reached maturity later than pure bred animals. This can have potential consequences for winter survival as well as decreased lifetime production of offspring (Berger & Cunningham, 1995). To examine this question in further detail, an increase in sample size per cross as well as parallel treatments using pure crosses would be necessary and could help to disentangle the effects of differences in life-history traits and an effect of population origin and sex on offspring production traits.

Differences in schooling behaviour between pure and hybrid offspring

As expected, newborn offspring from all tested populations displayed schooling behaviour, while significant differences between populations existed (see Magurran & Seghers, 1990). Oropuche newborns schooled significantly more than Guanapo offspring, while the time spent schooling was intermediate when newborns of both populations were tested together. This result was expected in a context where fish preferring different behavioural strategies had to find a compromise between their optimal schooling times.

Other than the hybrid behavioural impairment found in F_1 males (Russell & Magurran, 2006), schooling ability itself did not seem to be affected by mixed parentage in the F_1 generation. In all trials, hybrid offspring displayed schooling behaviour. However, the variation found in the times spent close to a companion fish was very large (Mean time: 142.2 sec. ± 74.2 S.D.). In contrast, the variation found in trials testing one Guanapo and one Oropuche together was less pronounced. Direct genetic impacts of mixed parentage on the schooling behaviour of newborn fish are therefore likely. This result is further strengthened when compared to schooling times in Turure offspring. They resembled the times measured in mixed offspring and were extremely variable (Mean time: 127.2 sec. ± 75.5 S.D.). The mothers of the Turure newborns used in this experiment came from the Valencia Road site. Genetic analysis using SNP markers had shown that at this site only 12.14% of nucleotides belonged to the original Turure genotype (see Chapter 2). This means that most alleles found in the current population are of Guanapo origin. Fish are therefore unlikely to have many Turure alleles and probably resemble more pure bred Guanapo fish than the mixed offspring in my experiment.

However, several possible theories could explain why the results of mixed newborns and Turure offspring still show strong similarities. It might be that several alleles on different linkage groups form the genetic basis of schooling behaviour and that already small changes in their composition can influence this trait. This could also explain the large variation found both in mixed and Turure offspring. Another possibility would be that any changes in the optimal schooling behaviour reduce an individuals chance of survival. For example, the anti-predator behaviour shown by farmed Atlantic salmon (*Salmo salar*) was significantly reduced compared to responses in wild fish. The hybrid offspring displayed intermediate behaviour to those of the parental populations (Houde et al., 2010). That means that a continuous influx of farmed fish into wild populations can influence anti-predator behaviour and shift the displayed response away from its optimum, with consequences for offspring survival. If these changes in schooling time originate from non-optimal combinations of alleles in fish, certain genotypes might be less likely to survive to adulthood and reproduce than others. It would therefore be possible that offspring with many Turure alleles display schooling behaviour that is further away from the optimum than newborn fish that mainly have Guanapo alleles. Hence, Turure newborns might be more likely to be preyed upon and therefore did not contribute a lot to the adult gene pool which was genetically analysed in Chapter 2. Unlike than their offspring, adult Turure females display a narrow range of schooling time (Mean time: 72.8 sec ± 17.6 S.D.). That means they either adapted to the predation regime they experience by learning or only those with optimal genotypes survived long enough to mature. This difference in the displayed range of schooling time between adult females and newborn guppies can be seen in several tested populations but is most obvious in Turure fish. It is therefore likely that a relative increase in individuals showing the optimal amount of schooling behaviour either by learning or selective survival is a general trend in guppies while fish mature.

To get a better insight into possible differences in the schooling behaviour of newborns due to population origin of their parents, newborns of mixed treatments were split into two groups depending on the origin of the mother and the father in a cross. A significant difference existed between the amount of time schooled by newborns with an Guanapo mother – Oropuche father and fish belonging to the reciprocal cross (Oro mother – Gua father). Offspring from the first treatment spent less time schooling and thereby resembled the result of pure bred Guanapo fish while newborns with a Oropuche mother and Guanapo father spent more time schooling, similar to pure bred Oropuche offspring. It seems therefore that not only the mixing of gene pools can influence this important behaviour, but that males and females contribute in an at least slightly different proportion to this trait in their offspring.

Taking the results displayed in this chapter together, it becomes obvious that a large amount of variation in schooling time existed in hybrid offspring compared to pure bred newborns. It also mattered for the display of schooling behaviour from which population the father and the mother within a cross came. There seemed to be a subtle effect of parental population origin in mixed fish. Individual populations differed quite remarkably from one another, but no real differences between drainage systems could be detected. While differences in schooling behaviour cannot therefore be used to distinguish between subspecies, it might still be interesting to do further crossing experiments including F_2 and back cross. The genetic basis of this important behaviour and how a mixed genetic background influences schooling ability and therefore survival rates in the face of different predation habitats should be further examined. This could then be used to disentangle the effect of inheritance, learning and different survival rates on the schooling times observed in Turure fish.

A next step would now be to raise pure and mixed offspring and test their schooling behaviour as adults. These results could be compared to the schooling times of wild caught females which would be particularly interesting for fish from the mixed treatments and the Turure. To gain a more complete insight into the consequences of an arising reproductive isolation between Caroni and Oropuche guppies, the crossing experiment should be taken further to the F_2 generation and back cross. This would help to investigate if a hybrid breakdown similar to the one discovered for male mating behaviour in the F_1, and brood sizes as well as sperm counts in the F_2 (Russell & Magurran, 2006) also affects the schooling ability in newborns and adults. Any genetically induced differences here that prevent fish from displaying the right schooling tactic according to the predation regime they are facing would have an impact on survival rates and therefore change the reproductive success of mixed fish relative to pure-bred individuals. Because, in accordance with Haldane's rule, male guppies seem to be more affected by mixed parentage in their reproductive traits, special attention should be paid to possible differences in the schooling behaviour of male and female offspring as well as adult fish. Another aspect worth looking at is the genetic basis of schooling behaviour and how it is influenced by population mixing.

Chapter 6 The effects of gene pool mixing on the genetic structure of two guppy populations in a mesocosm experiment

Abstract

A genetic/reproductive advantage such as higher offspring production held by one population over another could contribute to the success of invading individuals and increase the speed of gene pool mixing even when the invader's population size is still small. This experiment was meant to explore the importance of reproductive superiority vs. the proportion of introduced fish belonging to a certain population. SNP markers were used for the genetic analysis of fish from a mesocosm experiment, where two populations from the Guanapo and the Oropuche were mixed in different proportions. Invasive Guanapo fish did not contribute to the observed gene pool in a way as would have been expected in the presence of a genetic advantage over Oropuche fish. Nucleotide proportions belonging to both populations also did not resemble the original proportions with that each mesocosm was stocked. On the contrary, two out of three mixed treatments showed a clear excess of Oropuche alleles. The fact that fish belonging to pure Guanapo and Oropuche treatments were not completely homozygous for each SNP, together with the possibility of genetic drift due to a small initial population size, could have contributed to the observed domination of Oropuche type nucleotides, but can not fully explain them. An at least small reproductive advantage of Oropuche fish over Guanapo guppies must therefore have existed during the course of the experiment. Because this result is directly contrary to the arguments (such as the more successful securing of mating opportunities by males) employed for explaining the success of Guanapo fish in the Turure, more factors than just the simple mixing of populations are likely to have contributed to the present situation in this river. Differences between Haskins's introduction and the mesocosm experiment that could have influenced the results are addressed in the discussion.

Introduction

Biotic homogenisation as one of the most important factors that can reduce biodiversity and give rise to an increased similarity between different communities was referred to several times in previous chapters. Two distinct processes are responsible for biotic homogenisation: the extinction of native species and the invasion of alien populations into new areas (Olden & LeRoy Poff, 2003).

Intra-specific hybridisation can quickly homogenise the unique characteristics of geographically distinct populations as well as influence the fitness of individuals by disrupting local adaptations (Olden et al., 2004). With or without introgression, hybridisation between species or genetically distinct populations often endangers their survival in a wide variety of plant and animal taxa (Rhymer & Simberloff, 1996). When Hindar et al. (2006) simulated the future of wild Atlantic salmon (*Salmo salar*) populations using different intrusion rates of escaped farmed animals, the authors found that only in low-invasion scenarios would wild populations stay relatively unaffected by escaped farmed fish while with a medium intrusion rate of 20% extensive changes in the population structure of wild salmon would occur within around 10 generations. In case of persistent high-invasion their model suggests that populations found in the wild will consist of hybrid and farmed offspring eventually. In case of wild salmon this is particularly worrying because genetic effects on performance traits like offspring survival and growth appear to be negative when compared to unaffected native populations (Hindar et al., 1991).

A similar situation can be observed in the Turure. After more than 50 years of population mixing, the native genotype has nearly disappeared in long stretches (up to 5 km downstream the introduction site the proportion of the original genotype is below 15% of the total population) of the river, while invasive Guanapo fish dominate the present population (Shaw et al., 1992; Russell, 2004). Possible reasons other than differences in behavioural and life-history traits that could have played a role in the displacement of the native Turure population by invading Guanapo fish were therefore investigated in the following chapter.

As soon as Guanapo guppies started to invade the stretches of the Turure that were already occupied by a native population, spreading patterns and gene flow became important for population mixing and a successful further colonisation of the river. Male guppies frequently travel between different schools and were more likely to emigrate from their pool of release than females (27.3% vs. 6.9%, respectively) in a mark-recapture experiment by Croft et al. (2003). They are thereby covering distances that are great enough to prevent the evolution of reproductive barriers between populations inhabiting the same river, despite the strong influences that the existing predation regime has on morphology, life-history and behaviour. In a study using microsatellites, Crispo et al. (2006) showed that the amount of gene flow within predation regimes was the same as between predation regimes. Females, on the other hand, normally show site fidelity and usually return to their site of origin if replaced. However, only 12% of haplotypes found downstream of the barrier waterfall belonged to the original Turure matriline when the mitochondrial $NADH_2$-gene was analysed by Becher & Magurran (2000) which means that at least some Guanapo females must have overcome this barrier and contributed to the mixing of gene pools.

The importance of a reasonably high genetic diversity within a non-native population in adapting to novel biotic and abiotic factors was already mentioned. In their review, Lockwood et al. (2005) proposed that propagule pressure or the number of non-native individuals released in a region can explain significant variation in the establishment success of invasive species. Shortly afterwards, Roman & Darling (2007) found in an analysis of 43 introduced populations that less than 40% showed evidence of a significant loss of genetic diversity relative to their source population. This was mainly due to a high number of propagule vectors and multiple introductions. In the case of Haskins's introduction, 200 guppies were transferred to the upper Turure (Shaw et al., 1992). Because female guppies can store the sperm of several males, the number of fish genetically contributing to the newly establishing population might have been greater than 200. In any case, the genetic variability, where the mean number of alleles per locus was only slightly reduced compared to the source population (1.2 vs. 1.4, respectively) (Carvalho et al., 1996), proved high enough not only to establish a viable population but also to undergo fast changes in life-history and behaviour in relation to changing predation regimes (Magurran et al., 1992). A similar result concerning life-history and morphology was obtained in another introduction experiment by Endler (1980). As in Haskins's introduction, 200 guppies were transferred from a high predation habitat to a place (within the same river) where they were only facing minor predation. In this case no reduction in the mean number of allozymes per locus (1.2) could be detected between source and transplant population (Carvalho et al., 1996). As I showed in Chapter 2 in agreement with other authors (e.g. Shaw et al., 1992; Becher & Magurran, 2000), the entire Turure is by now populated by guppies of Guanapo ancestry, while the native genotype only survives in relatively small quantities in the lower parts of the river.

An important question now is whether the invasive success of Guanapo fish in the Turure was due to genetic or reproductive superiority or was simply a chance event. In Chapters 3 to 5 of this thesis I could already show that no behavioural differences seem to exist between fish from both populations that would give Guanapo guppies an advantage over their Oropuche (Turure) counterparts. But even without visible behavioural and life-history advantages, differences could still be present on a genetic basis, in the form of the production of more or superior sperm/offspring, better survival rates for offspring, or faster growth rates compared to Oropuche fish. Evans & Rutstein (2008) demonstrated that some male guppies always fathered more offspring, even when the amount of transferred sperm was held constant by artificial insemination. If this was the case with Guanapo males it could lead to an increased production of newborns carrying Guanapo alleles and therefore a quicker mixing of populations.

Another possibility for explaining changes in gene frequencies and the observed dominance of Guanapo alleles could be genetic drift or a change in allele composition due to random effects that are not influenced by selection. Because genetic drift is more likely to occur in small populations such as those inhabiting the upper stretches of rivers, it might have helped to increase the amount of Guanapo genes compared to the original Turure genotype simply by chance events. While no statements about the influence of genetic drift can be made for the original colonisation of the Turure by Guanapo fish, I have to take the possibility of drift in the following experiment into account when analysing the results.

Answering these questions by conducting yet another introduction experiment in the wild could once more endanger the survival of genetically distinct guppy populations, as was already pointed out by several authors (e.g. Magurran, 2005; Carvalho et al., 1996). Therefore I decided to carry out a population mixing experiment using mesocosms. These were large outside tanks filled with water and containing stones and water plants for shade and cover, thereby providing a relatively realistic imitation of natural habitats for the fish used in the following experiment. For this reason it can be assumed that the results obtained here would be similar to a study done in the wild.

In this long-term experiment that ran for over a year, I tested the importance of the initial proportion of individuals with that two populations come into first contact vs. genetic/reproductive superiority of one population over the other. Twenty fish from the Guanapo and Oropuche were introduced into mesocosm tanks in different proportions (100% Guanapo, Gua 80-20 Oro, Gua 50-50 Oro, Gua 20-80 Oro, 100% Oropuche) and left to interact freely for the next year. Beside the genetic analysis of offspring produced during the experiment using SNP markers, other variables like the number of fish per mesocosm at the end of the experiment after one year, their sex and age class as well as their size were investigated to test for any significant differences between treatments. If the proportion of fish belonging to a certain population was the main explanation for the distribution of population typical genes in a mixing event, I would expect to find the number of alleles specific for Guanapo and Oropuche fish at least roughly related to the proportion of initially introduced fish. If on the other hand any other factor like a higher reproductive output of fish belonging to one population is playing a major role, I expect that the amount of alleles belonging to either population will vary considerably from the proportions of fish originally introduced, but more or less point in the same direction. With the present situation in the Turure, I tested the hypothesis that a genetic/reproductive advantage of Guanapo fish over their Oropuche counterparts exists. A last possibility is that chance events or genetic drift due to the small number of initially introduced fish played a major roll in the outcome of this experiment. In that case the distribution of population specific alleles should vary unpredictably between treatments as well as between different mesocosms of the same treatment.

Methods

Twenty-four mesocosm tanks were set up in a fenced compound at UWI campus in St Augustine, Trinidad in December 2008. Each of them had a diameter of 1.2 m and height of 80 cm. An overflow in the form of a short tube covered with a fine mesh at the inner end. to hold water levels constant and prevent fish from being washed out during rainfall, was fitted to each mesocosm at a hight of 60 cm. A lid made out of mesh wire on top of all mesocosms was used to stop dead leaves from falling into the water as well as to avoid any bird predation on guppies. Tanks contained small and medium sized stones for cover especially of newborn fish and some plants to provide extra shade (Figure 28).

Figure 28: All mesocosms were set up in an outside compound at UWI campus. Several trees and hedges shades them throughout most of the day. Each tank contained some stones for cover and plants to provide extra shade.

This experiment consisted of five different treatments, each replicated three times (100% Oropuche; 100% Guanapo) or six times (Oro 20:80 Gua, 50:50, 80:20), respectively. Treatments were randomly assigned to mesocosms across the compound. Each tank was then stocked with 20 wild adult guppies from the Oropuche and/or Guanapo according to the treatments. The same number of males and females per population were used, e.g. a mesocosm containing an Oro 20:80 Gua treatment would be stocked with two Oropuche males and females and eight Guanapo males and females each. The proportions of fish used for each tank can be seen in Table 9.

Table 9: The proportion of fish (20 in total) from the Oropuche and the Guanapo with which each mesocosm was stocked in the beginning. The number of males and females per population was the same.

Mesocosm no	Proportion Oro:Gua (%)	Mesocosm no	Proportion Oro:Gua (%)
1	100:0	13	80:20
2	20:80	14	50:50
3	0:100	15	20:80
4	100:0	16	80:20
5	80:20	17	0:100
6	50:50	18	50:50
7	80:20	19	50:50
8	50:50	20	20:80

9	50:50	21	100:0
10	20:80	22	80:20
11	20:80	23	80:20
12	0:100	24	20:80

After their transfer to the mesocosms, fish were not fed, but lived on algae and small freshwater invertebrates. Several big trees and hedges at both sides of the compound provided shade or half-shade throughout most of the day. Figure 29 indicates the areas of shade (white) or exposed to the sun (coloured) at 8 am, 10 am, 12 am, 2 pm and 4 pm, respectively. No tank was exposed to the sun for more than 4 hours per day and the water temperature did not increase perceptibly during the day.

In January and February 2010, after 13 to 14 months and approximately 4-5 guppy generations, all fish in each mesocosm were caught using a hand net and the tank was carefully emptied using a small bucket, thereby checking for any fish that previously had escaped the net. Fish were transferred to the lab and pictures taken of the first 200 guppies (belonging to all age classes and both sexes) in the same way as mentioned in earlier chapters. Their total length was then measured on the computer using ImageJ. In the case that the fish number exceeded 200, the remaining animals were counted and sexed. 30 newborns or juveniles per mesocosm were randomly picked and killed with an overdose of Tricaine Methanesulfonate, fixed in 96% ethanol and stored at - 20°C. Juveniles were used to ensure a maximum chance for population mixing to have taken place since the start of the experiment. All remaining fish were released in an isolated artificial pond at UWI campus.

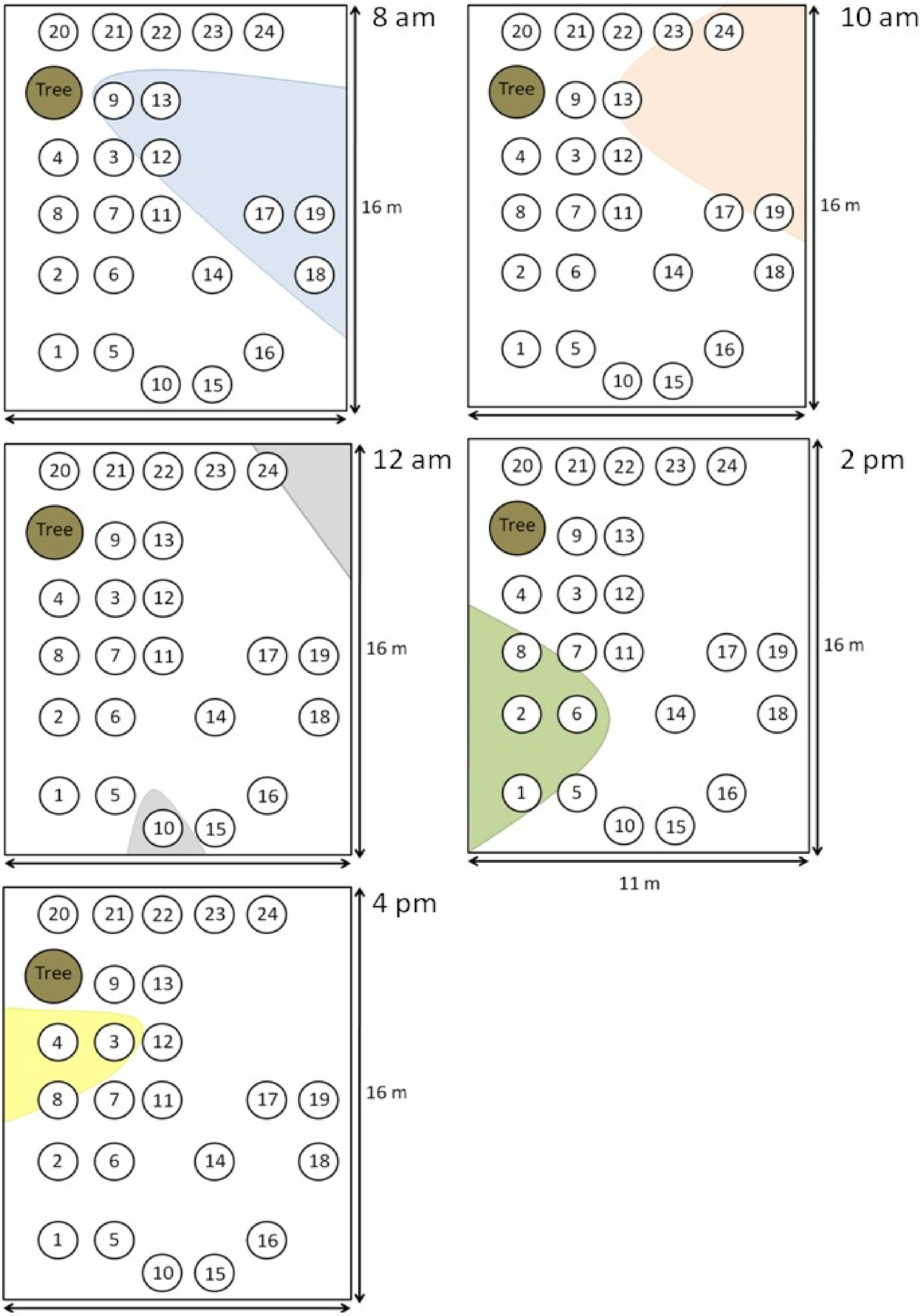


Figure 29: The amount of shade (no colour) and sunlight (coloured) each mesocosm received throughout the day at 8 am (top left), 10 am (top right), 12 am (middle left), 2 pm (middle right) and 4 pm (bottom left).

In June and July 2010, all fish samples were taken to the Max Planck Institute for Developmental Biology in Tuebingen, Germany, for genetic analysis, where population specific markers already existed due to a genome-wide SNP study of guppy population history in Trinidad and Venezuela (Willing et al., 2010).

DNA was isolated using the DNeasy Blood & Tissue Kit from Qiagen. 2 to 3 mg of dried body tissue (avoiding the gut) per baby were transferred into a 1.5 ml microcentrifuge tube. The tissue of five newborns was pooled in one tube, leading to six tubes per mesocosm, each containing around 10 to 15 mg of tissue. The rest of the protocol follows the steps already described in Chapter 2.

After isolation, the DNA concentration of all samples was measured and the two samples per site combined. Water was used to dilute each DNA sample to an end concentration of 25 ng/µl, each containing the DNA of 10 animals. All samples were stored at 4°C.

For DNA amplification the same protocol as in Chapter 2 was used. 28 different primer pairs were tested with newborns from the pure Oropuche and Guanapo mesocosms to identify the best markers. Out of these 28 markers only 3 resulted in useful PCR products and contained SNPs that were informative between both populations The following markers were therefore chosen to further test the remaining mixed mesocosm samples.

Table 10: Sequences of forward and reverse primer for each marker that produced clear results in pure Guanapo and Oropuche fish. The linkage group of all markers is shown as well. (Sequences from: Tripathi et al. (2009), Data supplement no 2 & 3).

Marker	Forward primer	Reverse primer	Linkage group	GenBank accession no
M 654	TTTACATCCCACACCTTCAATC	TGTGAATGCTCAACCAAACTC	LG02	FH890962
M 978	GGCCCATCTGGATAGAGTG	TTAACATCTTGTGGAGTTATGCTG	LG23	FH893187
M 1033	AATCAGTCAGTTTACAAAGTCTGGTC	TGGAGACGCAATCAGTGG	LG10	FH893635

- Tested markers that did not result in useful sequences:
  M 85, M 121, M 197, M 232, M 268, M 285, M 290, M 379, M 394, M 504, M 528, M 546, M 559, M 613, M 628, M 667, M 680, M 781, M 791, M 883, M 902, M 937, M 994, M 1033

Primer removal, the PCR preparation for sequencing, the sequencing and the SNP evaluation itself again followed the protocol mentioned in Chapter 2.

Results

The mean number of all fish per mesocosm did not differ between treatments (Kruskal-Wallis test, $\chi^2 = 6.24$, d.f. = 4, P = 0.182). Because of the small sample size especially for the pure Guanapo and Oropuche mesocosms, this result might not be very powerful and it would be possible to find a difference between Oropuche mesocosms and all other treatments when analysing a greater sample size (Figure 30).

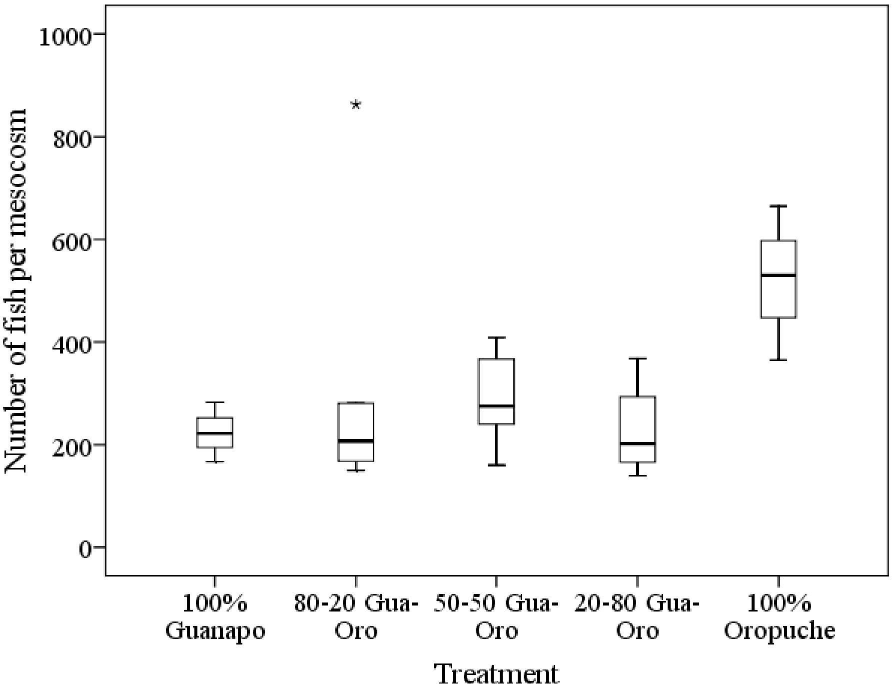


Figure 30: The number of mesocosm fish sorted by treatment. N = 6 for mixed treatments, n = 3 for pure Guanapo and Oropuche treatments. Medians, interquartile ranges and outliers are shown.

When looking at fish number separated by gender and age class, respectively, again there is no significant difference to be found between treatments for females (Kruskal-Wallis test, $\chi^2 = 7.58$, d.f. = 4, P = 0.102), males (Kruskal-Wallis test, $\chi^2 = 5.77$, d.f. = 4, P = 0.217) or newborns (Kruskal-Wallis test, $\chi^2 = 5.32$, d.f. = 4, P = 0.256), but the same limitations apply as mentioned before (Figure 31).

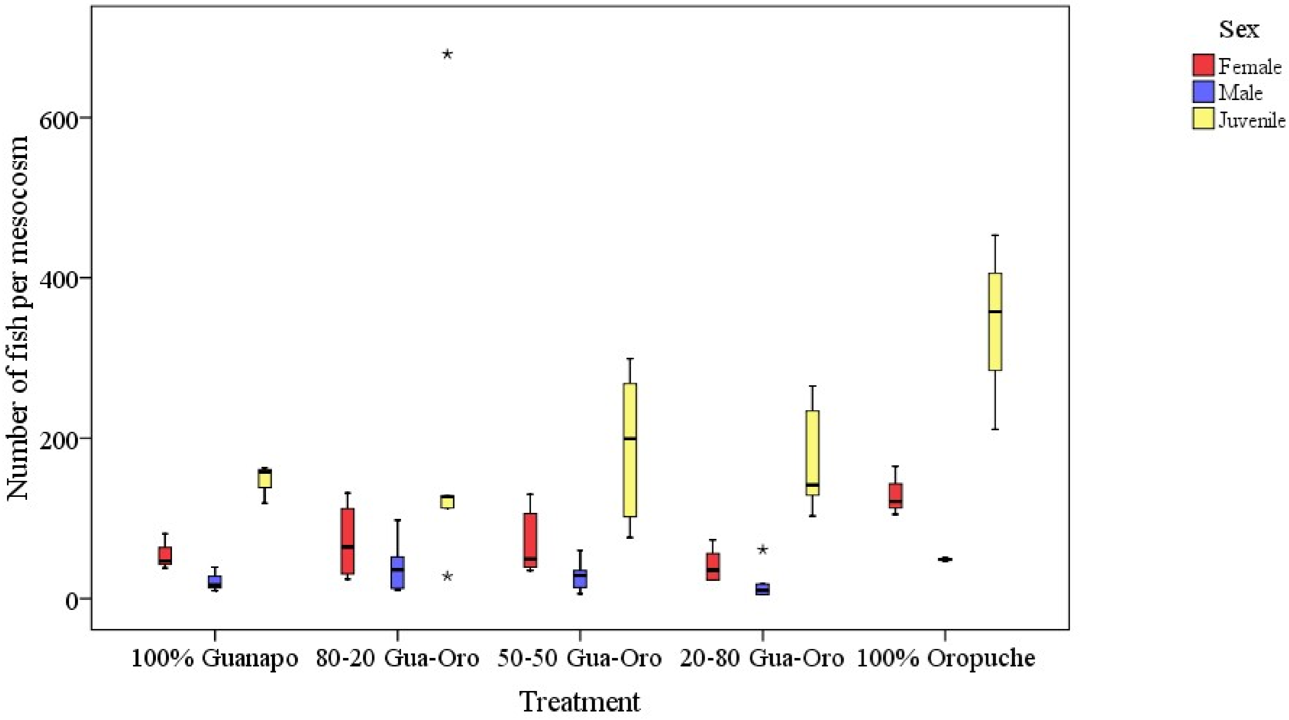


Figure 31: The number of mesocosm fish separated by age class and sex for all treatments. N = 6 for mixed treatments, n = 3 for pure Guanapo and Oropuche treatments. Medians, interquartile ranges and outliers are shown.

There was a significant size difference between female guppies belonging to different treatments (One-way ANOVA, F = 5.86, d.f. = 4, P < 0.001; Post Hoc Test: LSD) (Figure 32). Females from the Guanapo were significantly smaller than females from all other treatments, but results were not significant any longer when compared to pure Oropuche females after the significance level was adjusted using the sequential Bonferroni procedure. Females from the Oropuche were significantly smaller than females from the Gua 20:80 Oro treatment only before the adjustment of P-values. See Table 11 for all significant P-values and α-statistics.

Table 11: Significant results of a One-way ANOVA followed by a LSD Post Hoc test for female size between treatments. The significance level was adjusted using the sequential Bonferroni procedure.

Treatment	P-value	α
Gua tested with Oro	0.011	-
Gua – 80:20	0.001	$\alpha_3 = 0.0028$
Gua – 50:50	< 0.001	$\alpha_1 = 0.0025$
Gua – 20:80	< 0.001	$\alpha_2 = 0.0026$
Oro – 20:80	0.036	-

Similarly, male guppies in pure Guanapo mesocosms were significantly smaller than fish belonging to other treatments (One-way ANOVA, F = 9.87, d.f. = 4, P < 0.001; Post Hoc Test: LSD). Again all significant results are listed below in .Table 12

Table 12: Significant results of a One-way ANOVA followed by a LSD Post Hoc test for male size between treatments. The significance level was adjusted using the sequential Bonferroni procedure.

Treatment	P-value	α
Gua tested with Oro	< 0.001	$\alpha_1 = 0.0025$
Gua – 80:20	< 0.001	$\alpha_2 = 0.0026$
Gua – 50:50	< 0.001	$\alpha_3 = 0.0028$
Gua – 20:80	< 0.001	$\alpha_4 = 0.0029$

Lastly, guppies significantly differed in size between treatments (One-way ANOVA, F = 9.58, d.f. = 4, P < 0.001; Post Hoc Test: LSD), with fish from pure Guanapo and Oropuche mesocosms being smaller than mixed offspring. See Table 13 for all significant results of the Post Hoc test.

Table 13: Significant results of a One-way ANOVA followed by a LSD Post Hoc test for size between treatments. The significance level was adjusted using the sequential Bonferroni procedure.

Treatment	P-value	α
Gua tested with 80:20	0.030	-
Gua – 50:50	0.007	-
Gua – 20:80	< 0.001	$\alpha_1 = 0.0025$
Oro – 80:20	< 0.001	$\alpha_2 = 0.0026$
Oro – 50:50	0.001	$\alpha_4 = 0.0029$
Oro – 20:80	< 0.001	$\alpha_3 = 0.0028$
80-20 – 50:50	0.019	-
80-20 – 20:80	0.04	-

Again, after the adjustment of P-values using the sequential Bonferroni procedure, only some of the observed size differences between treatments remained significant.

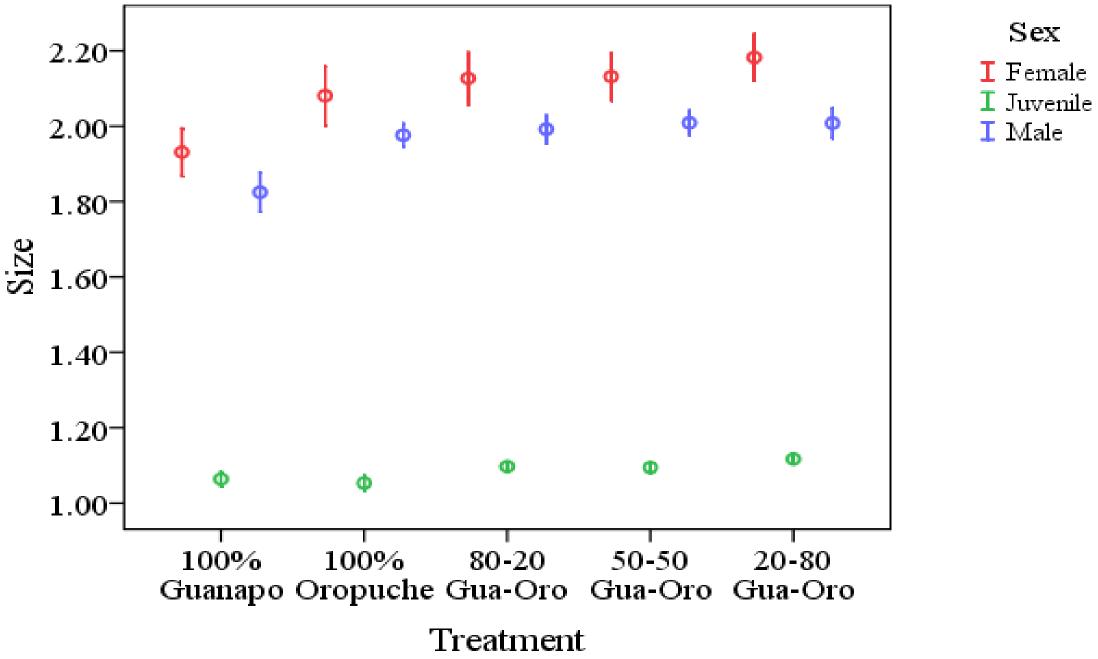


Figure 32: The mean size of mesocosm fish separated by age class and sex for all treatments. N = 6 for mixed treatments, n = 3 for pure Guanapo and Oropuche treatments. Error bars represent 95% CI.

Thirty fish per mesocosm were used for genetic analysis. This corresponded with a mean of 22.5% ± 19.9 S.D. of total offspring per mesocosm, ranging from 4.4% to, in one case, 100% (Mesocosm no 2, Gua 20:80 Oro). One sample t-tests were carried out for all five treatments to test for differences between the observed amount of Oropuche nucleotides found in 14 SNPs and a value expected according to the initial proportion of introduced fish from both populations (Figure 33).

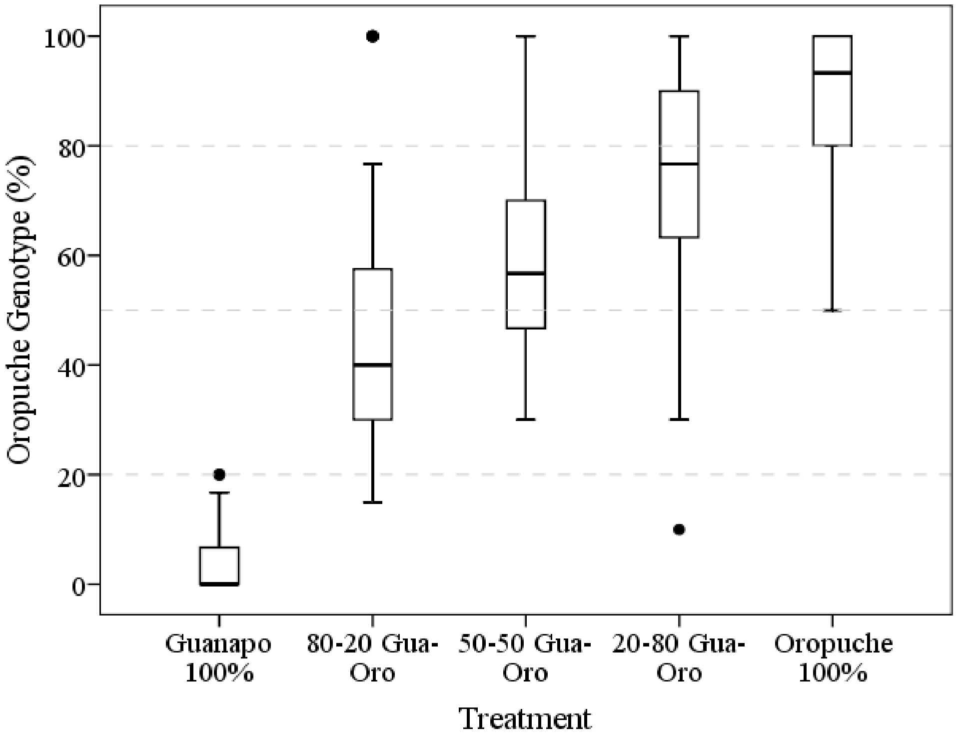


Figure 33: The percentage of Oropuche nucleotides found in 14 SNPs that distinguished between Guanapo and Oropuche origin for all treatments. Dashed lines at 20, 50 and 80% indicate where a theoretical mean for each treatment would be expected given that only the proportion of introduced fish is important for the distribution of Guanapo and Oropuche alleles. Medians, interquartile ranges and outliers are shown.

All treatments differed significantly from expected values. Neither pure Guanapo nor Oropuche mesocosms were homozygous for most SNPs but nearly always contained a small proportion of nucleotides mainly found in the other population (Guanapo mean = 3.97%; Oropuche mean = 88.77%). Both fish belonging to the Gua 80:20 Oro (Mean = 41.43% vs. 20%) and the Gua 50:50 Oro (Mean = 55.99% vs. 50%) treatments showed an excess of Oropuche type nucleotides while tested guppies from the Gua 20:80 Oro samples displayed a lack of Oropuche nucleotides in SNPs (Mean = 72.74% vs. 80%). See Table 10 for all test results and expected values.

Table 14: Results of one sample t-tests that compared the actual mean of Oropuche type nucleotides (in percent) to the theoretical value that would have been expected according to the initial proportions of Guanapo and Oropuche fish.

Treatment	t	d.f.	Mean (%)	S.D.	Expected mean (%)	P-value
Guanapo 100%	4.32	41	3.97	5.9470	0	< 0.001
Gua 80:20 Oro	12.16	81	41.43	15.9515	20	< 0.001
Gua 50:50 Oro	3.98	83	55.99	13.8009	50	< 0.001
Gua 20:80 Oro	- 3.31	78	72.74	19.4760	80	0.001
Oropuche 100%	- 6.03	41	88.77	12.0730	100	< 0.001

All informative SNPs found in the five treatments are displayed in Figure 34. Slight variations can be seen between SNPs found in fish belonging to the same treatment but overall patterns were the same across markers and no significant differences existed in any treatment (Guanapo 100%: One-way ANOVA, $F_{13,28} = 0.969$, $P = 0.503$; Gua 80:20 Oro: One-way ANOVA, $F_{13,68} = 0.872$, $P = 0.585$; Gua 50:50 Oro: One-way ANOVA, $F_{13,70} = 1.587$, $P = 0.110$; Gua 20:80 Oro: One-way ANOVA, $F_{13,65} = 0.535$, $P = 0.894$; Oropuche 100%: One-way ANOVA, $F_{13,28} = 1.142$, $P = 0.368$).

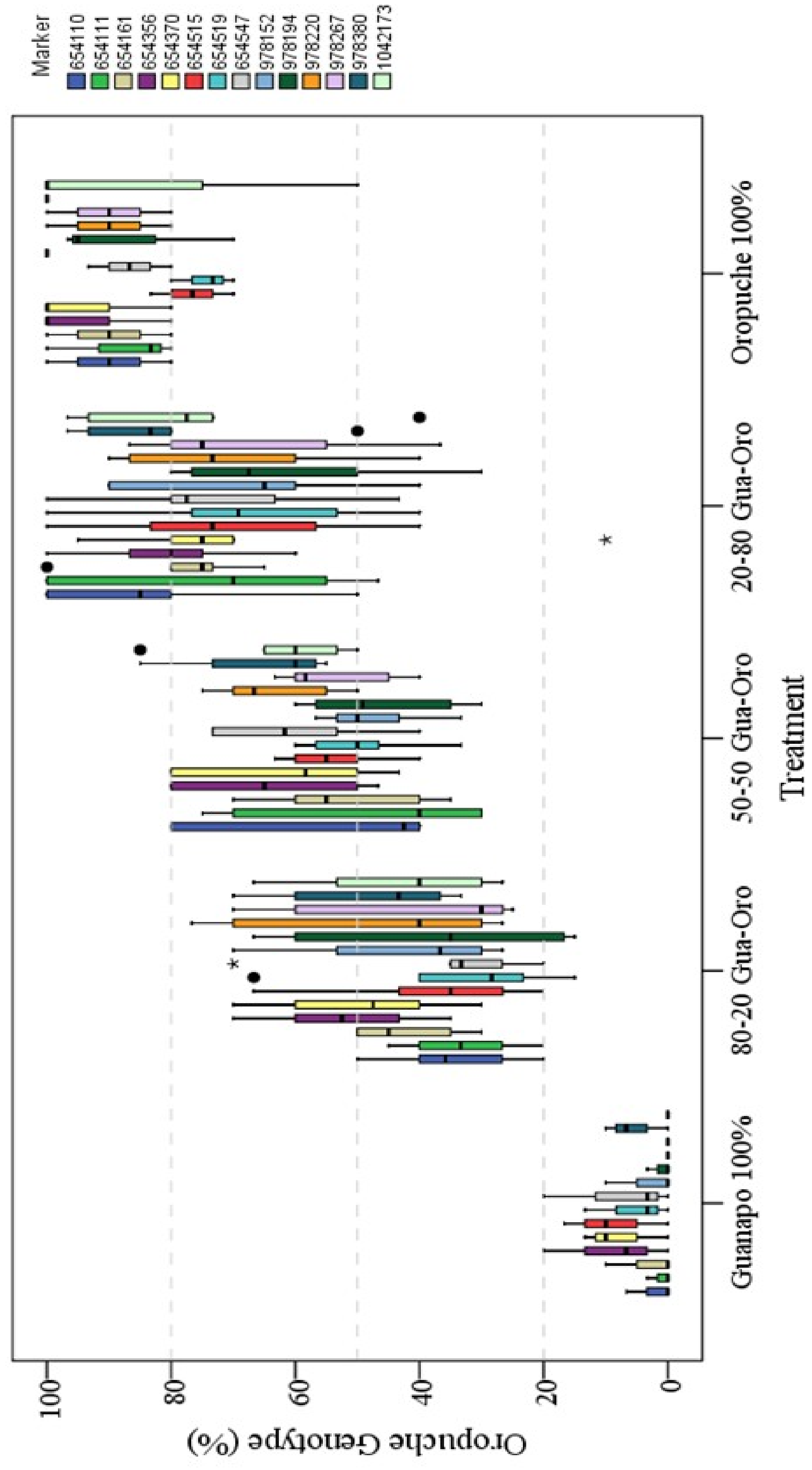


Figure 34: The percentage of Oropuche nucleotides per marker found in 14 SNPs that distinguish between Guanapo and Oropuche origin. As in Figure 33, dashed lines at 20, 50 and 80% indicate the expected values for each treatment in the absence of population differences concerning reproduction and survival. Medians, interquartile ranges and outliers are shown.

Discussion

The amount of Oropuche-typical SNPs significantly differed from the proportions expected in the total absence of population differences in reproduction. Pure Guanapo and Oropuche populations were not entirely homozygous for most SNPs, and the mixed treatments showed either higher (Gua 80:20 Oro: 41.4% Oropuche type nucleotides vs. of 20%; Gua 50:50 Oro: 56.0% vs. 50%) or lower (Gua 20:80 Oro: 72.7% vs. 80%) than expected values. No significant differences existed between different SNPs belonging to the same treatment. Numbers of fish per mesocosm did not differ between treatments while there existed a significant size difference between females, males and newborns belonging to different treatments. Guanapo fish generally were smaller than individuals from all other treatments and a slight, but mostly non-significant trend, was observed for mixed fish being largest.

No apparent reproductive advantage of Guanapo fish over their Oropuche counterparts could be detected in this experiment. In that case a obvious excess of Guanapo nucleotides would have been expected independent of treatment. Unlike than in my experiment, where fish of both populations came from high predation sites, Haskins introduced high predation Guanapo fish into a low predation environment. Because high predation guppies mature earlier and produce more offspring (Reznick & Bryga, 1987), Guanapo fish might have had an initial reproductive advantage over the slower reproducing Turure population. At the same time high predation males from the Aripo achieved greater reproductive success compared to their low predation counterparts not-withstanding the fact that females preferred the more colourful low predation males (Magellan & Magurran, 2007). Again, this could have meant another advantage for Guanapo fish and a quick population mixing. However, these advantages of Guanapo guppies over Turure fish are unlikely to have lasted very long. As Reznick & Bryga (1987) discovered in another introduction experiment involving the translocation of high predation guppies from the El Cedro River (Caroni drainage) to a low predation site in the same river, that within 4 years after introduction, males matured at a later age and greater size. Females produced fewer, larger offspring but did not yet show any sign of changes in other life-history traits. The authors suggested that males evolved more quickly compared to females. When a follow-up investigation was done 7.5 years after the introduction, females matured at a later age and larger size but other life-history traits had not changed (Reznick et al., 1997). 11 years after a similar introduction experiment in the upper Aripo (Caroni drainage) by Endler, life-histories of males and females completely resembled those of other low predation populations (Reznick & Endler, 1990). Any reproductive advantages of Guanapo fish over Turure guppies due to predation regime will therefore have lasted only for a few years after introduction until life-history traits of both populations matched. Genetic mixing of populations might have further eroded differences in reproductive output. The high-predation fish used in my mesocosm experiment resembled a later stage of the invasive event after life-history differences had already disappeared. Therefore only existing genetic or reproductive differences independent of predation regime could possibly have had an impact on the outcome of this experiment but results did not indicate the existence of any advantages of Guanapo fish over their Oropuche counterparts.

Data also did not match the values that would have been anticipated if only the initial proportion of fish introduced to the tanks were important for the end result. Genetic drift due to a very small initial population size as well as the short amount of time (≈ 4 guppy generations) will make it difficult to detect minor trends. Population mixing is unlikely to have reached a stable equilibrium or, in the case of a genetic advantage of one of the populations, time might have been too short for it to clearly show. On the other hand, the fact that no obvious difference existed between SNPs, even if these were found on different linkage groups, make it unlikely that genetic drift contributed markedly to the results. It seems, therefore, that at least at the Gua 80-20 Oro and 50:50 treatments, Oropuche fish are the more successful genotype and produced an excess of Oropuche typical nucleotides. Because neither fish from the Guanapo nor Oropuche were completely homozygous for all population typical alleles, some minor variations in mixed treatments could have been caused by this.

Another factor that could potentially have influenced the outcome of this experiment beside the proportion of fish belonging to each population are density effects. If a tank quickly reaches carrying capacity, population growth would slow down and mixing effects could take longer to show. However, it is unlikely that any of my mesocosms had reached or was close to carrying capacity as even in tanks containing a relatively high number of fish, these mainly were newborns and fish. That means that populations were still growing quickly and were probably mostly unaffected by density effects as competition for space and food or the easy spread of disease. In case of a longer running experiment and larger populations, density effects have to be accounted for.

Taking all results into account, a simple population mixing event alone is unlikely to fully explain the situation found in the Turure today. In an experiment looking at female mate choice and male mating success between fish belonging to the Tacarigua (Caroni drainage) and the Oropuche (Oropuche drainage), Magurran et al. (1996) found that while females did not prefer males from either population in a choice test, Tacarigua males secured all copulations in a mating experiment. The authors proposed that this dominance of Caroni type fish in achieving reproductive success could be an explanation for the success of Guanapo fish (Caroni drainage) over Turure fish (Oropuche drainage). In contrast to these results, I was not able to find any behavioural differences in the mating behaviour of males belonging to the Guanapo or the Oropuche. The non-existent excess of Guanapo type nucleotides in the analysed SNPs also makes a general superiority of Guanapo males unlikely. That my results differed from Magurran et al.'s proposal might be explained by a more natural setting of the mesocosm experiment where the same number of males and females was introduced in the beginning and females nearly always exceeded the number of males after a year. Because of this high proportion of females (compared to just one female tested with two males in Magurran's experiment), possible differences in dominance behaviour were probably less important. Other reasons than simply the introduction of a population and an ensuing gene pool mixing must exist to satisfactorily explain what happened in the Turure.

Beside an obviously much longer time scale and greater population size of guppies in the Turure, another factor differed between the mesocosm experiment and Haskins's introduction. My mesocosms were separated compartments where fish were unable to leave and no individuals from outside populations could enter the tank. Therefore there was no possibility for movement between populations and the corresponding gene flow this would create. The constant influx of Guanapo fish from above the barrier waterfall into the middle and lower parts of the Turure and the subsequent gene flow and population mixing over a sufficient amount of time might be another explanation for the situation found in the Turure today. This possibility will be further examined in the General discussion, taking all other results of my PhD into account.

This experiment being a preliminary study, some changes would be useful when repeating it at a larger scale. Because the influence of genetic drift cannot be excluded, especially while populations are still small, it would be interesting to see if and how allele frequencies shift during time and if the results I got in this experiment would be different after more generations where mixing could take place. Therefore a longer running time of the experiment, as was planned initially, would be useful, taking samples of fish every couple of months or once per year. It would also be interesting to investigate differences in the inheritance of the nuclear genome compared to the matri- and patriline to get further insight into eventual differences in the reproductive success of both sexes. As already mentioned in Chapter 2, more informative SNP markers as well as analysing individual fish instead of groupes of ten would give a more precise picture of the actual genetic distribution of invasive/native alleles in the mixing population. Furthermore, using size matched fish when stocking the tanks initially could decrease any bias for differences in offspring production.

Chapter 7 Speciation in guppies and the existence of *Poecilia obscura*

Abstract

For many years now, scientists have been trying to define exactly what a species is and how it is separated from other species. So far, no universal concept could be agreed upon, and the rise of genetic methods has not led to a better accepted species definition. Recently, Schories et al. (2009) proposed that the Trinidadian guppy be split into two separate species, *Poecilia reticulata* and *P. obscura*. The authors argued that the large amount of genetic difference between the two proved that they were two species that separated a long time ago rather than belonging to one species with considerable intraspecific variation. However, several earlier studies showed similar amounts of between population genetic variation without seeing the necessity for two different guppy species. This raises the question of the exact species status of the guppy. I investigated and compared the results and arguments of several studies concerning speciation in *P. reticulata*, as well as the results from my own work, where I compared two populations belonging to opposite Trinidadian drainage systems. No behavioural or life-history differences between populations could be found that would indicate the existence of two species. The inability or lack of interest of guppies to distinguish mating and schooling partners on the basis of population origin and the rapid production of fertile hybrid offspring both render it unlikely that the separation of the guppy into two species was necessary or useful to clarify the systematic situation in the genus *Poecilia*. In this chapter, I will give an overview of existing species definitions and concepts in the literature, and discuss the benefit of recognising two separate guppy species instead of one genetically very variable one.

What is a species? Definitions and concepts

Ever since Darwin published 'On the origin of species' in 1859, scientists have struggled to find a universal answer to the question what exactly defines a species and in what way species are separated from one another. Darwin's own definition of a species was that they represent morphologically distinguishable clusters of individuals and have few or no intermediate forms when in contact with another, similar cluster. Speciation therefore occurs when morphological gaps evolve between populations and do not disappear in sympatry. Hybridisation between species is possible, but will not change a species' distinctiveness. The only distinction between varieties or subspecies and species is, according to Darwin, that varieties are connected by intermediate forms, while species used to be connected in the same way in the past. He thereby treated species as relatively fluid congregations and pointed out that there might be no proper species concept at all, a suggestion Darwin was later criticised for by other biologists (see Mallet, 1995).

In the first half of the 20th century, Mayr, Dobzhansky and others came up with the Biological species concept that defined a species as a group of interbreeding natural populations which are reproductively isolated from all other groups (Mayr, 1940). Speciation in their eyes is the evolution of isolation mechanisms and is thought to be complete when reproductive barriers are strong enough to prevent gene flow between two newly evolved species. In contrast to Darwin, this concept does not allow for the production of fertile hybrids between species and does also not apply for asexual species. Several other concepts followed during the second half of the 20th century, including the Evolutionary species concept (Simpson, 1961) and Cracraft's Phylogenetic species concept (Cracraft, 1989) that defined a species as the smallest cluster of individuals that is distinct from other such clusters. Organisms within a cluster share the same ancestry and descent and are monophyletic. In the same year, Templeton (1989) proposed the Cohesion species concept that included reproductive isolation, ecological selection and reproductive compatibility and thereby combined several older species concepts.

The latest species definition by Mallet (1995), the Genotypic cluster definition, is based on Darwin's original thoughts on this topic, but takes the recent knowledge from genetics and morphology into account. Two species can be recognised by two identifiable genotypic clusters with no or few intermediates. Speciation is therefore the evolution of genotypic gaps between populations that, in the same way as morphological gaps, do not disappear in sympatry. Like Darwin, this definition allows hybridisation and the existence of subspecies that do not differ much from actual species except that they are connected by intermediate forms. Because the future is unpredictable, Mallet argues that it does not make sense to define species that show interspecific hybridisation as permanently separated. A change in selection pressure can or can not lead to the fusion of genotypic clusters. He therefore thinks it more important to have a species definition that leaves room for evolution in various ways than to force species into a concept that restricts their evolutionary potential (Mallet, 1995).

This long list of different attempts to define a species highlights the long and ongoing debate that evolved around this topic. A more complete list of existing definitions and concepts is shown in Table 15. The fact that there still is no consensus on what exactly a species is, makes it difficult to decide when two or more groups of organisms belong to separate species and when they are more likely to be varieties or subspecies. The recent split of *Poecilia reticulata* into two separate species, *P. reticulata* and *P. obscura*, by Schories et al. (2009) has to be thoroughly reviewed in this light while considering various aspects that can be used in favour or against the authors' decision. In the following chapter I discuss the likelihood that two separate guppy species exist in Trinidad, looking both at the literature as well as at the data I collected in several experiments throughout my PhD.

Table 15: A list of the different species concepts that exist in the literature and their definition of species and speciation sorted by publication date. If these concepts allow hybridisation between species and are valid for asexual species as well is stated in a separate column.

Species concept	Definition of species	Hybridisation /asexual species?	Reference
Morphological cluster concept	A species is a morphologically distinguishable group of individuals that has few or no intermediates when in contact with other such clusters Speciation: evolution of morphological gaps that do not dissolve in sympatry	+	Darwin, 1859
Biological species concept	Groups of actually and potentially interbreeding natural populations which are reproductively isolated from other such groups Speciation: evolution of isolation mechanisms	-	Mayr, 1940
Evolutionary species concept	A species is a single lineage of ancestral descendant populations of organisms, which maintains its own identity from other such lineages and which has its own evolutionary tendencies and historical fate	? / +	Simpson, 1961
Ecological species concept	A species is a lineage (or a closely related set of lineages), which occupies an adaptive zone minimally different from that of another lineage in its range and which evolves separately from all lineages outside its range	+	Van Valen, 1976
Recognition species concept	Species are the most inclusive population of individual biparental organisms, which share a common fertilisation system Speciation: evolution of different fertilization systems	-	Paterson, 1985

Species concept	Definition of species	Hybridisation /asexual species?	Reference
Cohesion species concept	A species is the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms Combines reproductive isolation, ecological selection, and reproductive compatibility	+	Templeton, 1989
Phylogenetic species concept	A phylogenetic species is an irreducible (basal) cluster of organisms that is distinct from other such clusters, and within which there is a parental pattern of ancestry and descent	- / ?	Cracraft, 1989
Genotypic cluster definition	A species is a (morphological or genetically) distinguishable group of individuals that has few or no intermediates when in contact with other such clusters Speciation: evolution of genotypic gaps that do not dissolve in sympatry	+	Mallet, 1995

Population variation in the guppy

Despite a large amount of behavioural, life-history and also some genetic variation between populations experiencing different predation regimes, no reproductive barriers have been developed in guppies inhabiting the same river. While strong sexual selection due to female choice can reinforce differences between populations caused by natural selection in the first place, reproductive isolation is unlikely to arise in the future. Forced copulations by males that increase with rising predation threat can potentially undermine female choice and act against reproductive isolation (Magurran, 1998). The strong tendency of male guppies to move relatively long distances (Croft et al., 2003) further increases gene flow between populations experiencing different selection pressures and weakens the strength of sexual selection operating in this species (Bisazza, 1993). In their analysis of mitochondrial DNA-sequences, Fajen & Breden (1992) discovered only a genetic differentiation of 0.0 – 1.0% between guppy populations within the same river. However, the same study revealed mtDNA variation of 3.9 – 5.6% between guppies belonging to different drainage systems. Several other studies came to the same result (see other chapters) and showed that populations inhabiting both drainage systems belong to genetically distinct groups. In 2006, Ludlow & Magurran estimated that guppies from the Caroni and Oropuche drainage have been separated from each other for up to 2 million years. But do the genetic differences between populations influence behavioural and life-history variations that could finally lead to the establishment of two separate species?

Several differences in behavioural, life-history and genetic traits exist between populations belonging to different rivers. These were investigated for possible differences between drainage systems in this chapter.

Behavioural differences

Behavioural traits can differ between populations due to genetic variation or differences in the experienced environment. Because of the close geographic proximity of the Caroni and Oropuche drainage system in Northern Trinidad, it is unlikely that populations inhabiting them face any large differences in biotic and abiotic factors (Magurran, 2005). Variations in behaviour might therefore be due to neutral selection or genetic drift and/or the fixation of different alleles in populations. One important behaviour frequently tested in guppies is the time individuals spend schooling to reduce predation threat. Seghers (1974) detected differences in the schooling behaviour of high and low predation fish, while Magurran & Seghers (1991) reported variations between several populations belonging to different rivers. In my own experiments, I affirmed the existence of differences in schooling time between individual populations of both drainage systems, however, no significant variations were found between drainage systems (Mann-Whitney U test, $Z = -1.484$, $n_{\text{Caroni}} = 71$, $n_{\text{Oro}} = 50$, $P = 0.138$). At the same time females from some but not all populations preferred to school with conspecifics rather than with *P. picta* females. Again, this trait did not seem to be drainage system dependent. Looking at the schooling abilities of newborn guppies, Magurran & Seghers (1990) discovered population differences in schooling behaviour. While I got similar results for testing different populations, no difference existed between populations belonging to different drainage systems (Mann-Whitney U test, $Z = -1.230$, $n_{\text{Caroni}} = 75$, $n_{\text{Oro}} = 50$, $P = 0.219$).

Mating behaviour and mate choice can play an important role in either supporting or weakening arising reproductive barriers between populations that can ultimately lead to the establishment of two separate species. Houde (1988) found a genetic difference in the mate choice preference of females belonging to two different populations (Aripo and Paria), depending on the amount of orange colouration displayed by males. However, this trait was not tested across drainage systems, so no final answer can be given where variations are to be found. A slight but significant preference of female guppies for males from their own population over males from alien populations was detected by Endler & Houde (1995) that could reinforce population differences. However, male guppies did not discriminate between females from different populations (Chapter 3). A study by Kodric-Brown (1992) found that dominant males can secure matings in the presence of another male even if they are not preferred by the female. But while Magurran et al. (1996) reported that Tacarigua males (Caroni drainage) secured all copulations in a mating experiment where they competed with Oropuche males (Oropuche drainage) for females from both populations, I did not find a difference in mating success or dominance behaviour when comparing Guanapo (Caroni drainage) and Oropuche males. Therefore it might be possible that the discovered variations again are found in individual populations but are not symptomatic for drainage systems. However, to clearly answer this question, more populations across drainage systems have to be compared in the same experiment.

Life-history differences

Many differences in life-history traits have been described in guppies, nearly all of them in populations from the same river that experience different predation regimes. As mentioned in previous chapters, individuals living in low predation habitats tend to mature at a later age and females give birth to fewer and larger offspring (Reznick & Endler, 1982), while guppies in high predation habitats reach maturity sooner and females produce more and smaller offspring (Reznick & Bryga, 1987). These traits can be reversed in a relatively short amount of time (years) after changes in the predation regime occurred (Reznick et al., 1990). Hence, life-history traits of fish facing comparable predation regimes are expected to be similar. In my experiments I could not detect any differences in brood size, offspring size or the number of days until a female gave birth between Guanapo and Oropuche individuals (see Chapter 5 for all results). The fact that only one population per drainage system was investigated will naturally reduce the power of these results, however, I do not expect to find major differences in life-history traits between drainage systems. Recently, a large study analysing multiple paternity patterns in broods from 10 populations across the Northern Mountain Range (including Tacarigua, Turure and Oropuche) revealed a skew in reproductive success among males. However, no difference in the skew was detected between males from low and high predation habitats or between drainage systems (Neff et al., 2008). All behavioural and life-history traits investigated during my PhD are listed in Table 16.

Table 16: Behavioural and life-history traits that were tested for population differences mainly between Guanapo (Caroni drainage) and Oropuche (Oropuche drainage) individuals during my doctoral research. Values in brackets indicate a low number of observed populations.

Trait	Populations tested	Difference between populations	Difference between drainage systems
Female schooling time	Gua, L.Tac, Quare, Oro, Tur	+	-
Schooling preference for own species over *P.picta*	Gua, L.Tac, Quare, Oro, Tur	+	-
Newborn schooling time	Gua, L.Tac, Quare, Oro, Tur	+	-
Male preference for own females	Gua, Oro	-	(-)
Male dominance behaviour	Gua, Oro	-	(-)
Days until birth	Gua, Oro	-	(-)
Brood size	Gua, Oro	-	(-)
Offspring size	Gua, Oro	-	(-)

Genetic differences

Other than with behavioural and life-history traits, considerable genetic differences have been detected between drainage systems in several studies. In their analysis of 23 allozymes, Carvalho et al. (1991) showed that the gene diversity based on Nei's gene diversity analysis was 66% due to differences between drainage systems, while 32% were due to within drainage differences and only 2% due to within river variation. In the same year, Shaw et al. (1991) found that populations divided in accordance with the drainage system they inhabited. Again, 25 allozymes were used for this study, 23 of them identical with the ones used in Carvalho et al. (1991). An investigation of mitochondrial DNA by Fajen & Breden (1992) showed a similar result. Most variation existed between drainage systems (3.9 to 5.6% sequence variation) where populations are believed to have been separated 600,000 to 1.2 million years ago. MtDNA variation between rivers within drainages ranged from 0.0 to 1.0%, with an estimated divergence time of 100,000 to 200,000 years for the most recently separated populations. All results mentioned here strengthen the 'two arcs hypothesis' that suggests that guppies and other freshwater species colonised Trinidad when the island was connected to the South American mainland and the Oropuche and Caroni drainage system belonged to different arms of the Orinoco. Genetically different guppy populations therefore spread across the separate systems and, due to lasting geographic isolation, can still be distinguished today (see Magurran, 2005). The most recent investigation of population history and genetic diversity in guppies by Willing et al. (2010), this time using SNP for a genome-wide analysis, confirmed the results of previous studies concerning population substructures due to geographic separation and the historic colonisation of Trinidad.

Different speciation models

Speciation is mainly a result of changes in the gene pool of a population and a reduction of gene flow between populations belonging to the same group of individuals. These changes can be caused by selection or genetic drift and lead to divergence between populations that might eventually end in speciation. Two main scenarios exist that would allow speciation to take place: allopatry and sympatry. In the end, speciation will be rendered complete if the newly established species can exist in sympatry with other closely related populations without losing their species specific characteristics.

Allopatric speciation

Allopatric speciation can occur if populations are separated by geographic barriers that prevent gene flow. Selection as well as genetic drift can then lead to genetic differentiation. If these species come into secondary contact after the removal of the barrier, several different scenarios are possible. Genetic, morphological and/or behavioural divergence might have been strong enough to prevent inter-breeding and both populations are fully reproductively separated. It might also be possible that populations can still interbreed but produce hybrids with reduced fitness. In this case, already existing weak reproductive barriers could be quickly reinforced by natural selection, a mechanism that finally isolates populations completely. A last possibility after secondary contact is that populations start reproducing until the mixing of gene-pools is complete again.

Sympatric speciation

Sympatric speciation on the other hand is the process of speciation in the absence of geographic barriers, meaning that individuals of a population are in direct spatial contact. However, partial isolation can be created by different geographical and ecological conditions. In this case gene flow between sub-populations can be reduced but does still exist. Disruptive selection for alternatively occurring adaptive models could now create phenotypes with differing preferences for food, habitat or host. If selection for extreme phenotypes over intermediate ones is strong enough, a population can eventually split into two (or more) separate species.

Poecilia obscura – a separate species?

Despite the large amount of genetic variation, the Trinidadian guppy, *Poecilia reticulata,* was treated as one species – albeit one potentially on its way to speciation - until the article published by Schories et al. (2009) that divided the guppy into two separate species. For this study, the mitochondrial control (D-loop) region and the cytochrome b gene as well as gonopodial characteristics were analysed. While no distinct differences in morphology could be detected that did not overlap between fish belonging to different drainage systems, the authors regarded the genetic variation as sufficient evidence that *Poecilia reticulata* and *P. obscura* belonged to separate and well established species. According to Schories et al., both species have been separated between 2.5 and 5 million years based on the cytochrome b sequences and between 0.4 and 4.2 million years based on the control region sequences and represent old species that have evolved in parallel evolution. Because they are lacking any obvious morphological differences, *P. reticulata* and *P. obscura* form a cryptic species complex together with *P. wingei*, who is given species status by the authors in the same paper. The genetic distance between these three putative guppy species is 0.029, and therefore in a range of values for morphologically very different species, e.g. in the genus *Xiphophorus* (e.g. was the genetic distance between Southern platyfish species and Southern swordtails the same as within the guppy species complex, i.e. 0.029) (Schories et al., 2009).

However, the genetic differences detected in this study were comparable to the results of former studies that did not conclude that the guppy should be split into two species. A close look at the arguments used by the authors of former studies and Schories et al. (2009) is therefore necessary to form a better understanding of this issue.

No full reproductive isolation exists between populations belonging to different rivers or drainage systems (Magurran, 1998), and, if kept together in the same tank, they mate and produce viable offspring (Houde, 1997). The high gene flow due to male sneaky mating behaviour is thought to prevent reproductive isolation and therefore speciation in the guppy (Magurran, 1998). Yet later studies revealed the existence of some reproductive barriers between populations of the Caroni and Oropuche drainage system. Ludlow & Magurran (2006) reported that sperm from native males had precedence over sperm of males belonging to the other drainage in a competitive context. This effect was symmetrical for both drainages. No disadvantage of foreign males could be discovered in a non-competitive experiment. The authors interpreted their results as evidence for the early stages of reproductive isolation between guppies. In the same year, Russell & Magurran (2006) detected the existence of some reproductive isolation between populations in the form of hybrid behavioural impairment of F_1 males that performed fewer mating behaviours compared to their parents. At the same time, there existed a hybrid breakdown for embryo viability, brood size and sperm count in F_2 and BC males. No effect on the reproductive performance of females was found. Again, the results of this study were interpreted as an indicator of an early stage of speciation.

Schories et al. (2009) on the other hand argued that the non-existence of obvious prezygotic isolation mechanisms between their guppy species are due to the fact that they evolved in allopatry and do not naturally co-occur. They see Haskins's introduction and the following mixing of gene pools as an artificial situation of sympatry, similar to enforced breeding in laboratory experiments. However, it is also possible to regard Haskins's introduction as the removal of a geographic barrier that separated two populations that now come into secondary contact, a scenario that could potentially happen to many geographically separated populations or species. The occurrence of gene flow will then show if and to what extent these populations have developed reproductive isolation. In the case that sufficient reproductive barriers exist or existing weak barriers are reinforced quickly, populations are likely to stay separate even if some hybridisation takes place. If, on the other hand, genetic barriers are not developed enough, hybridisation and gene flow between populations will be strong and eventually lead to the complete fusion of the diverging genotypes. If this happens, as was the case with Guanapo and Turure guppies after Haskins's introduction, and evolved genetic barriers are not strong enough to prevent a high amount of ongoing population mixing, the emerging species will fuse again and can therefore not be considered as separate. Even with some reproductive barriers already in place, the fact that especially male guppies do not distinguish between population origin of their mates, will lead to an increased amount of gene flow. Male guppies are able to differentiate between their own females and *P. picta*, a very similar looking species, as shown by Magurran & Ramnarine (2004). Males that occurred in sympatry with *P. picta* did not court heterospecifics while naïve males only ceased displaying to *P. picta* females after some days. It is very unlikely that the same preference for same-population females would develop in guppies belonging to different drainage systems, otherwise hardly any or at least a reduced amount of gene flow would be expected between populations of different drainages. To test this prediction, further mate choice experiments that follow the mating decisions of males and females over time would be necessary.

Returning to the different species definitions and concepts of the beginning of this chapter, most of them cannot be used to justify the existence of *P. obscura*. Both the Biological and Recognition species concept do not allow for hybridisation between species, which clearly takes place between different guppy populations. Because no ecological difference exists between both drainage systems and the ecological variation is much larger between the upper and lower parts of the same river than between different rivers, the Ecological species concept can also not fully explain the evolution of two separate species within both drainage systems. Both the Evolutionary species concept and the Genotypic cluster definition define species as lineages that maintain their identity from other lineages or clusters, in case of the later definition, even in the presence of gene flow. Because intermediate forms quickly form as soon as different populations come into contact, despite strong genetic differentiation, the term subspecies would be more appropriate to describe the situation found within the guppy. Some of the individual aspects joined in the Cohesion species concept (reproductive isolation, ecological selection and reproductive compatibility) are not met in *P. obscura*, this concept has to be excluded as a explanation as well, leaving only the Phylogenetic species concept. This concept defines a species as an irreducible cluster of organisms, with morphological and/or genetic characteristics distinct from other such clusters. Like Darwin's definition, the Phylogenetic species concept treats varieties as possible (future) species, but does not help with the decision where, i.e. at what amount of difference, to separate species (Mallet, 1995). With the help of ever better analysing methods it is therefore possible to find an increasing amount of genetic and morphological distinctions between individuals which would result in a growing number of species that differ very little from each other and might frequently only be distinguishable using molecular methods.

Conclusions

After taking various similarities and differences between Caroni and Oropuche populations into account, it seems unlikely to me that the split of guppies into *P. reticulata* and *P. obscura* is justified. Because of the absence of any clear morphological differences between both guppy species, Schories et al. (2009) argue that they form a cryptic species complex and can only be told apart by genetic analysis. However, not even members of both species distinguish between one another and seem to mate at random without regard to population origin. They thereby produce viable offspring and populations can mix quickly when coming into contact, as could be seen after Haskins's introduction in the Turure. The existence of some reproductive barriers seems more to indicate an early stage of the speciation process, as was suggested many times before by various authors (e.g. Ludlow & Magurran, 2006). Dividing species in ever smaller units, based on a probably vast amount of smaller and larger genetic differences that can be detected with increasingly precise molecular methods, will not add more clarity to systematics. It is more likely to shift the focus away from the species itself, how they stay separate from other groups despite possible overlaps and how they change throughout time to a mere appliance of definitions. Instead of seeing species as entities that can change through time in often unpredictable ways, this mostly phylogenetic approach would sort small groups of individuals into well described boxes. This might not always reflect the situation found in the real world where it will not be possible to apply the same narrow scheme to explain when a species is a species and when it is not. Furthermore, in the case of the guppy, the split into two species would take away the opportunity for examining the order of the arising and development of reproductive barriers within two subspecies, something that is nearly impossible to determine in already fully established species.

Chapter 8 General discussion

During my PhD, I investigated the consequences of Haskins's introduction and tried to find explanations for the invasive success of Guanapo guppies over their Turure counterparts. Several different aspects of guppy life, like behaviour, life-history and genetics, were examined and results compared between populations. However, no differences could be found that would explain any superiority of Guanapo fish that could have contributed to the disappearance of the original Turure genotype. Before I merge and discuss the results presented in the last 7 chapters, I briefly summarise my findings from each chapter of my thesis.

Summary

The genetic analysis of fish from several sites along the Turure using SNP markers showed that the invasive front of Guanapo alleles has travelled further downstream since the last investigation and probably has already passed the Turure's confluence with the Quare. Similar to previous studies, the original Turure genotype only occurred in traces close to the introduction site and in the middle parts of the river. The proportion of native Turure alleles then slowly grew with increasing distance to the barrier waterfall and reached 40% at the Turure's confluence with the Quare (Chapter 2).

Male mating behaviour did not differ between populations and both males from the Guanapo and the Oropuche did not distinguish between female origin when given the choice. No influence of Guanapo males on the mating behaviour of Oropuche males was found in a mating experiment. This makes a difference in dominance behaviour and therefore an advantage in securing matings for Guanapo fish over Oropuche males unlikely. When tested for their general ability to become familiar with females in a short amount of time (18 days), male guppies did not prefer to court unfamiliar females over familiar ones. Females, on the other hand, chose to stay close to familiar fish in a schooling experiment even after many years under laboratory conditions (Chapter 3).

In general, females in my experiments displayed a very low willingness to mate except when virgins, even after having been isolated from males for up to 6 months. This reluctance could possibly increase the importance of forced copulations for sperm transfer that reduce female choice and thereby sexual selection. They also seemed to be unable to distinguish between other females of differing population origin which will increase their likelihood forming mixed schools in the wild. These are then encountered by males that do not distinguish between female population origin but instead mate at random. Taken together, both behaviours could accelerate the speed with which populations mix in an invasive event. The amount of time spent schooling differed between populations, but no effect of drainage system could be detected. The same was true for a female's preference to school with conspecifics rather than *P. picta*, that existed in two out of five populations (Chapter 4).

No differences were found in life-history traits such as size, offspring size and number, or days until the birth of the first clutch between Guanapo and Oropuche fish. In a crossing experiment, the parents' population origin in a cross did not make a difference to the aforementioned traits except that offspring with a Oro mother – Gua father were significantly smaller than newborns from all other tested treatments and controls. This result might have been due to the relatively small sample size. However, population differences were found in the time newborn guppies spent schooling together, but could not be related to drainage system. Mixed fish showed more variation than pure-bred offspring in the amount of time they spent schooling. Furthermore, the effect of the mother's population origin seemed to be slightly stronger than that of the father with regard to schooling time (Chapter 5).

Contrary to expectations, Guanapo fish did not have a reproductive advantage over Oropuche guppies in a mesocosm experiment, in fact, the proportion of Oropuche alleles was slightly higher than the original proportion of stocked fish belonging to this population. Therefore it is unlikely that reproductive superiority of Guanapo fish was responsible for the present situation in the Turure (Chapter 6).

Lastly, all results obtained during my PhD, together with findings from the literature, were combined to discuss the benefits of splitting the guppy into two species, *Poecilia reticulata* and *P. obscura* (Schories et al., 2009). Because no obvious behavioural and life-history differences existed between drainage systems, and guppies mate willingly across drainage system borders if given the chance, thereby producing viable offspring, I concluded that it is unnecessary to have two separate species instead of one genetically very variable one. This split does not help to simplify the systematics within the genus and has no proper foundation in existing differences between species that would hold in the presence of population mixing (Chapter 7).

Guanapo guppies – an invasive population?

Are Guanapo guppies an invasive population after all? To answer this question, I will take the results of my PhD together and compare them to the definition of invasive species mentioned in Chapter 1. Furthermore, I will go back to some of the topics mentioned in the General Introduction, and especially discuss the importance of behaviour and genetic homogenisation of populations for the invasive success of Guanapo guppies. I will then discuss the wider implications of the results presented here for the understanding of mechanisms that can influence within-species invasions.

Steps to become an invader

When examining the establishment success of Guanapo guppies in the Turure, it is easy to see the first three of four important steps (Introduction, establishment, spread, impact) on the way to invasiveness fulfilled. C.P. Haskins collected around 200 guppies in the lower Guanapo, and, while releasing them in the upper Turure, transported them from one river (and drainage system) to the next. Here the introduced guppies quickly managed to establish a viable population and colonised the previously guppy-free parts of the Turure upstream of the introduction site. With the overcoming of the barriers waterfall, the Guanapo fish also managed to spread to new stretches of the middle and lower Turure, where they encountered the native Turure population (Shaw et al., 1992). However, the last step on their way to be called an invasive population, namely having a negative impact on other species/populations in their new environment, is less obvious to notice.

In the absence of any evident morphological, behavioural and life-history differences between Caroni and Oropuche guppies, it took 37 years to discover the implications of Haskins's introduction with the help of genetic analysis using allozymes (Shaw et al., 1991). Only then did it become apparent that Guanapo fish had succeeded in colonising the Turure and thereby reduced the amount of the original Turure genotype found in the river to less than 15% of all examined alleles. The colonisation and mixing with Turure fish is still an ongoing process today, as several authors (Shaw et al., 1992; Carvalho et al., 1996; Russell, 2004) and my own analysis showed. The nearly complete replacement of the original population by Guanapo guppies and the threat this introduction experiment still poses for other populations in adjacent rivers is enough evidence to finally call Guanapo guppies invasive in the Turure. However, their means of achieving this colonisation success have to be analysed carefully to see if this introduction experiment resulted in a typical and maybe even foreseeable invasion or if Haskins's introduction is an example of a less obvious form of invasive success that nevertheless poses a threat to biodiversity and the survival of populations.

Behaviour as a reason for invasive success

Holway & Suarez (1999) were among the first to point out the importance of behaviour for the invasive success of a population. They argued that behavioural variations can influence the invasive ability of a population and shifts in behavioural characteristics following introduction can enhance competitiveness. An example mentioned in the General introduction was that of invasive Argentine ants (*Linepithema humile*) in California, where this species forms extremely dense and large colonies that out-compete native ants. This kind of colony formation is possible due to the lack of intra-specific aggression in this species within its new environment and is contrasted by a strong exhibition of aggression in its native range (Suarez et al., 1999).

No behavioural differences were found between the invasive Guanapo guppies and Oropuche fish. When tested in the laboratory, Oropuche males did not reduce their amount of courtship behaviour directed towards females in the presence of Guanapo males compared to the presence of rivals from their own population. No other form of dominant or aggressive behaviour could be detected in Guanapo males. It is therefore unlikely that a more dominant behaviour expressed by Guanapo males reduced the mating success of native males. In contrast to Magurran et al. (1996), who found that while females did not prefer males from either population in a choice test, Tacarigua males (Caroni drainage) secured all copulations in a mating experiment when tested with rivals from the Oropuche drainage, no reproductive superiority of Guanapo males could be detected in the allele frequencies of the mesocosm experiment. Variation in behaviour or behavioural superiority of the invading population can therefore not be used to explain the success of Guanapo individuals in the Turure. However, some general behavioural patterns exist in guppies as a species that could help to increase the speed of population mixing after an introduction. Male guppies can overrule female choice with forced copulations (Houde, 1997) and do not seem to distinguish between the population origin of females (Chapter 3). Females similarly do not prefer to school with fish from the same population over fish from a different guppy population (Chapter 4). Therefore it is likely to find schools of mixed origin in the wild after the introduction of a different population. These mixed schools are then encountered by males that do not differentiate between population origin but try to mate with as many females as possible. This combination of behaviours found in male and female guppies could enhance gene pool mixing, without the need for any behavioural variations between populations.

The existence of invasive success of Guanapo guppies in the absence of behavioural differences, but still influenced by certain types of behaviour, again highlights the general need for detailed investigations of behavioural patterns in an invasive process, independent of species. Thereby it is important to examine invaders as well as native populations, especially in the case when population mixing can take place. Furthermore, these results show that the absence of any form of behavioural superiority does not mean behaviour itself is unimportant during the invasion of a population. Quite the contrary, behaviours that are not deemed invasive in the first place, like the ability to distinguish between their own and a different population and decisions based thereupon, could still influence the direction and speed with that two populations mix after coming into first contact.

Genetic homogenisation of populations

The reduction of genetic diversity, local extirpation of natives and genetic homogenisation are often the results of an invasion process and ultimately increase the risk of extinction for a native species or population (Olden & LeRoy Poff, 2003). Despite the absence of any detectable behavioural or life-history differences between Guanapo and Oropuche fish, that would explain the invasive success of the former population in the Turure since Haskins's introduction, the amount of genetic variation between these populations used to be very large. However, after more than fifty years of population mixing, the genetic differences have almost disappeared and the original Turure fish are nearly entirely displaced throughout the river by guppies of Guanapo ancestry. It was pointed out before by Daehler & Carino (2001) that intraspecific hybridisation can homogenise discrete characteristics of geographic isolated populations, and this is exactly what happened after Haskins's introduction. This transplant experiment is therefore another example of genetic homogenisation of populations and a reduction of biodiversity within a species by an invasive event. The existing, weak reproductive barriers between fish belonging to opposite drainage systems (Russell & Magurran, 2006; Ludlow & Magurran, 2006) were not strong enough to effectively prevent large-scale population mixing.

As seen in studies concentrating on other species [e.g. Atlantic salmon (Hindar et al., 2006), New Zealand grey duck (Rhymer et al., 1994)], the contact between genetically distinct populations or closely related species after an introduction can easily destroy genetic variation and blur the borders between separated populations or species. In the long term, and keeping the many invasive events worldwide in mind, population mixing and genetic homogenisation will lead to the creation of a more uniform and impoverished form of biodiversity (Olden et al., 2004). But the loss of distinctiveness between populations due to genetic homogenisation is more than just a threat to biodiversity. Taking away genetic variability can leave populations and species more vulnerable to environmental and climatic changes. Without a broad basis of genetic material to work on, some biotic and abiotic changes might be too extreme or appear too quickly for selection to shape new traits that help a species to adapt to its environment. However, in the case of the guppy, enough genetic variability remained in fish inhabiting the Turure to quickly adapt to changes and develop predation regime-specific schooling behaviour (Magurran et al., 1992).

Reasons for the success of Guanapo fish in the absence of population differences

But how is it possible to explain the invasive success of Guanapo guppies in the Turure in the absence of any discovered differences between these two populations? One possible answer to this question could be found within the geographic situation in that the introduction and following invasion took place. When Haskins introduced the Guanapo guppies, he chose a location above a barrier waterfall that was uninhabited by the native Turure population. Roman & Darling (2007) recently stated that high numbers of introduced individuals and multiple introductions reduced the risk of founder effects in most of 43 examined successful aquatic invasions. The release of 200 fish seems to have been enough to prevent larger bottlenecks. In the newly founded population of

Guanapo fish the mean number of alleles per locus was only slightly lower than in the source population (1.2 vs. 1.4) (Carvalho et al., 1996). Guanapo fish established a population, overcame the barrier and came in contact with the original Turure fish. Gene pool mixing took place, while the invasive guppies spread downstream, thereby encountering more and more native individuals.

Every time fish from the Guanapo and Turure mated and produced hybrid offspring, both genotypes became a bit more diluted. While the death of Turure guppies would take native alleles out of the gene pool, no reservoir of original Turure fish could supply this population with new, unmixed individuals. Guanapo fish, on the other hand, always had a constant supply of pure Guanapo alleles from above the waterfall, so that the impact that death and population mixing had on the genetic composure of these fish could be balanced by the addition of new, unmixed individuals. Over the course of 37 years, until the genetic results of this transplant experiment were discovered by Shaw et al. (1991), this constant addition of Guanapo alleles to the mixing population in the middle and lower parts of the Turure might have added enough genetic material to explain the successful spread of Guanapo guppies in this river in the absence of any superior behavioural, life-history or genetic traits. A quick initial establishment and spread of Guanapo guppies in the parts of the Turure below the waterfall could also have been supported by a temporal extirpation of native Turure guppies in these places. Population density in the upper regions of rivers can be low at times, and when I first sampled the Turure in 2009, I could not find any guppies above and directly below the waterfall. One year later, however, it was possible to catch enough fish for genetic analysis. One reason for a sudden decline in population density in the upper parts of rivers are floods that can remove between 22 - 92% of individuals (Grether et al., 2001).

To investigate this theoretical explanation of the present Turure situation, it would be necessary to model the introduction event and following spread of Guanapo fish using different population sizes both of Guanapo and Turure guppies as well as differing numbers of pure Guanapo fish that are added to the mixing gene pool each year. This would help to find the minimum number of individuals required to overcome the barrier waterfall each year to explain the dominance and spread of Guanapo alleles in the Turure in the absence of any other differences between populations. See Figure 35 for a schematic illustration on the colonisation event in the Turure since Haskins's introduction in 1957. A description of a possible model to further investigate gene pool mixing between two populations is found in the Future research section at the end of this chapter.

When trying to understand the outcome of Haskins's introduction, it might help to divide it into two separate events. The original transplant of Guanapo fish to the site above the barrier waterfall can be seen as a single introduction where enough fish were released to prevent a strong bottleneck and founder effect. In the theoretical case that this first site had been populated by a native population, I would expect to find population mixing and the reaching of a stable equilibrium between alleles belonging to both populations similar to the results of the mesocosm experiment. This hypothesis of a stable equilibrium was originally proposed for the entire Turure (Becher & Magurran, 2000), but does not seem very likely now in this context. In the mesocosms, no reproductive advantages existed for Guanapo fish. On the contrary, the proportions of alleles belonging to either the Guanapo or the Oropuche indicated a slight advantage for the latter after one year of population mixing (Chapter 6).

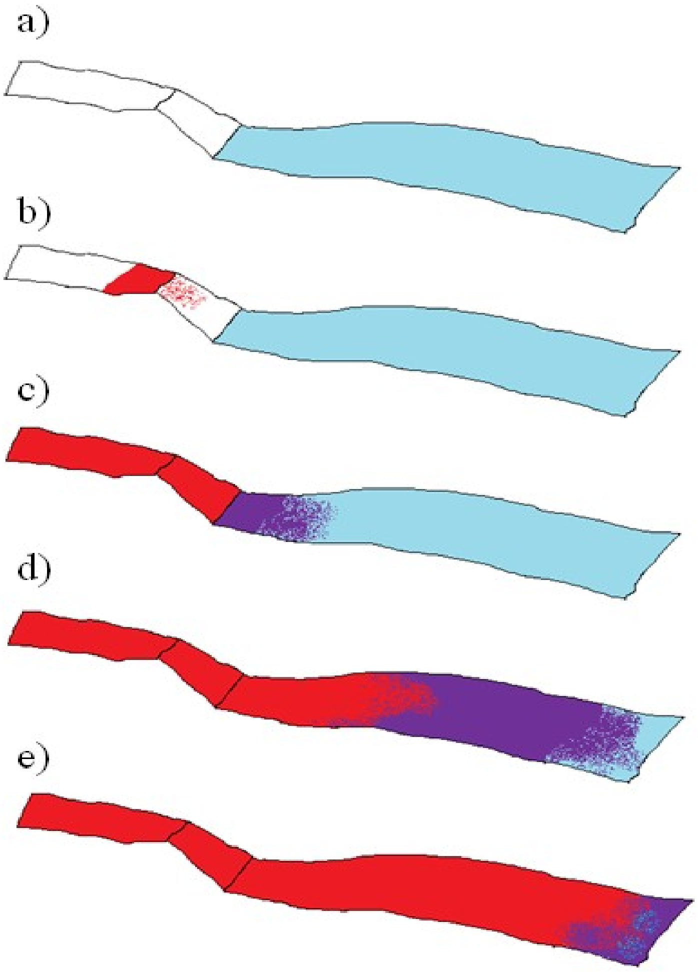


Figure 35: Schematic illustration of the colonisation event in the Turure after Haskins's introduction, starting with the original 'Turure fish only' situation (a). After the introduction event, fish start to colonise the upper regions of the Turure and overcome the barrier waterfall (b). Guanapo fish now begin to mix with the natives, and hybrid fish start to spread downstream (c). Because of the ongoing addition on new, unmixed Guanapo guppies into the mixing zone, the native genotype slowly disappears while the invasive front moves further downstream (d). Eventually, the entire river is populated by Guanapo fish, while the original Turure alleles only survive in traces (e).

However, the existence of the barrier waterfall adds an additional layer to the whole process. The constant introduction of pure Guanapo fish from above the waterfall to the middle and lower parts of the Turure can be seen as a series of several introductions with animals from the same source population. Because the number of individuals per propagule and the number of release events can significantly influence the establishment success of a population (Lockwood et al., 2005), the situation at the Turure is likely to have had a positive influence on the spread of Guanapo guppies. As time passed, this continuous adding of unmixed Guanapo guppies to the mixing population below the barrier might have been enough to genetically swamp the entire river with the invasive genotype while natural processes like mortality slowly reduced the amount of Turure alleles found in this stream.

Conclusions

To sum up, the successful spread of Guanapo guppies in the Turure does not seem to be due to behavioural, life-history or genetic superiority of the invaders. This population is probably not more or less invasive than others, and the situation found in the Turure today can be best explained by the special geographic features of this river. If a different population had been released instead of Guanapo fish, I would expect to get a similar result of population mixing and reduction of the native Turure genotype.

This result underlines that even a population or species that does not display any traits that make it superior to others, can still threaten the survival or genetic integrity of close relations or genetically distinct populations in certain circumstances. Translocating individuals from their native habitat and introducing them into new places will therefore always pose potential threats to other species and ultimately to biodiversity. Closely related species or subspecies are especially vulnerable to genetic homogenisation, as happened within the guppy, because of non-existing or weak reproductive barriers that could prevent hybridisation. Initial gene flow after the introduction of an alien population will lead to the mixing of possibly distinct genotypes and can in the worst case end in the disappearance of one of them. This reduction of genetic variety may destroy specific local adaptations and reduce the possibility of a population to adapt quickly to changing environmental conditions, therefore reducing its chances of survival. But even without an actual threat for survival could the mixing of once separated gene pools lead to an increase of genetic homogenisation and a loss of biodiversity. This means that even small-scale and short-range translocations of individuals belonging to the same species can have an impact on, and threaten the existence of native populations. The effects Haskins's introduction had on Turure guppies were probably only detected because the guppy is an intensely studied species and receives a lot of scientific attention. This might of course be one reason for the frequent transfer of individuals belonging to different populations in the first place, but also highlights another problem. Other, less well-studied populations might be in a similar situation as the Turure fish investigated here, regarding the amount of gene pool mixing and genetic homogenisation, but are simply overlooked by biologists. It is therefore possible that a considerable amount of genetic diversity vanishes without somebody knowing about it.

For that reason introductions of animals or plants for a scientific purpose should be carried out with a lot more caution than it is presently done. While it might be difficult nowadays to reduce or prevent the unintentional spread of species around the world, scientists should bear in mind that it is easy to endanger biological diversity even with small-scale experimental introductions, and that the absence of traits that are commonly thought of as invasive in a species is no guarantee that this species does not have a negative effect on its new environment. The results of my thesis highlight the general need to include behaviour when investigating potentially or actually invasive populations. Special attention has to be paid to behavioural traits that could influence the speed and direction of population mixing despite not being regarded as “invasive” behaviours in the first place. Not being able to distinguish between members of their own and a different population as well as any behaviour that would increase cross-population matings are particularly important to analyse. A thorough and detailed investigation of potential risk factors should precede every plan to translocate species for scientific or other reasons. Routinely screening imported animals or plant species before their release and producing a risk assessment regarding their potential impact on the environment should become a lot more common than it is today and should also include the release of individuals for scientific purposes. Next to an investigation of a species' traits, the ecological circumstances of the introduction should be analysed as well. The outcome of the translocation of species could be influenced by geographic or other factors scientists are not aware of, as was shown by some of my results, and should therefore be included in the general assessment before a release event. Of course this will be time-consuming and might be expensive, but not trying everything to reduce the potential risk of endangering a population's survival by scientific experiments would be hypocritical and unethical.

In the case of the guppy, Haskins's introduction is unlikely to endanger the survival of the species. However, some of the genetic diversity that guppies became famous for has been irretrievably lost due to an experiment that was carried out to understand exactly this diversity. Back then, the existing strong genetic differences between populations were not as well known. But even today, with the knowledge of what has happened in the past, some researchers persist on introducing species, including guppies in Trinidad, into new locations in the name of science. In the end, scientists have to ask themselves if to answer a question by any means is ethical if at the same time it might endanger the species/population under study or any other part of its surroundings. A general obligation to obtain permission for all species transfers for scientific purposes in form of a permit might help to rise awareness and caution of potential negative influences each translocation can have on the survival of native species, genetic integrity of local gene pools and biodiversity.

Future research

To further investigate the reasons that, after Haskins's introduction, led to the present situation in the Turure, several experiments looking at different aspects of the invasion would be useful. Both the current invasive front and rate of spread as well as consequences for the speciation process between Caroni and Oropuche guppies should be further investigated. Finally, modelling the introduction event could help to understand more about the numbers of individuals needed to explain the nearly complete colonisation of Guanapo fish in the Turure in the absence of population differences.

Present location and future spread of the invasive front in the Turure

Because the colonisation of the Turure by Guanapo fish seems to be an ongoing process, there is a need for repeatedly sampling the Turure and adjacent rivers like the Quare or Oropuche. Then the invasive process could be followed through time and space, giving detailed information about the speed with which Guanapo guppies spread as well as patterns of mixing between populations. Always using the same set of markers for this analysis would furthermore increase the comparability between the studies done in different years. It would also be useful to look for differences in the spread of male and female alleles through the mixing population. This could finally answer the question to what extent the present situation found in the Turure is due to behavioural differences of the opposite sexes or if the sex of an individual does not play much of a role during the invasion process. Because male guppies are more likely to travel large distances

(Croft et al., 2003), it might be that they are more important for a quick spread of Guanapo alleles in the Turure, while females later catch up when the growing population spreads.

Consequences of invasion for the speciation process

The amount of population mixing between Guanapo and Turure fish that followed Haskins's introduction could pose a serious threat for the possible speciation process observed between Caroni and Oropuche populations. Because of the nearly complete disappearance of the original Turure genotype and an ongoing spread of Guanapo guppies downstream, the emerging reproductive barriers like hybrid breakdown in the F_2 generation (Russell & Magurran, 2006) and gametic isolation of guppies belonging to different drainage systems (Ludlow & Magurran, 2006) could disappear again. It seems unlikely that Guanapo fish will colonise the entire Oropuche system, and especially above barrier waterfalls and in rivers further away from the Turure unmixed Oropuche guppies will continue to exist. Nevertheless, the reduction in genetic variation between drainage systems reduces or at least sets back the chances for entire reproductive isolation to appear within the foreseeable future. A series of investigations would therefore be useful to monitor the amount and direction of gene flow between populations at increasing distances from the introduction site. Laboratory experiments could further shed light on the importance of the already existing reproductive barriers in the presence of different proportions of invaders. Taking the crossing experiments done by Russell & Magurran (2006) further and carefully following the spread of the invasive genome as well as observing consequences for courtship behaviour and offspring production could help to find a threshold up to which reproductive barriers are effective in reducing gene flow and prevent population specific genetic patterns to disappear.

Modelling of the invasive process

A simulation of the spread of Guanapo guppies and population mixing with Turure fish following Haskins's introduction could help to further understand the principles behind this invasive process. With the help of a model it would be possible to calculate the minimum number of Guanapo fish that have to overcome the barrier waterfall and contribute to gene pool mixing in the Turure to explain their success in the absence of all other differences between both populations. Several parameters could be included into this simulation (see Table 17 for a possible list).

Table 17: A set of possible parameters that could be included to model the invasion of the Turure by Guanapo fish.

Parameter	
Population size Gua/Tur	Flexible, to allow for different scenarios to take place
Survival rate	From literature
No. of produced offspring	From literature
No. of fish added from above waterfall per time step	Flexible, to allow for different scenarios to take place
No. of alleles	Simplest case: 2 alleles, 3 conditions: Gua-Gua, Gua-Tur, Tur-Tur
Rate of movement	Optional, if distance is included in model as a parameter

To resemble the geographical conditions that exist in the Turure, two scenarios seem possible. The waterfall could be added as a barrier between the introduced Guanapo fish and the middle parts of the river where population mixing can take place. Movement of fish and therefore gene flow could only take place in one direction down the waterfall. A second, easier option would be to define the number of Guanapo guppies that are added to the model per time step, without the need for the existence of two separate zones.

If the rate of movement of individuals is included as a parameter, the model needs a distance function. In this case it would be possible to simulate the gradual increase of Guanapo fish and the distance covered by the invasive front per time. However, the inclusion of this parameter is optional; in the simplest case it would be sufficient to simulate the mixing of two populations in a confined space.

A series of actions would then have to take place with every time step (e.g. months):

1. Pre-mating round: Take fish that die out of model
   Add Guanapo guppies from above the waterfall
   [Fish movement downstream]
2. Mating round: Based on existing proportions of alleles in the population

Add Guanapo, Turure or mixed fish that are born
Calculate new proportions

3. 2nd pre-mating round
4. ...

The varying proportions of pure Guanapo and Turure fish as well as mixed offspring could then be followed through time. To make this model even simpler, it would be possible to only use Guanapo and Turure individuals with no possibility of forming hybrids. In this case there would be no need for separate alleles within each individual and the simulation would only concentrate on the change in proportions of fish from both populations. In the end, this should lead to a similar result as when allowing population mixing. By changing the parameters 'Size of the original Turure population' and 'Number of added Guanapo per time step', it would be possible to experiment with different scenarios and see how these parameters influence the success and speed of colonisation. This type of model could of course also be used examine other invasive or mixing events, and to compare general processes underlying invasions.

Outlook

So far the guppy, *Poecilia reticulata,* has been used as a model species for a variety of different behavioural, life-history and evolutionary aspects, including familiarity, sperm competition and fast evolutionary change in the face of changing ecological conditions. Based on the results of my thesis, further use of the guppy as a model species for intra-specific invasion and its consequences would certainly help to get a better understanding of mechanisms that influence the outcome of an invasion in not fully reproductively isolated populations and subspecies. Several aspects recommend the use of the guppy for this important field in ecology. Because this species has been investigated for decades, it's behavioural, life-history and genetic aspects are well described and understood, which would be of advantage for a better understanding of the mechanisms underlying invasion. The guppy can easily be observed both in the field and the lab, and reproduces fast enough to obtain large sample sizes in studies. Several well documented introductions involving different guppy populations exist across Trinidad that could be used for ongoing research on the effects of translocations in the wild without the need for further introductions. And lastly, the relatively easy way to set up mesocosms, where experiments involving more than one population can be carried out under semi-natural circumstances, offers the chance for detailed and controlled studies involving large numbers of fish that resemble small populations. In this way it might be possible to test for effects that can neither be investigated in the wild where no further introductions should be carried out, nor in the artificial surroundings of a lab. I therefore highly recommend the use of the guppy to get a better inside into the consequences of invasions in nature and to find answers to the question how to prevent and control them more efficiently.

Literature

Arim, M., Abades, S.R., Neill, P.E., Lima, M. & Marquet, P.A. 2006. Spread dynamics of invasive species. Proceedings of the National Academy of Sciences, 103, 374-378.

Becher, S.A. & Magurran, A.E. 2000. Gene flow in Trinidadian guppies. Journal of Fish Biology, 56, 241-249.

Becher, S.A. & Magurran, A.E. 2004. Multiple mating and reproductive skew in Trinidadian guppies. Proceedings of the Royal Society London B, 271, 1009-1014.

Berger, J. & Cunningham, C. 1995. Multiple bottlenecks, allopatric lineages and Badlands bison *Bos bison* – consequences of lineage mixing. Biological Conservation, 71, 13-23.

Berger, J., Suzuki, T., Senti, K.-A., Stubbs, J., Schaffner, G. & Dickson, B.J. 2001. Genetic mapping with SNP markers in *Drosophila*. Nature Genetics, 29, 475-481.

Bisazza, A. 1993. Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. Behavioural ecology of fishes, 23, 257-286.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia, 144, 1-11.

Breden, F. & Stoner, G. 1987. Male predation risk determines female preference in the Trinidad guppy. Nature, 329, 831-832.

Brown, J.A. & Colgan, P.W. 1986. Individual and species recognition in centrarchid fishes: evidence and hypotheses. Behavioural Ecology Sociobiology, 19, 373-379.

Carlton, J.T. 1999. The scale and ecological consequences of biological invasions in the world's oceans. In: Invasive species and biodiversity management. (Ed. by O.T. Sundlund, P.J. Shei & A. Viken), pp. 195-212. Dordecht: Kluwer Academic.

Carvalho, G.R., Shaw, P.W., Magurran, A.E. & Seghers, B.H. 1991. Marked genetic divergence revealed by allozymes among populations of the guppy *Poecilia reticulata* (Poeciliidae), in Trinidad. Biological Journal of the Linnean Society, 42, 389-405.

Carvalho, G.R., Shaw, P.L. & Hauser, L. 1996. Artificial introductions, evolutionary change and population differentiation in Trinidadian guppies (*Poecilia reticulata*: Poeciliidae). Biological Journal of the Linnean Society, 57, 219-234.

Cassey, P., Blackburn, T.M., Duncan, R.P. & Chown, S.L. 2005. Concerning invasive species: Reply to Brown and Sax. Austral Ecology, 30, 475-480.

Chivers, D.P., Brown, G.E. & Smith, R.J.F. 1995. Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas)* – implications for antipredator behaviour. Canadian Journal of Zoology, 73, 955-960.

Clavero, M. & García-Berthou, E. 2005. Invasive species are a leading cause of animal extinctions. TRENDS in Ecology and Evolution, 20, 110.

Cohen, A.N. & Carlton, J.T. 1998. Accelerating invasion rate in a highly invaded estuary. Science, 279, 555-558.

Convention on Biological Diversity. 1993. [online]. Available from:

http://www.cbd.int/convention/articles.shtml?a=cbd-02 [Accessed 17th May 2010].

Cracraft, J. 1989. Speciation and its ontology: The empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In: Speciation and its consequences (Ed. by D. Otte & J.A. Endler), pp. 28-59. Sunderland, MA: Sinauer Associates.

Crispo, E., Bentzen, P., Reznick, D.N., Kinnison, T. & Hendry, A.P. 2006. The relative influence of natural selection and geography on gene flow in guppies. Molecular Ecology, 15, 49-62.

Croft, D.P., Albanese, B., Arrowsmith, B.J., Botham, M., Webster, M. & Krause, J. 2003. Sex-biased movement in the guppy (*Poecilia reticulata*). Oecologia, 137, 62-68.

Croft, D.P., Arrowsmith, B.J., Bielby, J., Skinner, K., White, E., Couzin, I.D., Magurran, A.E. Ramnarine, I. & Krause, J. 2003b. Mechanisms underlying school composition in the Trinidadian guppy, *Poecilia reticulata*. OIKOS, 100, 429-438.

Croft, D.P., Arrowsmith, B.J., Webster, M. & Krause, J. 2004. Intra-sexual preferences for familiar fish in male guppies. Journal of Fish Biology, 64, 279-283.

Daehler, C.C. 2009. Short lag times for invasive tropical plants: evidence from experimental plantings in Hawaii. PloS ONE, 4, 1-5.

Daehler, C.C. & Carino, D.A. 2001. Hybridization between native and alien plants and its consequences. In: Biotic Homogenisation. (Ed. by J.L. Lockwood & M.L. McKinney), pp. 81-102. Alphen aan den Rijn: Kluwer Academic/Plenum Publishers.

Darwin, C. 1859. On the origin of species. London: John Murray.

Davis, M.A. 2003. Biotic globalization: does competition from introduced species threaten biodiversity? BioScience, 53, 481-489.

Dosen, L.D. & Montgomerie, R. 2004. Female size influences mate preferences of male guppies. Ethology, 110, 245-255.

Dugatkin, L.A. & Alfieri, M. 1991. Guppies and the TIT FOR TAT strategy: preference based on past interaction. Behavioural Ecology and Sociobiology, 28, 243-246.

Echelle, A.A. & Echelle, A.F. 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs pupfish and Sheepshead minnow. Conservation Biology, 11, 153-161.

Elton, C.S. 1958. The ecology of invasions by animals and plants. Chicago: The University of Chicago Press.

Endler, J.A. 1980. Natural selection on colour patterns in *Poecilia reticulata*. Evolution, 34, 76-91.

Endler, J.A. 1983. Natural and sexual selection on colour patterns in poeciliid fishes. Environmental Biology of Fishes, 9, 173-190.

Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. TRENDS in Ecology and Evolution, 10, 22-29.

Endler, J.A. & Houde, A.E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution, 49, 456-468.

Evans, J.P. 2010. Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. Proceedings of the Royal Society Series B, doi: 10.1098/rspb.2010.0826, published online, downloaded on June 8, 2010.

Evans, J.P. & Magurran, A.E. 2000. Multiple benefits of multiple mating in guppies. Proceedings of the National Academy of Sciences, 97,10074-10076.

Evans, J.P. & Magurran, A.E. 2001. Patterns of sperm precedence and predictors of paternity in the Trinidadian guppy. Proceedings of the Royal Society London B, 268, 719-724.

Evans, J.P. & Rutstein, A.N. 2008. Post-copulatory sexual selection favours intrinsically good sperm competitors. Behavioral Ecology and Sociobiology, 62, 1167-1173.

Evans, J.P., Pilastro, A. & Ramnarine, I.W. 2003. Sperm transfer through forced matings and its evolutionary implications in natural guppy (*Poecilia reticulata*) populations. Biological Journal of the Linnean Society, 78, 605-612.

Evans, J.P., Zane, L., Francescato, S. & Pilastro, A. 2003. Directional postcopulatory sexual selection revealed by artificial insemination. Nature, 421, 360-363.

Evans, J.P., Kelley, J.L., Bisazza, A., Finazzo, E. & Pilastro, A. 2004. Sire attractiveness influences offspring performance in guppies. Proceedings of the Royal Society Series B, 271, 2035-2042.

Fajen, A. & Breden, F. 1992. Mitochondrial DNA sequence variation among natural populations of the Trinidad guppy, *Poecilia reticulata*. Evolution, 45, 1457-1465.

Galil, B.S. 2007. Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. Marine Pollution Bulletin, 55, 314-322.

Garcia-Vazquez, E., Moran, P., Perez, J., Martinez, J.L., Izquierdo, J.I., de Gaudemar, B. & Beall, E. 2002. Interspecific barriers between salmonids when hybridisation is due to sneak mating. Heredity, 89, 288-292.

Goldschmidt, T., Witte, F. & Wanink, J. 1993. Cascading effects of the introduced Nile Perch on the detritivorous/phytoplanktivorous species in the sublittoral areas of Lake Victoria. Conservation Biology, 7, 686-700.

Grether, G.F., Millie, D.F., Bryant, M.J., Reznick, D.N. & Mayea, W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology, 82, 1546-1559.

Griffiths, S.W. & Magurran, A.E. 1997a. Familiarity in schooling fish: how long does it take to acquire? Animal Behaviour, 53, 945-949.

Griffiths, S.W. & Magurran, A.E. 1997b. Schooling preferences for familiar fish vary with group size in a wild guppy population. Proceedings of the Royal Society Series B, 264, 547-551.

Griffiths, S.W. & Magurran, A.E. 1998. Sex and schooling behaviour in the Trinidadian guppy. Animal Behaviour, 56, 689-693.

Guevara-Fiore, P., Skinner, A. & Watt, P.J. 2009. Do male guppies distinguish virgin females from recently mated ones? Animal Behaviour, **77**, 425-431.

Hastings, A., Cuddington, K., Davies, K.F., Dugaw, C.J., Elmendorf, S., Freestone, A., Harrison, S., Holland, M., Lambrinos, J., Malvadkar, U., Melbourne, B.A., Moore, K., Taylor, C. & Thomson, D. 2005. The spatial spread of invasions: new developments in theory and evidence. Ecology Letters, 8, 91-101.

Hindar, K., Ryman, N. & Utter, F. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences, 48, 945-957.

Hindar, K., Fleming, I.A., McGinnity, P. & Diserud, O. 2006. Genetic and ecological effects of salmon farming on wild salmon: modelling from experimental results. ICES Journal of Marine Science, 63, 1234-1247.

Holldöbler, B. & Wilson, E.O. 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften, 64, 8-15.

Holway, D.A. & Suarez, A.V. 1999. Animal behaviour: an essential component of invasion biology. TRENDS in Ecology and Evolution 14, 328-330.

Houde, A.E. 1988. Genetic difference in female choice between two guppy populations. Animal Behaviour, 36, 510-516.

Houde, A.E. 1997. Sex, colour and mate choice in guppies. Princeton: Princeton University Press.

Houde, A.E. & Endler, J.A. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata*. Science, 248, 1405-1408.

Houde, A.L.S., Fraser, D.J. & Hutchings, J.A. 2010. Reduced anti-predator responses in multi-generational hybrids of farmed and wild Atlantic salmon (*Salmo salar* L.). Conservation Genetics, 11, 785-794.

Hughes, K.A., Du, L., Rodd, F.H. & Reznick, D.N. 1999. Familiarity leads to female mate preferences for novel males in the guppy, *Poecilia reticulata*. Animal Behaviour, 58, 907-916.

Jirotkul, M. 1999a. Operational sex ratio influences female preference and male-male competition in guppies. Animal Behaviour, 58, 287-294.

Jirotkul, M. 1999b. Population density influences male-male completion in guppies. Animal Behaviour, 58, 1169-1175.

Johnson, L.E. & Carlton, J.T. 1996. Post-establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel, *Dreissena polymorpha*. Ecology, 77, 1686-1690.

Kelley, J.L. & Magurran, A.E. 2003a. Learned predator recognition and antipredator responses in fishes. Fish and Fisheries, 4, 216-226.

Kelley, J.L. & Magurran, A.E. 2003b. Effects of relaxed predation pressure on visual predator recognition in the guppy. Behavioral Ecology and Sociobiology, 54, 225-232.

Kelley, J.L., Graves, J.A. & Magurran, A.E. 1999. Familiarity breeds contempt in guppies. Nature, 401, 661-662.

Kelly, C.D. & Godin, J-G.J. 2001. Predation risk reduces male-male sexual competition in the Trinidadian guppy (*Poecilia reticulata*). Behavioral Ecology and Sociobiology, 51, 95-100.

Kodric-Brown, A. 1992. Male dominance can enhance mating success in guppies. Animal Behaviour, 44, 165-167.

Krause, J., Butlin, R., Peuhkuri, N. & Pritchard, V.L. 2000. The social organisation of fish schools: a test of the predictive power of laboratory experiments for the field. Biological Reviews, 75, 477-501.

Kydd, E. & Brown, C. 2009. Loss of shoaling preference for familiar individuals in captive-reared crimson spotted rainbowfish *Melanotaenia duboulayi.* Journal of Fish Biology, 74, 2187-2195.

Laurie, C.C. 1997. The weaker sex is heterogametic: 75 years of Haldane's rule. Genetics, 147, 937-951.

Lewis, M.A. & Kareiva, P. 1993. Allee dynamics and the spread of invading organisms. Theoretical Population Biology, 43, 141-158.

Liley, N.R. 1966. Ethological isolating mechanisms in four sympatric species of Poeciliid fishes. Behaviour (Suppl.), 13, 1-197.

Liley, N.R. & Seghers, B.H. 1975. Factors affecting the morphology and behaviour of guppies in Trinidad. In: Function and Evolution in Behaviour. (Ed. by G.P. Baerends, C. Beer & A. Manning), pp. 92-118. Oxford: Oxford University Press.

Lockwood, J.L., Cassey, P. & Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. TRENDS in Ecology and Evolution, 20, 223-228.

Lockwood, J.L., Hoopes, M.F. & Marchetti, M.P. 2007. Invasion Ecology. Malden, MA: Blackwell Publishing.

Ludlow, A.M. & Magurran, A.E. 2006. Gametic isolation in guppies (*Poecilia reticulata*). Proceedings of the Royal Society B, 273, 2477-2482.

Luyten, P.H. & Liley, N.R. 1985. Geographic variation in the sexual behaviour of the guppy, *Poecilia reticulata* (Peters). Behaviour, 95,164-179.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications, 10, 689-710.

Magellan, K. & Magurran, A.E. 2007. Mate choice, sexual coercion and gene flow in guppy populations. Journal of Fish Biology, 71, 1864-1872.

Magurran, A.E. 1996. Battle of the sexes. Nature, 383, 307.

Magurran, A.E. 1998. Population differentiation without speciation. Philosophical transactions of the Royal Society of London. B, 353, 275-286.

Magurran, A.E. 2005. Evolutionary Ecology: The Trinidadian Guppy. New York: Oxford University Press.

Magurran, A.E. & Phillip, D.A.T. 2001. Evolutionary implications of large-scale patterns in the ecology of Trinidadian guppies, *Poecilia reticulata*. Biological Journal of the Linnean Society, 73, 1-9.

Magurran, A.E. & Ramnarine, I. 2004. Learned mate recognition and reproductive isolation in guppies. Animal Behaviour, 67, 1077-1082.

Magurran, A.E. & Seghers, B.H. 1990. Population differences in the schooling behaviour of newborn guppies, *Poecilia reticulata*. Ethology, 84, 334-342.

Magurran, A.E. & Seghers, B.H. 1991. Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. Behaviour, 118, 214-234.

Magurran, A.E. & Seghers, B.H. 1994. Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. Proceedings of the Royal Society London B, 255, 31-36.

Magurran, A.E., Seghers, B.H., Carvalho, G.R. & Shaw, P.W. 1992. Behavioural consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad: evidence for the evolution of anti-predator behaviour in the wild. Proceedings of the Royal Society B, 248, 117-122.

Magurran, A.E., Seghers, B.H., Shaw, P.W. & Carvalho, G.R. 1994. Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. Journal of Fish Biology, 45, 401-406.

Magurran, A.E., Paxton, C.G.M., Seghers, B.H., Shaw, P.W. & Carvalho, G.R. 1996. Genetic divergence, female choice and male mating success in Trinidadian guppies. Behaviour, 133, 503-517.

Mallet, J. 1995. A species definition for the modern synthesis. TRENDS in Ecology and Evolution, 14, 328-330.

Mayr, E. 1940. Speciation phenomena in birds. The American Naturalist, 74, 249-278.

Mienis, H.K., Galili, E. & Rapoport, J. 1993. The Spiny Oyster, *Spondylus spinosus*, a well-established Indo-Pacific bivalve in the Eastern Mediterranean off Israel (Mollusca, Bivalvia, Spondylidae). Zoology in the Middle East, 9, 83-91.

Mooney, H.A. & Cleland, E.E. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences of the United States of America, 98, 5446-5451.

Neff, B.D., Pitcher, T.E. & Ramnarine, I.W. 2008. Inter-population variation in multiple paternity and reproductive skew in the guppy. Molecular Ecology, 17, 2975-2984.

Nei, M., Maruyama, T. & Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. Evolution, 29, 1-10.

Ojanguren, A.F. & Magurran, A.E. 2004. Uncoupling the links between male mating tactics and female attractiveness. Proceedings of the Royal Society London B, 271, S427-S429.

Olden, J.D. & LeRoy Poff, N. 2003. Towards a mechanistic understanding and prediction of biotic homogenisation. American Naturalist, 162, 442-460.

Olden, J.D., LeRoy Poff, N., Douglas, M.R., Douglas, M.E. & Fausch, K.D. 2004. Ecological and evolutionary consequences of biotic homogenisation. TRENDS in Ecology and Evolution, 19, 18-24.

Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., Williamson, M.H., Von Holle, B., Moyle, P.B., Byers, J.E. & Goldwasser, L. 1999. Impact: toward a framework for understanding the ecological effects of invaders. Biological Invasions, 1, 3-19.

Paterson, H.E.H. 1985. The recognition concept of species. In: Species and Speciation. Transvaal Museum Monograh No. 4 (Ed. by E.S. Verba), pp. 21-29. Pretoria.

Pilastro, A. & Bisazza, A. 1999. Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). Proceedings of the Royal Society London B, 266, 1887-1891.

Pilastro, A., Evans, J.P., Sartorelli, S. & Bisazza, A. 2002. Male phenotype predicts insemination success in guppies. Proceedings of the Royal Society London B, 269, 1325-1330.

Pilastro, A., Mandelli, M., Gasparini, C., Dadda, M. & Bisazza, A. 2007. Copulation duration, insemination efficiency and male attractiveness in guppies. Animal Behaviour, 74, 321-328.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience, 50, 53-65.

Pitcher, T.J. & Parrish, J.K. 1993. Functions of shoaling behaviour in teleosts. In: Behaviour of Teleost Fishes. (Ed. by. : Pitcher, T.J.), pp. 363-439. London: Chapman & Hall.

Pitcher, T.J., Magurran, A.E. & Winfield, I.J. 1982. Fish in larger shoals find food faster. Behavioural Ecology Sociobiology, 10, 149-151.

Pitcher, T.E., Neff, B.D., Rodd, F.H. & Rowe, L. 2003. Multiple mating and sequential mate choice in guppies: females trade up. Proceedings of the Royal Society Series B, 270, 1623-1629.

Price, A.C. & Rodd, F.H. 2006. The Effect of social environment on male-male competition in guppies (*Poecilia reticulata*). Ethology, 112, 22-32.

Reynolds, J.D. & Gross, M.R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. Proceedings of the Royal Society London B, 250, 57-62.

Reznick, D.N. & Bryga, H. 1987. Life-history evolution in guppies (*Poecilia reticulata*): 1. Phenotypic and genetic changes in an introduction Experiment. Evolution, 41, 1370-1385.

Reznick, D.N. & Endler, J.A. 1982. The impact of predation on life-history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution, 36, 160-177.

Reznick, D.N. & Endler, J.A. 1990. Experimentally induced life-history evolution in a natural population. Nature, 346, 357-359.

Reznick, D.N. & Miles, D.B. 1989. Review of life-history patterns in poeciliid fishes. In: Ecology and evolution of livebearing fishes (Poeciliidae) (Ed. by G.K. Meffe & F.F. Snelson), pp. 125-148. Englewood Cliffs, New York: Prentice Hall.

Reznick, D.N., Bryga, H. & Endler, J.A. 1990. Experimentally induced life-history evolution in a natural population. Nature, 346, 357-359.

Reznick, D.N., Shaw, F.H., Rodd, F.H. & Shaw, R.G. 1997. Evaluation of the rates of evolution in natural populations of guppies (*Poecilia reticulata*). Science, 275, 1934-1937.

Rhymer, J.M. & Simberloff, D. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics, 27, 83-109.

Rhymer, J., Williams, M.J. & Braun, M.J. 1994. Mitochondrial analysis of gene flow between New Zealand mallards (*Anas platyrhynchos*) and grey ducks (*Anas superciliosa*). The Auk, 111, 970-978.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, 43, 223-225.

Rodd, F.H. & Reznick, D.N. 1997. Variation in the demography of guppy populations: the importance of predation and life histories. Ecology, 78, 405-418.

Roman, J. & Darling, J.A. 2007. Paradox lost: genetic diversity and the success of aquatic invasions. TRENDS in Ecology and Evolution 22, 454-464.

Russell, S.T. 2004. Evolution of reproductive isolation in the Trinidadian guppy, *Poecilia reticulata*. Ph.D. thesis. University of St Andrews.

Russell, S.T. & Magurran, A.E. 2006. Intrinsic reproductive isolation between Trinidadian populations of the guppy, *Poecilia reticulata*. Journal of Evolutionary Biology, 19, 1294-1303.

Russell, S.T., Ramnarine, I.W., Mahabir, R. & Magurran, A.E. 2006. Genetic detection of sperm from forced copulations between sympatric populations of *Poecilia reticulata* and *Poecilia picta*. Biological Journal of the Linnean Society, 88, 397-402.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. 2001. The population biology of invasive species. Annual review of ecology and systematics, 32, 305-332.

Schories, S., Meyer, M.K. & Schartl, M. 2009. Description of *Poecilia* (*Acanthophacelus*) *obscura* n. sp., (Teleostei: Poeciliidae), a new guppy species from western Trinidad, with remarks on *P. wingei* and the status of the "Endler's guppy". Zootaxa, 2266, 35-50.

Seghers, B.H. 1974. Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary response to predation. Evolution, 28, 486-489.

Serbesoff-King, K. 2003. Melaleuca in Florida: A literature review on the taxonomy, distribution, biology, ecology, economic importance and control measures. Journal of aquatic plant management, 41, 98-112.

Seghers, B.H. 1974. Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary response to predation. Evolution, 28, 486-489.

Shaw, P.W., Carvalho, G.R., Magurran, A.E. & Seghers, B.H. 1991. Population differentiation in Trinidadian guppies (*Poecilia reticulata*): patterns and problems. Journal of Fish Biology, 39, 203-209.

Shaw, P.W., Carvalho, G.R., Seghers, B.H. & Magurran, A.E. 1992. Genetic consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad. Proceedings of the Royal Society B, 248, 111-116.

Simberloff, D. 1997. Eradication. In: Strangers in paradise. (Ed. by D. Simberloff, D.C. Schmitz & T.C. Brown), pp. 221-228. Washington, D.C.: Island Press.

Simpson, G.G. 1961. Principles of Animal Taxonomy. New York: Columbia University Press.

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Conservation Biology, 87, 173-180.

Suarez, A.V., Tsutsui, N.D., Holway, D.A. & Case, T.J. 1999. Behavioural and genetic differentiation between native and introduced populations of the Argentine ant. Biological Invasions, 1, 43-53.

Suk, H.Y. & Neff, B.D. 2009. Microsatellite genetic differentiation among populations of the Trinidadian guppy. Heredity, 102, 425-434.

Templeton, A.R. 1989. The meaning of species and speciation: A genetic perspective. In: Speciation and its consequences (Ed. by D. Otte & J.A. Endler), pp. 3-27. Sunderland, MA: Sinauer Associates.

Theodorakis, C.W. 1989. Size segregation and the effects of oddity on predation risk in minnow schools. Animal Behaviour, 38, 496-502.

Tripathi, N., Hoffmann, M., Willing, E.M., Lanz, C., Weigel, D. & Dreyer, C. 2009. Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation. Proceedings of the Royal Society B, 276, 2195-2208.

Vamosi, S.M. & Schluter, D. 1999. Sexual selection against hybrids between sympatric stickleback species: Evidence from a field experiment. Evolution, 53, 874-879.

van Valen, L. 1976. Ecological species, multispecies and oaks. Taxon, 25, 233-239.

van Wilgen, B.W., Cowling, R.M. & Burgers, C.J. 1996. Valuation of ecosystem services. Bioscience, 46, 184-189.

Verlaque, M., Afonso-Carrillo, J., Candelaria Gil-Rodríguez, M., Durand, C., Boudouresque, C.F. & Le Parco, Y. 2004. Blitzkrieg in a marine invasion: *Caulerpa racemosa* var *cylindracea* (Bryopsidales, Chlorophyta) reaches the Canary Islands. Biological Invasions, 6, 269-281.

Vermeij, G.J. 1991. When biotas meet: Understanding biotic interchange. Science, 253, 1099-1104.

Ward, A.J.W. & Hart, P.J.B. 2003. The effects of kin and familiarity on interactions between fish. Fish and Fisheries, 4, 348-358.

Ward, A.J.W. & Hart, P.J.B. 2005. Foraging benefits of shoaling with familiars may be exploited by outsiders. Animal Behaviour, 69, 329-335.

Ward, A.J.W., Axford, S. & Krause, J. 2003. Cross-species familiarity in shoaling fishes. Proceedings of the Royal Society Series B, 270, 1157-1161.

Ward, A.J.W., Holbrook, R.I., Krause, J. & Hart, P.J.B. 2005. Social recognition in sticklebacks: the role of direct experience and habitat cues. Behavioural Ecology Sociobiology, 57, 575-583.

Ward, A.J.W., Webster, M.M. & Hart, P.J.B. 2007. Social recognition in wild fish populations. Proceedings of the Royal Society Series B, 274, 1071-1077.

Ward, A.J.W., Webster, M.M., Magurran, A.E., Currie, S. & Krause, J. 2009. Species and population differences in social recognition between fishes: a role for ecology? Behavioural Ecology, 20, 511-516.

Way, M., Banks, C. 1967. Intra-specific mechanisms in relation to the natural regulation of numbers of *Aphis fabae*. Annals of Applied Biology, 59, 189–205.

Williamson, M. & Fitter, A. 1996. The varying success of invaders. Ecology Special Feature, 77, 1661-1665.

Willing, E.-M., Bentzen, P., van Oosterhout, C., Hoffmann, M., Cable, J., Breden, F., Weigel, D. & Dreyer, C. 2010. Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. Molecular Ecology, 19, 968-984.

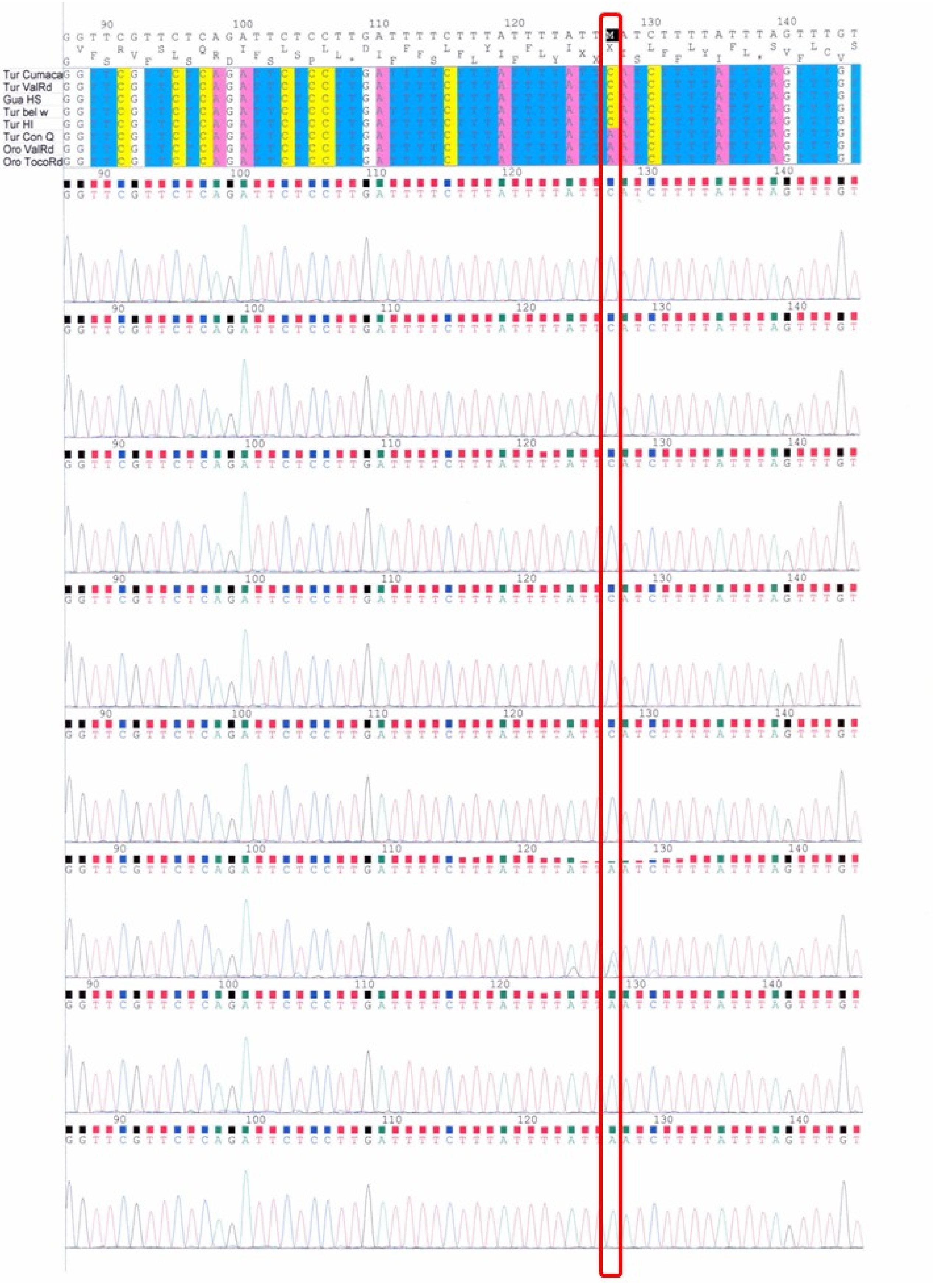
Winge, Ö. 1922. A peculiar mode of inheritance and its cytological explanation. Journal of Genetics, 12, 137-144.

Zajitschek, S.R.K., Evans, J.P. & Brooks, R. 2006. Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. Behavioral Ecology, 17, 911-916.

Appendices

Appendix I

Alignment of the consensus sequence for marker 978 (see Chapter 2).



Alignment showing the SNP at base pair 127 (152 in Table 2, Chapter 2) of the consensus sequence for marker 978. The original Oropuche nucleotide is an A which is expressed in both Oropuche samples and at the Turure's confluence with the Quare. Fish from all other sites along the Turure contain the invasive Guanapo C.