CAUSES AND CONSEQUENCES OF MOVEMENT IN SPACE AND TIME: A MULTI-LEVEL APPROACH IN TRINIDADIAN GUPPIES

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ABSTRACT

Movement through dispersal and gene flow drives the distribution and diversity of organisms. Movement in freshwater ecosystems is shaped by the characteristics of the river network. In rivers, water flow and barrier waterfalls dictate the direction of fish dispersal, which has consequences for population persistence over waterfalls, gene flow in the downstream direction, meta-population genetic structure, and local adaptation. In my thesis, I used the Trinidadian guppy *Poecilia reticulata* as a model species to investigate these topics. Although well studied independently, an integrative view of the causes and consequences of guppy movement at different levels is lacking. In the first half of my thesis, I investigated the population genetic structure of guppy populations located in two watersheds using microsatellite markers. I started by exploring locations where gene flow happened across watersheds and the relationship between patterns of genetic and phenotypic diversity. I found that despite gene flow, local adaptation is effective at creating variation in the adaptive trait studied in this chapter. Secondly, in the same two watersheds, I asked how stable population genetic structure and phenotypic diversity are over time after being hit by two rare, but massive, flood events. I showed that resistance of genetic structure is high in most of the sites, but that phenotypic diversity shows resilience after being impacted more strongly. In the second half of my thesis, I investigated at a smaller spatial scale a behavior by which guppies can avoid being displaced downstream. To maintain their position in the stream, fish possess a response to align against the flow, which is called positive rheotaxis. I started by characterizing the level of positive rheotaxis of two populations located in the upper reaches of their stream. I found that indeed populations located above waterfalls show strong positive rheotaxis, but also that the two populations evolved different behaviors to achieve the same performance. I finally explored how disturbance by ectoparasites *Gyrodactylus turbulli*

influence guppy rheotaxis by looking at the impact of presence and parasite load at the individual level. I demonstrated that with increasing parasite load, guppies avoid high flows and cover less distance in the upstream direction. Together, this research highlights the importance of integrating the mechanisms, causes and consequences of movement in riverine ecosystems to increase our understanding of the distribution and diversity in fish and the impact of disturbances on their movement.

RESUME

Le mouvement des organismes, que ce soit leur dispersion ou leur flux de gènes, permet d'expliquer la distribution et la diversité des espèces. Le mouvement dans les écosystèmes d'eau douce est façonné par les caractéristiques du réseau riverain. Dans les rivières, le débit d'eau et les chutes d'eau qui crée une barrière dictent la direction de la dispersion des poissons, ce qui a des conséquences sur la persistance de la population au-dessus des chutes, le flux génétique vers l'aval, la structure génétique de la métapopulation et l'adaptation locale. Dans ma thèse, j'utilise les guppies de Trinidad *Poecilia reticulata* comme espèce modèle pour étudier ces sujets. Quoique bien étudiés indépendamment, une approche intégrative des causes et des conséquences du mouvement des guppies à différentes échelles fait défaut. Dans la première moitié de ma thèse, j'étudie la structure génétique des populations de guppy localisées dans deux bassins versants à l'aide de marqueurs microsatellites. Je commence par explorer les endroits où le flux génétique a eu lieu d'un bassin versant à l'autre, puis la relation entre la distribution des diversités génétique et phénotypique. J'ai trouvé que malgré les flux génétiques, l'adaptation locale prédomine dans la variation d'un trait adaptatif. Deuxièmement, dans les deux mêmes bassins versants, je demande dans quelle mesure la structure génétique de la population et la diversité phénotypique sont stables au fil du temps, après avoir été frappées par deux inondations rares mais massives. Je montre que la résistance de la structure génétique est élevée dans la plupart des sites, mais que la diversité phénotypique montre de la résilience après avoir été plus fortement impactée. Dans la seconde moitié de ma thèse, j'étudie à une échelle spatiale plus fine un comportement par lequel les guppies peuvent éviter d'être déplacés vers aval. Pour maintenir leur position dans la rivière, les poissons possèdent une réponse pour s'aligner contre le courant, qui est appelée la rhéotaxie positive. Je commence par caractériser le niveau de rhéotaxie positive de deux

populations situées dans le cours supérieur de leur cours d'eau. J'ai constaté qu'en effet les populations situées au-dessus des chutes d'eau présentent un fort niveau de rhéotaxie positive, mais aussi que les deux populations ont évolué des comportements différents pour atteindre les mêmes performances. J'explore enfin comment la perturbation par les ectoparasites *Gyrodactylus turbulli* influence la rhéotaxie du guppy en examinant l'impact de la présence et de la charge parasitaire au niveau individuel. Je démontre qu'avec l'augmentation de la charge parasitaire, les guppies évitent les courants forts et parcourent moins de distance dans la direction de l'amont. Ensemble, ces recherches soulignent l'importance d'intégrer les mécanismes, les causes et les conséquences des mouvements dans les écosystèmes fluviaux pour mieux comprendre la distribution de la diversité des poissons et l'impact des perturbations sur leur mouvement.

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These past years did I not only learn that it takes a village to raise a child, but that it also takes one to complete a thesis. I want to say thank you to all the people who helped me directly or indirectly and that made me who I am now. I'll try to keep the list shorter than the actual thesis.

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Contribution to Original Knowledge

All chapters of my thesis constitute original scholarship performed in partial fulfillment of the requirements for the degree of Doctor of Philosophy at McGill University.

In my first chapter, I analyzed two microsatellite datasets to infer population genetic structure of guppy populations located in two watersheds. The first dataset is a re-analysis of a master thesis work, but that was never published in a peer-review journal. The second dataset was analyzed for the first time in this study. Together, they provide a comprehensive description of the population genetic structure of the two Trinidadian watersheds studied there. In this chapter we document previously unknown instances of recent and historical gene flow at different places where the two watersheds come into close proximity.

In my second chapter, I assess the resistance and resilience of guppy populations in the same two watersheds after rare but intense disturbances. Evidence of disturbance is generally difficult to obtain as pre-disturbance data is often missing. This study is the first to quantify population genetic structure and phenotypic variation of guppy populations before and immediately after two massive flood events (four time points in total).

In my third chapter, I explore the rheotactic behavior (response to water flow) of introduced and native guppy populations above and below waterfalls. This chapter is the first study that shows how individuals from the same species but not from the same population achieve similar levels of positive rheotaxis using different behavioral strategies.

In my fourth and last chapter, I evaluate the impact of ectoparasite presence and parasite load on guppy rheotaxis. This chapter is the first study to quantify rheotaxis of descendants of upstream guppies that have been infected with *Gyrodactylus turnbulli*. It is also the first multiple test of individual rheotaxis in guppies.

Contribution of Authors

All chapters of my thesis are either published, submitted, or in the preparation for submission. I am the first author for all the chapters. For chapters 1 and 2, all co-authors and publishers have granted permission to use the manuscripts in this thesis. I am the only author on all non-manuscript parts of this thesis (introduction, linking statements and general conclusion).

Chapter 1 has been published in *Ecology and Evolution* in February 2019. As stated in the manuscript, I designed the study with help from Andrew P. Hendry and Paul Bentzen; Paul Bentzen, Ian Paterson and Andrew P. Hendry contributed to sample collection; Lyndsey Baillie, Jessica Quinton and Ian Paterson genotyped the fish; Jahson B. Alemu made the GIS map; I analyzed the data and wrote the manuscript with inputs from all co-authors and three anonymous reviewers.

Chapter 2 was submitted to Molecular Ecology in August 2020. As stated in the manuscript, I designed the study with help from Andrew P. Hendry and Paul Bentzen. Andrew P. Hendry collected the samples. I genotyped the fish with the help of Ian Paterson. I performed data curation and analyses. I wrote the manuscript with inputs from all co-authors.

Chapter 3 has been published in *Genes* in January 2020. As stated in the manuscript, I designed the study with help from Andrew P. Hendry and Marilyn E. Scott. I collected the samples. I conducted the experiments with Sandra Klemet-N'Guessan. I performed data curation and analyses. I wrote the manuscript with inputs from all co-authors and three anonymous reviewers.

Chapter 4 is in preparation for submission. This chapter was originally an independent research project conducted by Sandra Klemet-N'Guessan under the supervision of Andrew P. Hendry and me. I designed the study with help from Andrew P. Hendry and Marilyn E. Scott. Sandra Klemet-N'Guessan and I performed the infections and the experiments. Sandra

Klemet-N'Guessan extracted the data from the video recordings. I performed the analyses and wrote the manuscript with the inputs of all co-authors.

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INTRODUCTION

"You cannot step twice into the same river."
– Heraclitus

What is movement?

Movement plays a critical role in shaping patterns of distribution and diversity in living organisms (Dingle, 1996). Movement takes different forms and can be studied through dispersal or gene flow. Dispersal is the movement of gametes or organisms, from a birth area to a breeding area, or between two breeding areas (Clobert *et al.*, 2001). Gene flow is a consequence of dispersal, and is the change in allele frequency due to the movement of gametes (Slatkin, 1987). Together, the study of dispersal and gene flow inform us on processes that are happening at the individual level, such as the physiological mechanisms that are enabling individual dispersal (Dufty Jr & Belthoff, 2001; Ronce & Clobert, 2012). Further, dispersal and gene flow provide insights into entire populations, including their genetic diversity and capacity for adaptation (Lenormand, 2002; Garant *et al.*, 2007). Given that species around the world face increasing risks of habitat loss, fragmentation, and extreme weather (IPBES, 2019), it is thus essential to understand the interplay between multiple components of animal movement, as well as their causes and consequences.

What are the causes and consequences of movement?

The likelihood that an organism will disperse is influenced by both individual traits and the environment. Organisms might show a suite of correlated traits favoring their dispersal (Ronce & Clobert, 2012). For example, dispersers of the large white butterfly exhibit longer wings and are better flight performers than residents (Legrand *et al.*, 2015). Dispersal can also be triggered

to avoid unfavorable conditions in the environment, either from abiotic (e.g. shortage of resources) or biotic (e.g. inbreeding) factors. For instance, male brown bears disperse further than females, leading to inbreeding avoidance (Shirane *et al.*, 2019). External forces like wind and water flow can also lead to passive dispersal and displace organisms to a different environment, such as the high rate of black flies neonates dispersal that can be explained by water currents (Fonseca & Hart, 1996).

Individuals can disperse in new patches, resulting in the colonization of new areas, or disperse into already occupied environments, potentially leading to gene flow among populations. This influx of genes can either dampen or have little effect on local adaptation (Garant *et al.*, 2007): for example, gene flow between populations of lake threespine sticklebacks constrained adaptation of the outlet population (Moore *et al.*, 2007). On the another hand, gene flow between high and low predation guppies did not influence the maintenance of adaptations to local conditions (Fitzpatrick *et al.*, 2015). These two examples also emphasize the particularity of streams as metapopulation networks, where gene flow is often asymmetrical. As my thesis will show, this characteristic strongly influences how and why freshwater organisms move, and shapes gene flow and adaptation across populations.

What are the characteristics of movement in rivers?

Riverine environments are dendritic networks (Fagan, 2002), characterized by tributaries connected to a main stem and unidirectional water flow. This unidirectional flow creates a gradient in abiotic conditions from upstream to downstream, which imposes selective pressures on riverine organisms (Vannote *et al.*, 1980). Similarly, along this river gradient, gene flow is going mostly in the downstream direction due to physical constraints. These constraints have several implications: strong currents, or any barrier along the river, such as waterfalls or dams,

can isolate the most upstream populations, and mechanisms have to exist to ensure population persistence above barriers. In stream invertebrates, the 'drift paradox' (Müller, 1954) asks why aquatic invertebrates that are drifting downstream with the current are able to persist upstream. This is resolved by recolonization of upstream habitats by flying adults, small scale movements along the riverbed, coupled with density-dependence (Anholt, 1995; Humphries & Ruxton, 2002). In fishes, one of the main mechanisms that allows population persistence in isolated upper reaches of streams is the ability to swim against the current and maintain their position, also known as positive rheotaxis (Lyon, 1904; Arnold, 1974).

Rheotaxis has many fitness advantages. When facing upstream, fish maximize the detection of cues in the water and the interception of prey (Kanter & Coombs, 2003), and avoid costs associated with downstream emigration (Montgomery *et al.*, 1995). Rheotaxis is a multi-trait behavior. The importance of visual and tactile cues was first investigated by Lyon (1904) in a series of experiments where he used different set-ups to test for fish orientation with moving backgrounds or darkened labyrinths. Later, the key role of lateral line in the mediation of rheotaxis was highlighted by the study of superficial neuromasts cells (Montgomery *et al.*, 1997). In salmonids, fish located above a waterfall or a dam have been shown to express higher positive rheotaxis behavior compared to fish located below (Jonsson, 1982a; Morita & Yamamoto, 2001; Northcote, 2010). However, the link between variation in rheotaxis and its consequences for gene flow and meta-population structure is still unclear.

The study of animal movement in rivers benefits from a multi-level approach because numerous questions that involve multiple components still remain. From a spatial perspective, what factors influence dispersal, which mechanisms and behaviors are underlying movement and in which direction? From a temporal perspective, is gene flow constant through time and what are the long-term consequences of movement on population genetic structure? The

overarching goal of my thesis is to understand the factors that drive dispersal and gene flow in a riverine fish: the Trinidadian guppy, *Poecilia reticulata*.

How do guppies move?

Since the species was described in 1859 by Wilhelm Peters, guppies have been studied by numerous scientists to advance scientific knowledge on ecology, evolution, behavior, and physiology (Magurran, 2005). Of particular interest within the framework of this thesis, movement of guppies has been studied both in nature (Croft *et al.*, 2003a; van Oosterhout *et al.*, 2007) and in the lab (Ghalambor *et al.*, 2004; Mohammed *et al.*, 2012). For example, the population genetics of guppies located in the Northern Mountain range has received extensive attention (Shaw *et al.*, 1991, 1994; Alexander *et al.*, 2006; Crispo *et al.*, 2006; Barson *et al.*, 2009; Suk & Neff, 2009). These studies revealed that genetic diversity is usually lower in the upper reaches of rivers, and that waterfalls are creating barriers to upstream gene flow. In my thesis, I leverage the guppy system to explore some of the causes and consequences of movement.

Gene flow is an evolutionary force that is either promoting or reducing local adaptation (Lenormand, 2002; Garant *et al.*, 2007). In rivers, gene flow is expected to occur mainly within watersheds from upstream to downstream. In my first chapter, I described the genetic structure of populations located in two neighboring watersheds, quantifying gene flow both within and between watersheds. I then compared genetic and phenotypic diversity to determine if movement between watersheds was influencing local adaptation.

Disturbance from natural disasters such as seasonal flooding are predicted to increase due to climate change (Milly *et al.*, 2002; van Aalst, 2006). In the second chapter of my thesis, I investigate the impact of rare but massive floods on the population genetic structure and the

phenotypic diversity of guppy populations located in two watersheds. I quantified the impact of two major floods that happened in 2005 and 2016 on fish movement and tested whether populations were resistant (no change after disturbance) or resilient (impacted but go back to pre-flood level of genetic and phenotypic variation).

Guppies have been shown to respond differently to water flow depending on whether they originate from lake, or stream environments, with fish from upstream riverine habitats showing more pronounced positive rheotactic behavior (Mohammed *et al.*, 2012). Therefore, the presence of barrier waterfalls that isolate upstream population from downstream migrants represent an excellent opportunity to study the evolution of rheotaxis as a function of dispersal. In the third chapter of my thesis, I quantified the rheotactic behavior of upstream populations of guppies from two different rivers to infer their level of positive rheotaxis and explore the evolution of this dispersal trait.

Locally adapted individuals have a higher fitness than individuals that are displaced to a different environment (Kawecki & Ebert, 2004). There are several factors that can influence the passive dispersal of an organism, and parasites that impact their host physiology, morphology, and behavior can also influence their movement. Ectoparasites *Gyrodactylus sp*. have been found to negatively correlate with recapture rate of guppies in nature (van Oosterhout *et al.*, 2007). In my last chapter, I tested the effect of the presence and abundance of *Gyrodactylus* on guppy rheotaxis.

Trinidadian streams are often described as a "natural laboratory" to test theories in ecology and evolution (Magurran, 2005). With the guppy as a model system, the multiple streams and their series of waterfalls make it a perfect opportunity to study movement in dendritic networks. My thesis seeks to deepen our understanding in this topic and focuses on the mechanisms that enable population persistence over waterfalls, and the consequences of dispersal and gene flow

in entire watersheds. Understanding how fish move in rivers and what constraint or promote movement is necessary to apprehend how increasing disturbances will affect population diversity and distribution.

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CHAPTER 1: Evidence for contemporary and historical gene flow between guppy populations in different watersheds, with a test for associations with adaptive traits[1]

Abstract

In dendritic river systems, gene flow is expected to occur primarily within watersheds. Yet, rare cross-watershed transfers can also occur, whether mediated by (often historical) geological events or (often contemporary) human activities. We explored these events and their potential evolutionary consequences by analyzing patterns of neutral genetic variation (microsatellites) and adaptive phenotypic variation (male color) in wild guppies (*Poecilia reticulata*) distributed across two watersheds in northern Trinidad. We found the expected signatures of within-watershed gene flow; yet we also inferred at least two instances of cross-watershed gene flow – one in the upstream reaches and one further downstream. The upstream cross-watershed event appears to be very recent (41 ± 13 years), suggesting dispersal via recent flooding or undocumented human-mediated transport. The downstream cross-watershed event appears to be considerably older (577 ± 265 years), suggesting a role for rare geological or climatological events. Alongside these strong signatures of both contemporary and historical gene flow, we found little evidence of impacts on presumably adaptive phenotypic differentiation, except perhaps in the one instance of very recent cross-watershed gene flow. Selection in this system seems to overpower gene flow – at least on the spatiotemporal scales investigated here.

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Introduction

Historical and contemporary patterns of dispersal and gene flow are key components shaping population genetic structure (Slatkin, 1987; Bohonak, 1999). From an historical perspective, different colonization routes and times, and different patterns of starting genetic variation can leave signatures that persist for millennia (Avise, 2000). From a contemporary perspective, recent fluctuation in population sizes and patterns of dispersal can strongly shape genetic similarity among populations (Endler, 1977; Slatkin, 1987; Bohonak, 1999). These historical and contemporary effects can interact to shape the population structure over small and large spatiotemporal scales. For example, studies of isolation-by-distance often find that genetic differences between populations are correlated with geographic distances, because gene flow is reduced over longer distances (e.g. Castric *et al.*, 2001; Pogson *et al.*, 2001; Crookes & Shaw, 2016). Yet, such studies also often detect discontinuities between geographically-proximate populations that have different historical origins, such as different colonization events (e.g. Cuenca *et al.*, 2003). Our goal will be to disentangle the roles of ongoing contemporary and historical gene flow in a classic evolutionary model system – Trinidadian guppies *Poecilia reticulata* (Peters, 1859).

Gene flow, whether contemporary or historical, can play an important role in the ability of populations in different places to adapt to their local environments (Slatkin, 1987; Lenormand, 2002; Garant *et al.*, 2007). In particular, studies focusing on *contemporary* gene flow have shown that populations exchanging more genes are often less able to diverge in adaptive traits (reviews: Räsänen & Hendry 2008; Hendry 2017), although other studies have found limited – or even positive – effects of gene flow on adaptive divergence (e.g., Hemmer-Hansen *et al.*, 2007; Fitzpatrick *et al.*, 2015). The importance of *historical* gene flow for ongoing adaptation is less well understood. On the one hand, we might expect the power of

selection to quickly overcome any historical legacy, such that adaptive trait divergence bears little relationship to neutral genetic marker divergence (Merilä & Crnokrak, 2001). On the other hand, some studies have suggested that populations coming from different colonization events can maintain important differences in adaptive traits despite long-term occupancy of similar environments, an effect referred to as historical contingency (Travisano *et al.* 1995; Losos *et al.* 1998; Taylor & McPhail 2000). Although numerous studies have investigated whether phenotypic variation correlates with contemporary selection or with historical contingency (Thorpe *et al.*, 1995; Hoekstra *et al.*, 2005; Alexander *et al.*, 2006), the focus has not been on effects of contemporary versus historical gene flow on adaptive traits. To gain insight into such effects, we combine genetic inferences about historical and contemporary gene flow in guppies from two adjacent watersheds in Trinidad, with information on an important class of adaptive traits – male color.

Several previous studies have examined population structure in Trinidadian guppies (Carvalho *et al.*, 1991; Fajen & Breden, 1992; Alexander *et al.*, 2006; Crispo *et al.*, 2006; Barson *et al.*, 2009; Suk & Neff, 2009; Willing *et al.*, 2010), typically revealing that patterns of neutral genetic variation within watersheds are strongly influenced by the distance between sites and by physical barriers to movement, such as waterfalls (Crispo *et al.*, 2006; Primmer *et al.*, 2006; Gomez-Uchida *et al.*, 2009). In keeping with the expectation that the greatest barrier to dispersal in such systems is dry land, greater genetic differences are usually found among rather than within watersheds (Carvalho *et al.*, 1991; Barson *et al.*, 2009; Suk & Neff, 2009; Willing *et al.*, 2010). However, exceptions are known wherein guppies occupying some tributaries in one watershed can show surprising genetic similarity to particular populations in other watersheds (Willing *et al.*, 2010). These cross-watershed affinities could reflect historical or contemporary gene flow owing natural events, such as earthquakes or severe

flooding, or human-mediated transport. The best predictors of such cross-watershed gene flow events are expected to be similar elevations and geographic proximity, except in the case of some longer-distance human-mediated transfers.

Our focal study system

Our work focused on guppies located on the north slope of the Northern Mountain Range in Trinidad, where multiple streams run roughly parallel to each other from the mountains over a series of waterfalls to the ocean. We studied two neighboring watersheds, the Marianne and the Paria (Figure 1.1). Gene flow within these watersheds is expected to be relatively high, at least in the downstream direction, as their total lengths are only 10.69 km for the Marianne and 9.22 km for the Paria. However, gene flow can be reduced in the upstream direction owing to the direction of water flow and to physical features such as waterfalls (Crispo *et al.* 2006). Waterfalls are present throughout the entire course of the Marianne, but there is no major waterfall along the Paria watershed that could prevent upstream migration. Gene flow would seem less likely between the two watersheds, and yet still might be possible owing to their close proximity at two elevation ranges: 50-100 m (downstream area) and 550-600 m (upstream area) (Figure 1.1). To examine patterns of gene flow with these expectations and possibilities in mind, we analyzed guppies from several sites for variation at 10 and 42 microsatellite loci.

To see how gene flow might relate to adaptation, we focused on male guppy color, which has a known genetic basis (Lindholm & Breden, 2002; Gordon *et al.*, 2017) and evolves in response to sexual selection favoring conspicuousness and natural selection favoring crypsis (Endler, 1980a; Reznick & Endler, 1982; Reznick *et al.*, 1990). Most obviously, populations in different predation environments show dramatic color differences

that reflect adaptation to the local balance between natural and sexual selection (Endler, 1980a; Millar *et al.*, 2006; Kemp *et al.*, 2008). These color patterns often (but not always) evolve quickly (Karim *et al.*, 2007; Kemp *et al.*, 2008; Gordon *et al.*, 2017) and differences among populations are stable over time (Gotanda & Hendry, 2014). Large variation also exists among populations within a given predation regime, reflecting the specific types and densities of local predators (Endler, 1978; Millar *et al.*, 2006; Kemp *et al.*, 2008; Weese *et al.*, 2010; Millar & Hendry, 2012), canopy cover (Grether *et al.*, 2001; Schwartz & Hendry, 2010), and sexual selection (Houde & Endler, 1990; Schwartz & Hendry, 2007). Divergence in male color also has been argued to be influenced – both positively (increased divergence) and negatively (decreased divergence) – by gene flow (Endler 1978; Fitzpatrick *et al.*, 2015, 2017). We therefore compare patterns of gene flow with patterns of neutral genetic differentiation to infer the potential role of historical and contemporary gene flow in shaping adaptive trait divergence.

In summary, our goals were to (i) investigate population genetic structure of guppies in the two watersheds, (ii) infer the existence and timing of gene flow events between sites within and between watersheds, and (iii) test for associations between gene flow and differences in male color. Our interpretations proceed as follows:

i. If current watershed structure is the primary determinant of gene flow, samples should cluster by watershed; and gene flow estimates should be higher within than between watersheds. Deviations from this expectation (e.g., some clustering and inferred gene flow between watersheds) would indicate cross-watershed genetic exchange.

ii. If inferred gene flow between watersheds was due to historical – and presumably rare – events, such as earthquakes or floods, estimated divergence times between sites should be older than a few centuries. Deviations from this expectation (e.g., more

recent divergence) would suggest the importance of contemporary factors, such as recent natural or human-mediated gene flow.

iii. If gene flow among sites is influencing adaptation, we expect patterns of male color divergence among sites to be associated with patterns of neutral genetic divergence. Deviations from this expectation (e.g., limited or no correspondence between neutral and adaptive divergence) would inform the extent to which local selection overcomes historical and contemporary gene flow, or would indicate genetic drift.

Methods

Fish sampling

We sampled fish along the Marianne and Paria watersheds in northern Trinidad over several years (2002-2014; average of 38 individuals per year and site, min=18, max=50; details in Table S1.1). At each site, we used butterfly nets to capture guppies that were then transported to our laboratory in Trinidad. The fish were euthanized with a solution of tricaine methanesulfonate (MS222) and preserved in 95% ethanol for genotypic analysis. A subset of the fish was also photographed following a standard method (details below).

Genotypic data

Two data sets were generated using different methods implemented at different times. One dataset has fewer loci (10) but more sites (20), whereas the other dataset has more loci (42) but fewer sites (12). The term "site" refers to a specific discrete sampling location, and site numbers correspond to those reported in previous work on these watersheds (Crispo *et al.*, 2006; Millar *et al.*, 2006; Schwartz & Hendry, 2010; Gotanda *et al.*, 2013; Gotanda & Hendry, 2014). We here analyze both data sets because they represent two independent

efforts, with different strengths and weaknesses, to quantify genetic population structure for the same watersheds. We analyze the two data sets separately because few of the loci and only some of the sites were in common.

For the first data set ("10loci-20sites"), DNA was extracted using a modified glassmilk protocol (Elphinstone *et al.*, 2003) from fins of sampled fish. DNA was amplified by PCR and then visualized by capillary gel electrophoresis. Microsatellite markers comprised four tetranucleotide loci (*Pre9, Pre13, Pre15* and *Pre26*: Paterson et al. 2005) and six dinucleotide loci (*Pret27, Pret28, Pret38, Pret46, Pret80* and *G145*: Watanabe *et al.* 2003; Shen *et al.* 2007).

For the second dataset ("42loci-12sites"), DNA was extracted using the same method for 42 di- and tri-nucleotide microsatellite loci selected from the guppy genome (NCBI BioProject PRJNA238429). The 42 loci were multiplexed in a single PCR, and indexing sequences were subsequently added to the PCR products using a second PCR. The index PCR used oligonucleotides composed of Illumina annealing adapter sequences, a 6b index (barcode), and the Illumina sequencing primers. DNA was then sequenced on an Illumina MiSeq. Individual genotypes were characterized using MEGASAT, a program that reads sequence files and automatically scores microsatellite genotypes. Full laboratory and bioinformatic methods are presented in Zhan *et al.* (2017).

MICRO-CHECKER (van Oosterhout *et al.*, 2004) was used to test for potential genotyping errors. GENEPOP (version 4.2; Raymond & Rousset 1995) was used to test for deviations from Hardy-Weinberg Equilibrium (HWE) for each locus in each "sample" (i.e., a microsatellite data set at a particular site in a particular year) and to test for linkage disequilibrium between loci within each sample. LOSITAN was used to check if loci were potentially under selection based on an F_{ST} outlier test (Antao *et al.*, 2008).

We used the R software with R studio (RStudio Team, 2016; R Core Team, 2018) to calculate basic population genetic measures. The package *pegas* (Paradis, 2010) and *hierfstat* (Goudet, 2005) were used to calculate the number of alleles per site, as well as observed heterozygosity and gene diversities (H_o and H_s respectively). The package *hierfstat* (Goudet, 2005) was also used to calculate pairwise F_{ST} between samples.

STRUCTURE (version 2.3.4; Pritchard *et al.* 2000) was used to infer genetic population structure and to find the appropriate number of clusters (*K)* that best explain the genotypic distribution. Three iterations were run for each *K*, from 1 to 28 or from 1 to 19 (total number of samples from the two data sets). Burn-in length and run length of the program were each set at 100,000 using the admixture model and the correlated alleles model. We used the Evanno et al. (2005) method implemented in STRUCTURE HARVESTER to find the best *K*. We generated STRUCTURE plots using the R package *pophelper* (Francis, 2017). These analyses included multiple years of sampling for a given site so as to help assess the temporal consistency of among-site patterns.

We estimated long term gene flow by calculating migration rates ($M = m/\mu$) between sites using MIGRATE (version 3.6; Beerli 2009). In cases where a data set included multiple years from a single site, we kept – for ease of estimation – only one year per site by choosing samples with the minimum length of time between them (i.e., temporal outliers were more likely to be excluded). We used an MCMC with Bayesian inference coalescent approach that employed a Brownian model approximating a single-step mutation model and default values from the software. A mutation rate of 5×10^{-4} was chosen because it is the mutation rate commonly used in other microsatellite fish studies (Lippé *et al.*, 2006; Barson *et al.*, 2009). For each dataset, a first run determined F_{ST} parameters that were then used as start values for three more runs. The number of runs was dictated by when the mean across runs was stable.

We explored recent (over the last few generations) migration rates using BAYESASS (version 3; Wilson & Rannala 2003). For each data set, we only kept one year per site, and we first adjusted the mixing parameters to meet acceptance rates. The burn-in period of the model was then set at $1x10^6$, while MCMC iterations were set at $1x10^7$. We ran several instances of the model with different starting seeds: results were similar among runs and so we here report only values from the first run. Model convergence was also tested using TRACER (version 1.6; Rambaut & Drummond 2013). Values calculated with this method represent the fraction of individuals in a population that are migrants derived from another population.

We used DIYABC (version 2.1.0; Cornuet *et al.* 2014) to estimate divergence time between pairs of sites across watersheds – the level at which such inferences were desired. This analysis was done using the 42loci-12sites dataset, with only one year per site. For each pair of sites, we tested a simple model of two populations having diverged *t* generations in the past from a common ancestral population (Figure S1.2), a reasonable approximation of a discrete cross-watershed gene flow event. Our models thus simplify a complex scenario of watershed colonization with multiple sites but allows the comparison of pairs of sites across watersheds. The mutation model was left as the default in the program (mean mutation rate: 5×10^{-4}). We generated $1x10^6$ simulated datasets to estimate the divergence time between each pair of sites. As guppies can have 2-3 generations per year (Magurran, 2005), we assumed a value of 2.5 generations per year.

Phenotypic data

Differences in color among guppy populations in the studied watersheds are remarkably consistent through time (Gotanda & Hendry, 2014), and so we were able to use phenotypic data (male color) from years other than the genetic data. Specifically, the data re-analyzed

here were previously published in Millar et al. (2006), wherein details are provided. In brief, we extracted color information from standardized digital pictures of male guppies. Scion Image (Scion, 2001) was used to measure body size (area, length, and depth) and each color spot (area) on the left side of the body. Each color spot was classified into a color category: orange (includes red), black, fuzzy black, yellow, blue (includes purple), green, violet-blue, bronze-green, and silver. For simplicity, these categories were then further grouped into three more inclusive categories: melanic (black and fuzzy black), carotenoid (orange and yellow), and structural (blue, green, violet-blue, bronze-green and silver). These categories and labels are only general as, for example, the "carotenoid" colors include additional compounds influencing color (Grether *et al.*, 1999). For the present analysis, we used – for each individual fish – the total number of spots and the relative total spot area (total spot area divided by the total body area of the fish) for each color category.

We used a MANOVA to detect differences in male color between predation regimes in the Marianne watershed. We calculated pairwise P_{ST} as a measure of the phenotypic (color) distance between guppies at each pair of sites. Following Phillimore *et al.* (2008), we used the formula $P_{ST} = \delta^2_{GB} / (\delta^2_{GB} + 2\delta^2_{GW}h^2)$, where δ^2_{GB} and δ^2_{GW} are the between and within group variance and h^2 is the heritability. Given the established very strong genetic basis for the sorts of traits measured here (Karino & Haijima, 2001; Lindholm & Breden, 2002; Tripathi *et al.*, 2009; Gordon *et al.*, 2017), we made the assumption that $h^2=1$, meaning that all variance is genetic. Choice of a different value for heritability would not have influenced conclusions, which are based on *relative* differences between various types of population pairs. Following Phillimore *et al.* (2008), we conducted pairwise MANOVA for all sites across watersheds using the R package *stats*. Variance-covariance matrices were then summed to estimate δ^2_{GB} and δ^2_{GW}.

Comparison of genotypic and phenotypic data

To enable direct comparisons of population structure between the genetic (from both datasets) and phenotypic (male color) data, we analyzed both types of data using discriminant analysis on principal components (DAPC; Jombart *et al.* 2010) implemented in the R package *adegenet*. This method infers individual exchangeability between sites and allows evolutionary inferences from the classification of each individual into different categories of sites (Hendry *et al.*, 2013). For each data type (genetic or phenotypic), we recorded the probability that each individual is assigned to (i) its site of origin, (ii) a site from the same watershed at the same elevation (upstream vs. downstream), (iii) a site from the same watershed but with a different elevation, (iv) a site from the other watershed with the same elevation, and (v) a site from the other watershed with a different elevation. We then recorded "classification" as the highest assignment of an individual to its own site or in any other site, and "cross-classification" as the highest assignment to any other site apart from the site of origin (Hendry *et al.*, 2013).

We calculated F_{ST} (each dataset separately) and P_{ST} means and confidence intervals to allow comparisons and used a Mantel test in the R package *vegan* (Oksanen *et al.*, 2018) to statistically compare these measures. Here we used only F_{ST} measures from the 10loci-20sites dataset, because insufficient overlap occurred between sites in the 42loci-12sites dataset and the male color dataset.

Results

We start with a brief summary of the main findings and the analyses supporting them before moving to specific presentation of the specific analyses. Overall, we found strong evidence of

gene flow not only within watersheds but also between them – as supported by five analyses. First, STRUCTURE most strongly supported four clusters for the 10loci-20sites dataset and three clusters for the 42loci-12sites dataset, with one of the clusters in each STRUCTURE analysis including sites from both watersheds. Second, DAPC for the genetic data showed that, although individuals were mostly classified to their site of origin, a reasonable number were classified into sites in the same watershed (especially at the same elevation), and some were even classified into sites in the other watershed (almost exclusively at the same elevation). Third, MIGRATE suggested considerable historical gene flow both within and between watersheds, with estimates between watersheds often higher than some of those within watersheds. Fourth, BAYESASS suggested very recent migration events among some sites within watersheds, and even from the Paria to Marianne (especially at higher elevations). Fifth, DIYABC inferred historical gene flow between the two watersheds at low elevations and recent gene flow between the watersheds at higher elevations. Finally, we found that male color showed little or no correspondence with neutral genetic variation, suggesting that selection tends to erase the effects of gene flow in these particular comparisons.

Genetic variation

In the 10loci-20sites dataset, 33 out of 128 tests showed departures from HWE equilibrium after sequential Bonferroni correction ($\alpha = 0.05$). Although these deviations were haphazardly distributed across loci and samples, we nevertheless searched for correlations between F_{IS} and F_{ST} at the level of individual loci (Waples & Allendorf 2015). We did not find any positive relationships between these two measures (10loci20sites: $r^2 = 0.08$; 42loci12sites: $r^2=0.01$), ruled out a Wahlund effect. F_{IS} values for *Pre26* were very positive compared with other loci (*Pre26*: $F_{IS} = 0.23$; median for the other loci: $F_{IS} = 0.04$), reflecting heterozygote deficiency.

For this locus, MICROCHECKER indicated potential null alleles in 11 of the 28 samples. This locus was thus excluded from further analyses. In the 42loci-12sites dataset, 55 out of 798 tests showed departures from HWE after sequential Bonferroni correction. Because these departures only constituted 6% of the tests, and were haphazardly distributed across loci and sites, we did not exclude any loci from this dataset.

In the 10loci-20sites dataset, 3 out of 1260 tests showed evidence of linkage disequilibrium after sequential Bonferroni correction. All significant tests were for site P13, which could indicate a small effective population size (N_e), or could show admixture of different lineages for guppies at that site. In the 42loci-12sites dataset, 15 out of 8436 tests showed evidence of linkage disequilibrium after sequential Bonferroni correction. However, physical linkage is unlikely given that the loci are known (i.e., specifically developed) to be widely distributed in the guppy genome.

The F_{ST} outlier method implemented in LOSITAN detected six loci potentially under selection in the 10loci-20sites dataset, and 16 loci in the 42loci-12sites dataset. For the 10loci-20sites dataset, we did not eliminate any loci, because of low information with only four remaining loci for the analysis. For the 42loci-12sites dataset, we ran STRUCTURE with and without the potentially selected loci and obtained the same results. Hence, we kept all loci for subsequent analyses.

The total number of alleles per site ranged from 34 to 141 for the 10loci-20sites dataset, and from 54 to 262 for the 42loci-12sites dataset (Table S1.1). Mean number of alleles per locus was 27.11 for the 10loci-12sites dataset and was 13.02 for the 42loci-12sites dataset. Observed heterozygosity ranged from 0.292 to 0.752 for the former and from 0.073 to 0.573 for the latter (Table S1.1). Average observed heterozygosity was higher in downstream sites ($H_o = 0.73 \pm 0.06$; 10loci-20sites dataset) than in upstream sites ($H_o = 0.54 \pm 0.14$;

10loci-20sites dataset). F_{ST} values were higher between sites that were geographically more distant (Table S1.4; Table S1.5).

Population structure and gene flow

STRUCTURE: The most likely number of clusters was four for the 10loci-20sites dataset and three for the 42loci-12sites dataset (Figure 1.2; Figure S1.1). Of particular note, both datasets revealed a cluster composed of eastern upstream Marianne sites (M3 and M4) and western upstream Paria sites (P8, P7, P15, and P17 in both datasets; P16 in the 42loci-12sites dataset). Both datasets also revealed a cluster composed of several western Marianne (M16, M1, and M15) sites. The remaining cluster in the 42loci-12sites dataset was composed of eastern downstream Marianne sites (M7, M8, M9, M10) and western downstream Paria sites (P1 and P18). This last cluster was further split into two clusters in the 10loci-20sites dataset: one cluster composed of sites from the downstream Marianne (M9, M10, M11) and the other of sites from the downstream Paria (P1, P3, P12, P13, P14, P16 and P18). For sites that were sampled multiple times, we found consistent patterns between years. Considerable admixture between the clusters was inferred for P1, P18, M7, and M15, and admixture increased between years for M7 in the 10loci-20sites dataset. Summarizing these patterns, sites did not cluster together exclusively by watershed but rather also according to their geographic position (upstream vs. downstream; and eastern vs. western in the Marianne). We also found moderate support for a structure of 15 clusters for the 10loci-20sites dataset and 10 clusters for the 42loci-20sites dataset (Figure 1.2). These clusters are much more conservative; i.e., for both datasets each site often constitutes its own exclusive cluster, with the notable exception of sites located in a portion of the river that is called the “Petite Marianne” (M9, M10 and M11), which cluster together in both datasets.

DAPC: For both genetic datasets, classification was highest to the site of origin (Figure 1.3: Figure 1.4), indicating that each site constitutes its own guppy population at least partially isolated from other guppy populations. Some individuals were also assigned to sites from the same watershed at the same elevation, presumably reflecting the easiest contemporary routes for ongoing gene flow. For cross-classification (i.e., assigning all individuals away from their population of origin), the highest classification was generally to sites in the same watershed at the same elevation, then to the same watershed at a different elevation or instead to the other watershed at the same elevation (Figure 1.4).

MIGRATE: Both datasets suggested historical gene flow within and between watersheds (Figure S1.3). Overall, migration rates were roughly similar among all elevations and between watersheds, suggesting either similar genetic exchange at each of these levels or low power to detect any differences. Despite this absence of large differences in inferred gene flow among site pairs, we draw attention (for the purposes of later discussion) to the relatively high migration rates suggested between the eastern downstream Marianne (M9, M10) and the western downstream Paria (P18, P14, P12, P3 and P1), and between the eastern upstream Marianne (M3, M4) and the western upstream Paria (P7, P8).

BAYESASS: Both datasets suggested reasonable levels of contemporary gene flow between pairs of sites in the same watershed (Table S1.6; Table S1.7). Some sites obviously received considerable migrants from neighboring sites; for example (10loci-20sites) from P1 to P3, P7 to P17, P13 to P12 and P14, P15 to P17, M10 to M9 and M11; and (42loci-12sites) from P1 to P18, P7 to P15 and P7, P15 to P7, M7 to M8, M8 to M7 and M10, and M10 to M7. Evidence of cross-watershed contemporary gene flow was also apparent in the 42loci-12sites dataset, with some sites apparently receiving relatively recent migrants from sites in the adjacent watershed; for example, from P7 to M3 and M4 (upstream Paria to upstream

Marianne), from P18 to M7 and M8 (downstream Paria to downstream Marianne), and from M9 to P18 (downstream Marianne to downstream Paria; Table S1.7). We are not certain whether these reflect actual contemporary gene flow events or rather the continued signature of past gene flow events.

DIYABC: Divergence time estimates between watersheds differed greatly among the various pairs of sites (Figure 1.5). The shortest divergence time (41 ± 13 years) was estimated between nearby sites located in the upstream reaches of the two rivers. The second shortest divergence times were estimated between the downstream Paria and the upstream Marianne (533 ± 167 years) and between the adjacent downstream reaches of the two watersheds (577 ± 265 years). The longest divergence times (2,803 ± 470 years) were estimated between M16 in the western Marianne and various sites in the Paria.

Genetic versus phenotypic patterns

General patterns here were several. First, male color patterns significantly differed between predation regimes (MANOVA; Wilks' $\lambda = 0.766$, df = 290, $P < 0.001$). Second, classification in DAPC was always highest to the site of origin in all datasets (Figure 1.4), indicating that each is a unique "population" to at least some extent. Third, populations differed less phenotypically than genetically at all levels, especially across watersheds (Figure 1.3; Figure 1.6; Table S1.3). This outcome was mostly driven by variation in neutral genetic differentiation (Figure 1.6). Together these results suggest that phenotypic differentiation, while present among all sites, is ultimately more "constrained" in the magnitude of divergence. Third, no correspondence was seen between patterns of neutral genetic differentiation and patterns of phenotypic differentiation (Figure 1.3), and the comparison between F_{ST} and P_{ST} matrices was not significant (Mantel test: r= 0.17, p= 0.27). The only

potential indication of an effect of gene flow was that male guppies from sites where we detected a recent gene flow event were very similar in color (Figure 1.6).

Discussion

Many studies have emphasized the particular nature of connectivity in streams through ideas such as the "river continuum concept" (Vannote *et al.*, 1980). Exchanges (of nutrients, individuals, gametes, and genes) along these networks is envisioned to occur primarily within watersheds and to be biased in the downstream direction owing to water flow and barriers such as waterfalls. Genetic data consistent with these interpretations have emerged from a large number of studies of aquatic organisms in rivers, including fishes (e.g. Sivasundar *et al.*, 2001), aquatic invertebrates (e.g. Monaghan *et al.*, 2002; Alp *et al.*, 2012), and plants (e.g. Nilsson *et al.*, 2010). Previous work on guppies has also provided evidence for this type of genetic structure. For example, different watersheds tend to have genetically divergent guppy populations (Carvalho *et al.*, 1991; Alexander *et al.*, 2006; Barson *et al.*, 2009; Suk & Neff, 2009; Willing *et al.*, 2010) and upstream guppy populations show reduced genetic variation consistent with rare colonization events, limitations to upstream gene flow, and possible frequent bottlenecks due to floods (Crispo *et al.*, 2006; van Oosterhout *et al.*, 2006; Barson *et al.*, 2009). At a first cut, our analyses suggest much the same, with the largest among-site genetic differences occurring between watersheds, with upstream versus downstream populations in a given watershed often differing genetically (Figure 1.2), and with lower genetic variation in upstream than downstream populations (Table S1.1).

At the same time, our results revealed unexpected levels of cross-watershed gene flow. Guppy populations in different watersheds were often more closely related genetically than were some guppy populations in the same watershed (Figure 1.2, Table S1.1). These findings

are consistent with other studies of guppies that have found indications of cross-watershed gene flow. Some of these linkages can be attributed to known human-mediated introductions (Shaw *et al.*, 1991), whereas others are more mysterious (Suk & Neff, 2009; Willing *et al.*, 2010). In our case, cross-watershed gene flow was found in two areas, both closely adjacent tributaries at the same elevation. This finding is reminiscent of a recent study of Atlantic salmon (*Salmo salar*), where fish located at the same elevation but in different rivers were more related to each other than to fish in the same river but at a different elevation (Cauwelier *et al.*, 2018). Uniquely in our study, the two cross-watershed genetic linkages seemed to have occurred on different time-scales (historical and contemporary) in different parts of the watersheds – neither of which are associated with any known human-mediated introductions.

Contemporary and historical cross-watershed gene flow

We found likely signatures of very recent and probably high gene flow between adjacent headwater tributaries of the Marianne and Paria watersheds. These populations were characterized by very close genetic affinity (Figure 1.2), high gene flow estimates (Table S1.4; Table S1.5), recent estimated dates of divergence (41 ± 13 years; Figure 1.6), and even possible ongoing gene flow (from upstream Paria to upstream Marianne; Table S1.7). Of all potential sites for cross-watershed gene flow, this area is perhaps the least surprising owing to close physical proximity (only a few hundred meters), an only minor elevational barrier, and a well-traveled road linking them (around 2 km between the two sites). It is particularly tempting to infer human-mediated causes for the transfer (e.g., we sometimes see children carrying buckets full of guppies); although natural flooding events, perhaps accentuated by deforestation, are a reasonable alternative.

We also found signatures of historical gene flow between the two watersheds. Such signatures have been documented for some other systems and have been attributed to rare and severe events such as ice dams, earthquakes, or volcanic activity (Burridge *et al.*, 2006, 2007; Gelmond *et al.*, 2009; Lescak *et al.*, 2015). In Trinidad's Northern Range, we inferred historical gene flow between one western downstream tributary of the Paria (called the Jordan River) and one eastern downstream tributary of the Marianne (called the Petite Marianne). We suggest, based on several lines of evidence, that the latter was actually colonized at the inferred time from the former. First, Petit Marianne guppies cluster genetically with the adjacent Jordan River guppies. Second, the Petit Marianne is physically isolated by an approximately 10 m waterfall that likely prevents migration from the rest of the Marianne River. Third, guppies are currently found in the headwaters of the Jordan River, less than 50 m of horizontal distance, with an only minor elevational change, from a steep slope down to the Petite Marianne (A. Hendry and P. Bentzen, pers. obs.), which might have allowed Jordan River fish to colonize the Petite Marianne.

Divergence time estimates indicate old (577 years ± 265) connectivity between the Petite Marianne and Jordan River guppies. Several old, but rare, events could explain this historical cross-watershed linkage. First, indigenous people present on the island since around 1000 BC could have moved fish from one watershed to the other. However, this explanation seems unlikely given the remote location of these small tributaries, and the fact that indigenous people relied mainly on fish from the ocean rather than fresh water (Newson, 1976). Second, Trinidad is located on the Caribbean tectonic plate, and major earthquakes have been reported since written history of the island (Shepard & Aspinall, 1983). Such earthquakes, violent hurricanes, or massive flooding could have led to river capture (Bishop, 1995), i.e. "the transfer of part or all of a (generally well established) river's flow to another

river", causing the movement of Paria guppies from the Jordan River into the Petite Marianne.

Once cross-watershed gene flow occurs, a logical question is whether that influence spreads far beyond the site of origin. Several studies have shown that experimental introductions of guppies have genetic consequences that spread downstream, including over waterfalls and into different predation environments (Becher & Magurran, 2000; Fitzpatrick *et al.*, 2015; Fraser *et al.*, 2015). For our non-experimental, whether natural or anthropogenic, cross-watershed transfers, we also see signatures of downstream consequences. For instance, several main-stem Marianne populations (M7 and M8) immediately below the Petite Marianne show a signature of downstream gene flow from the putative Paria-origin Petit Marianne fish.

Consequences for adaptive traits

We uncovered signatures of gene flow within and between riverine networks reflecting a complex combination of water flow (biased downstream), barriers (waterfalls), physical proximity, potential recent human introductions, and past geological or climatological events. To what extent has this gene flow influenced adaptive trait variation? A classic theoretical expectation would be reduced divergence in the case of very high, and especially recent, gene flow (Hendry *et al.*, 2001; Lenormand, 2002). On the other hand, some theoretical treatments suggest a potential positive role for gene flow in facilitating local adaptation (review: Garant et al. 2007).

Previous work on guppies has thus far emphasized strong adaptive divergence among guppy populations in diverse traits such as male color (Endler, 1980a; Millar *et al.*, 2006; Kemp *et al.*, 2008; Gotanda & Hendry, 2014), body shape (Hendry *et al.*, 2006; Burns *et al.*,

2009), life history (Reznick *et al.*, 1996), parasite resistance (van Oosterhout *et al.*, 2003; Fraser & Neff, 2010; Pérez-Jvostov *et al.*, 2015), and behavior (Magurran & Seghers, 1991; Jacquin *et al.*, 2016). Yet a few studies have also hinted that closely adjacent populations can be more phenotypically similar than expected given their environmental differences (Endler, 1978), while others have failed to find such a signature (Fitzpatrick *et al.*, 2015). Given the diverse outcomes of these previous studies, we considered to what extent the patterns of contemporary and historical gene flow we documented might carry over to any signature in adaptive traits, specifically male color.

Overall, male color was quite location-specific (Figure 1.4), suggesting adaptation to local conditions. Some of this variation was associated with differences in predation regime (high versus low) within the Marianne, as described in previous analyses of this system (Millar *et al.*, 2006; Gotanda & Hendry, 2014). Yet considerable variation was also seen between our study sites within a given predation regime (Figure 1.3; Figure 1.4), which previous studies have attributed to these site-specific factors such as specific predator identities and densities (Millar et al. 2006), canopy cover (Grether *et al.*, 2001; Schwartz & Hendry, 2010), and sexual selection (Schwartz & Hendry, 2007). However, differences among sites in color were generally less that differences among sites in neutral markers (Figure 1.6; Table S1.3). This result likely reflects some level of convergent stabilizing selection on male color owing to constraints on the range of possible color space and the need to be attractive to females but also cryptic to predators. By contrast, neutral markers are free to diverge to an extent (mostly) unconstrained by selection, instead being limited only by time and gene flow.

Importantly, we see little evidence that the constraint imposed on divergence for male color reflects gene flow – given the overall lack of correspondence between genetic and

phenotypic divergence (Figure 1.3; Figure 1.4; Mantel test: r= 0.17, p= 0.27). However, one detailed local comparison hinted at potential gene flow effects: populations from the upstream Paria and upstream Marianne were extremely similar in color (Figure 1.6). In this particular instance, recent gene flow might have left a signature on male color differentiation. An alternative possibility is that the environments experienced by these two populations were exceptionally similar, and thus favored similar phenotypes; although habitat data does not suggest such exceptional similarity (e.g., Millar et al. 2006). Furthermore, we cannot rule out that gene flow constrains or facilitates adaptation for other traits or in other contexts. For instance, Fitzpatrick et al. (2017) found evidence for trait-specific constraining and diverging effects in an experimental manipulation of gene flow.

Divergence time

Divergence time between the Paria and Marianne guppies was previously estimated to be approximately 100,000 years based on mitochondrial DNA data (Fajen & Breden, 1992). Our multi-locus estimates suggest much more recent connections between the two watersheds, ranging from a maximum of a few thousand years between isolated portions of the watersheds, up to contemporary gene flow between proximate portions of the watersheds at the same elevation. These results have to be tempered because we only tested very simple models of divergence and because homoplasy occurs with microsatellite markers, which could create noise in our results (Estoup *et al.*, 2002). However, in the light of our findings, we would still like to discuss that divergence time between other watersheds extensively studied in Trinidad might also be more recent than previously estimated, a possibility that has important implications for our understanding of adaptive evolution and early speciation in this system. For instance, the general lack of speciation in guppies is often considered surprising

(Magurran, 1998) given their ancient divergence – but perhaps gene flow has been much more recent. Also, although we know through experiments that contemporary evolution is common in guppies (Reznick *et al.*, 1990), perhaps even naturally established populations have evolved on much shorter than expected time frames.

Conclusion

Our findings are broadly consistent with previous population genetic work for riverine organisms in general, and for guppies in particular. Specifically, we confirmed within-watershed gene flow in which upstream populations are less genetically diverse and more isolated than are downstream populations. However, we also discovered levels of cross-watershed gene flow, to the extent that some populations are more closely related to populations in the adjacent watershed than they are to some populations within their own watershed. Although surprisingly genetic similarities between the Paria and the Marianne watersheds have been previously suggested (Willing *et al.*, 2010), our much more detailed sampling was able to infer where, when, and in what directions these genetic exchanges took place. In one case, cross-watershed linkages were recent and, in the other case, they occurred centuries ago, suggesting different contributions from geological, climatological, or anthropogenic drivers. However, none of these gene flow patterns seemed to have any major consequence for adaptive trait variation – although our findings do not rule out effects for other traits or on smaller spatial scales. Dispersal, and thus subsequent gene flow, clearly paves the way for colonization of new environments, but it did not seem to here substantially constrain adaptation by guppies to those environments.

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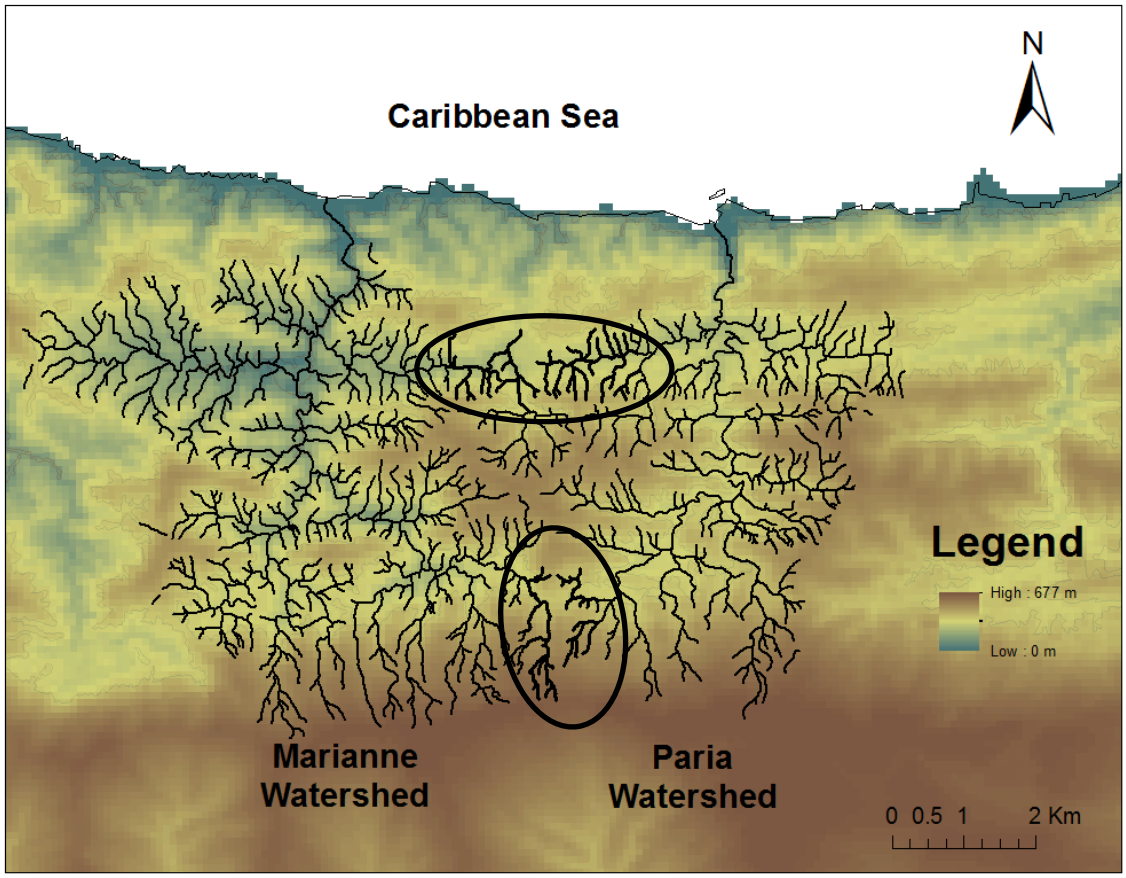
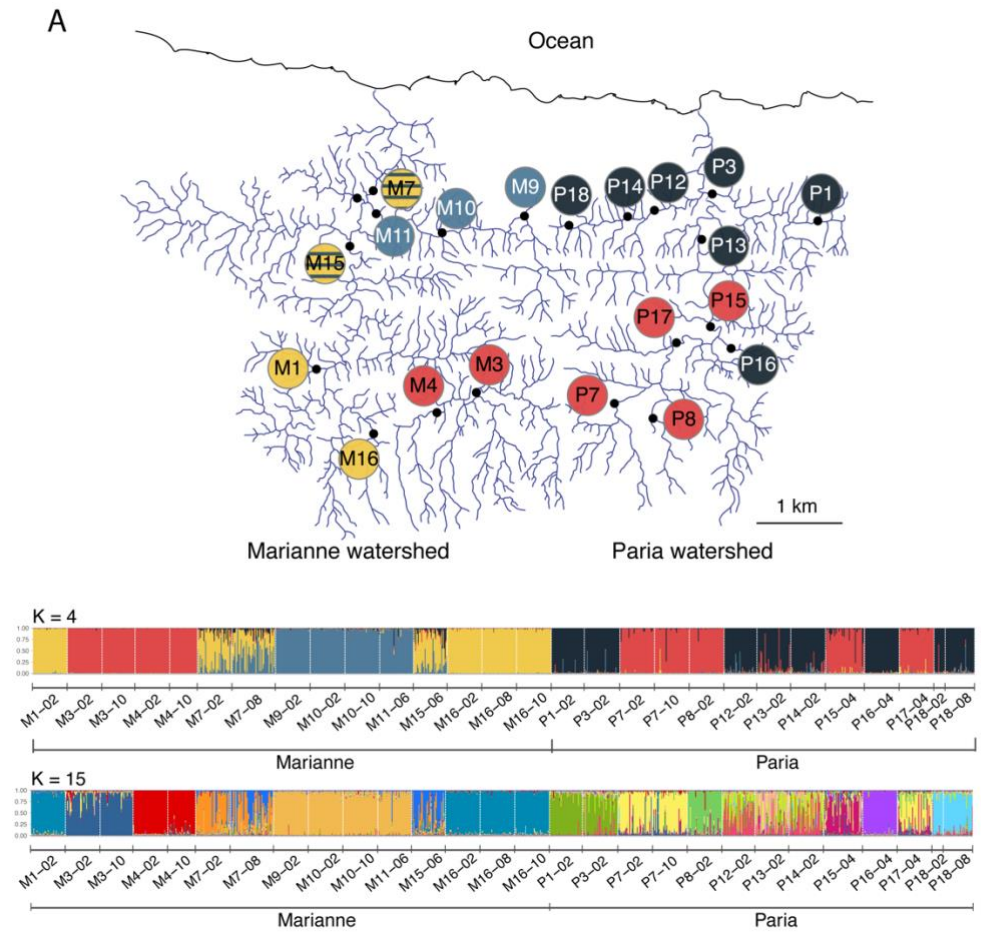


Figure 1.1 Topographic map of the Marianne and Paria watersheds. Bold and circled sections of the rivers indicate potential gene flow zones between watersheds, located at different elevations.


A
Ocean
M7
M10
M9
P18
P14
P12
P3
P1
M11
P13
M15
P17
P15
M1
M3
P16
M4
P7
P8
M16
1 km
Marianne watershed
Paria watershed
K = 4
M1–02 M3–02 M3–10 M4–02 M4–10 M7–02 M7–08 M9–02 M10–02 M10–10 M11–06 M15–06 M16–02 M16–08 M16–10 P1–02 P3–02 P7–02 P7–10 P8–02 P12–02 P13–02 P14–02 P15–04 P16–04 P17–04 P18–02 P18–08
Marianne
Paria
K = 15
M1–02 M3–02 M3–10 M4–02 M4–10 M7–02 M7–08 M9–02 M10–02 M10–10 M11–06 M15–06 M16–02 M16–08 M16–10 P1–02 P3–02 P7–02 P7–10 P8–02 P12–02 P13–02 P14–02 P15–04 P16–04 P17–04 P18–02 P18–08
Marianne
Paria

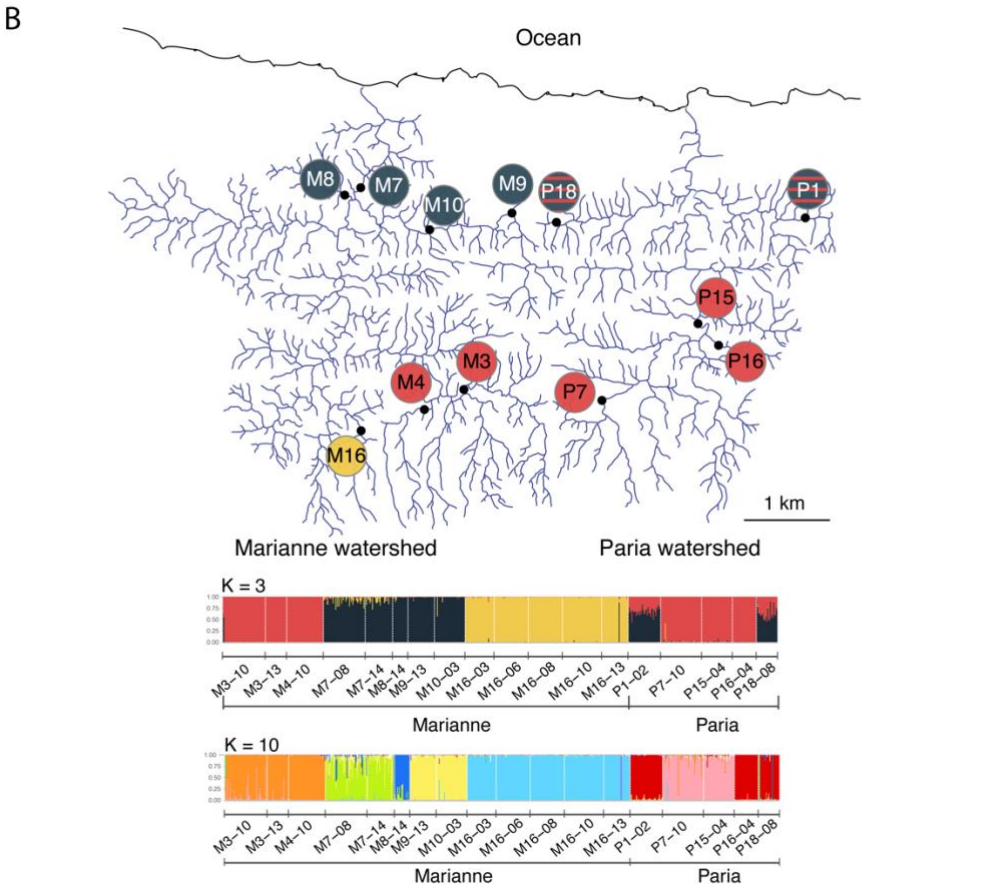

B
Ocean
M8
M7
M9
P18
P1
M10
P15
P16
M3
M4
P7
M16
1 km
Marianne watershed
Paria watershed
K = 3
M3–10 M3–13 M4–10 M7–08 M7–14 M8–14 M9–13 M10–03 M16–03 M16–06 M16–08 M16–10 M16–13 P1–02 P7–10 P15–04 P16–04 P18–08
Marianne
Paria
K = 10
M3–10 M3–13 M4–10 M7–08 M7–14 M8–14 M9–13 M10–03 M16–03 M16–06 M16–08 M16–10 M16–13 P1–02 P7–10 P15–04 P16–04 P18–08
Marianne
Paria

Figure 1.2 Distribution of clusters inferred by STRUCTURE analysis and their corresponding maps based on (A) 10loci-20sites and (B) 42loci-12sites datasets. For (A) K=4 (mean ΔK = 378.42 among three replicates); For (B) K=3 (mean ΔK = 87.71 among three replicates). Sites on the map are colored according to the highest assignment to a cluster. When individuals of a site show admixture, site symbol is filled with stripes of the corresponding color. Additional support for K=15 (10loci-20sites) and K=10 (42loci-12sites) is also represented.

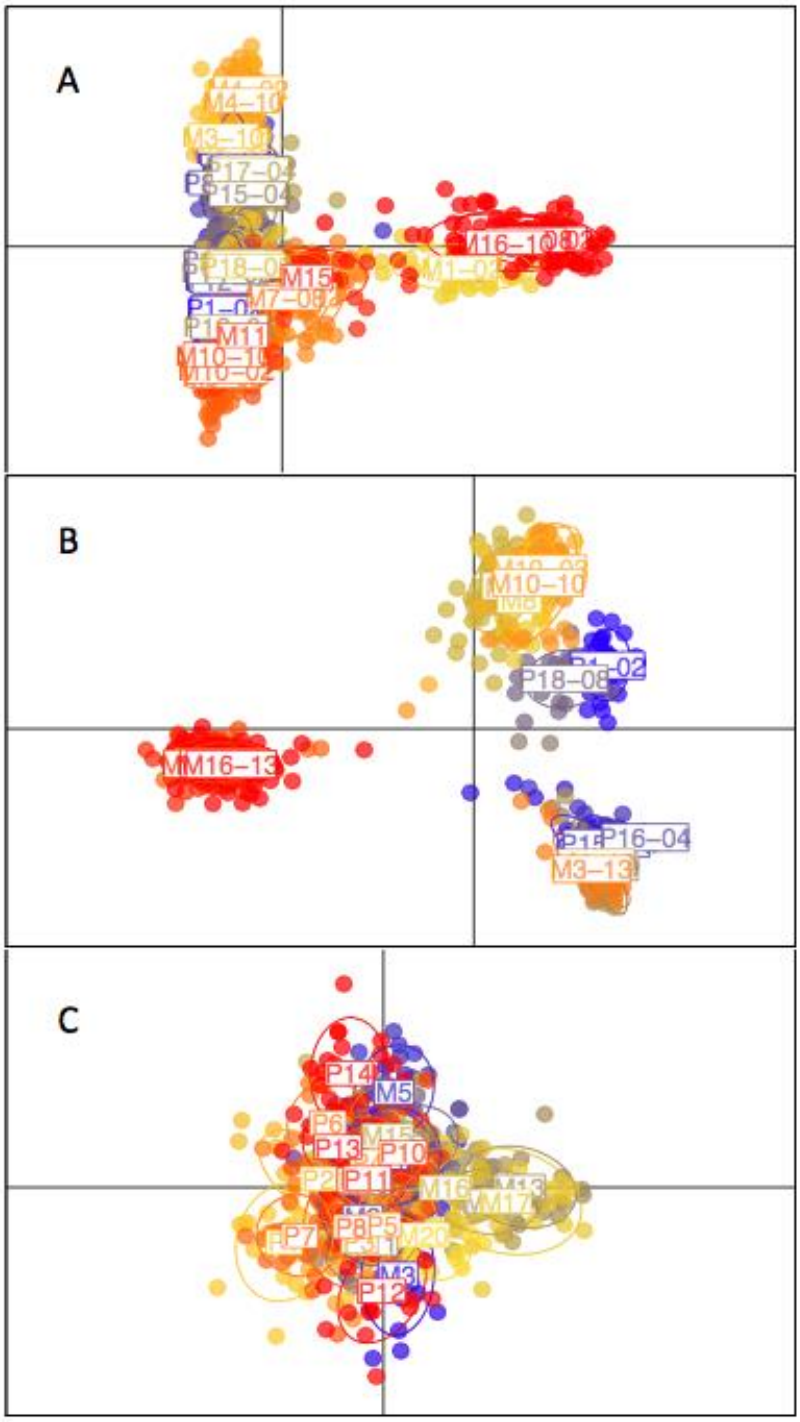


Figure 1.3 Scatter plot based on discriminant analysis of principal components for (A) 10loci-20sites neutral markers, (B) 42loci-12sites neutral markers, and (C) male color traits. Colors correspond to a posteriori groups defined by the DAPC analysis. Individuals are represented as dots and groups as inertia ellipses.

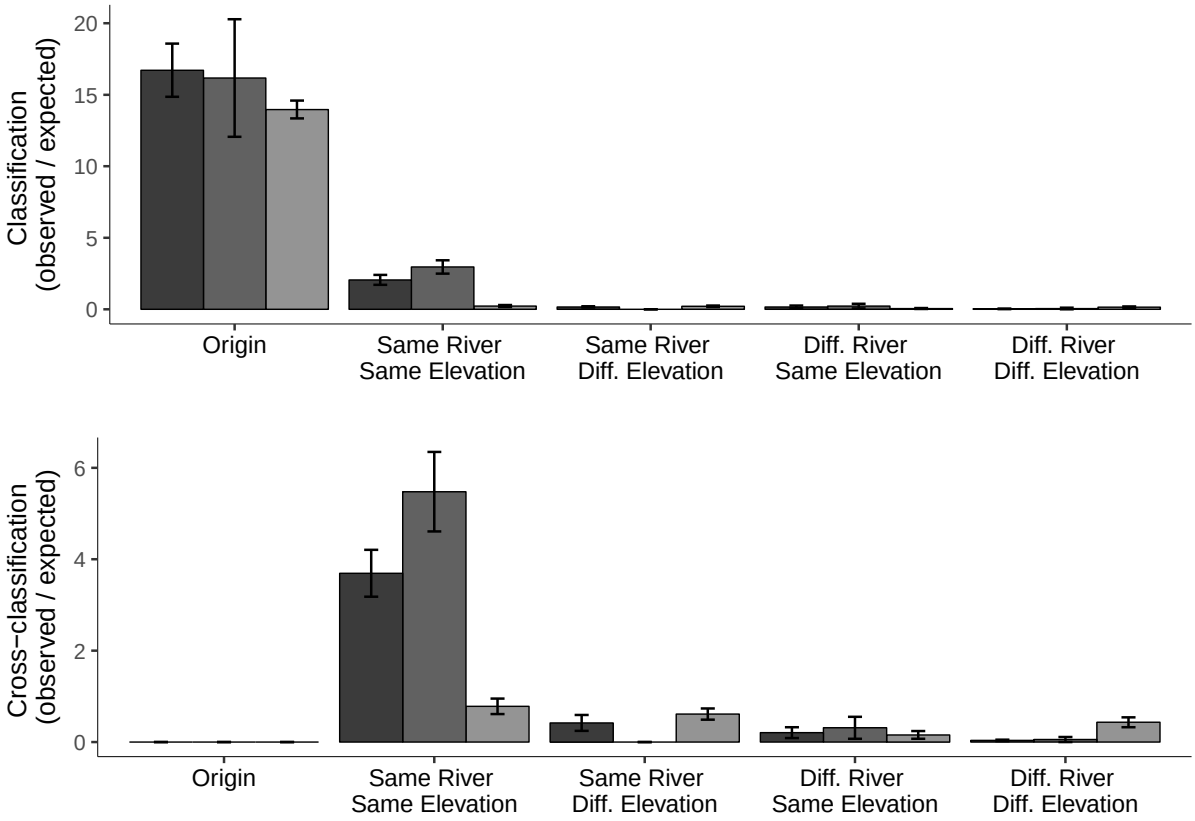


Figure 1.4 Ratio of the mean number of individuals classified into each category as indicated on the x axis to the mean number expected to be classified into these categories by chance. Upper panel shows the classification for the 3 datasets (10loci-20sites in dark grey, 42loci-12sites in medium grey, and male color in light grey), lower panel shows cross-classification.

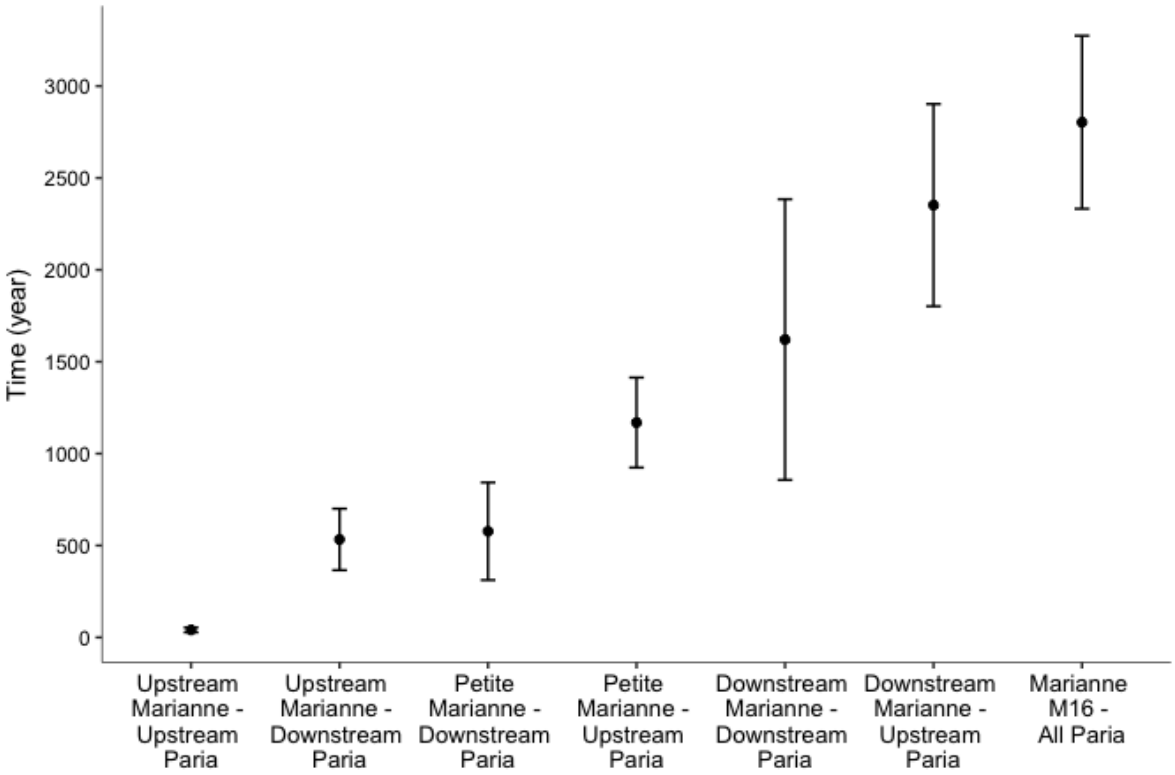


Figure 1.5 Mean divergence time estimates from pairwise comparison of sites across watersheds, calculated using DIYABC. Errors bars represent standard variation in each group. Groups are as follow: Upstream Marianne (M3, M4); Upstream Paria (P7, P15); Downstream Paria (P1, P16, P18); Petite Marianne (M9, M10); Downstream Marianne (M7, M8); Marianne M16 (M16).

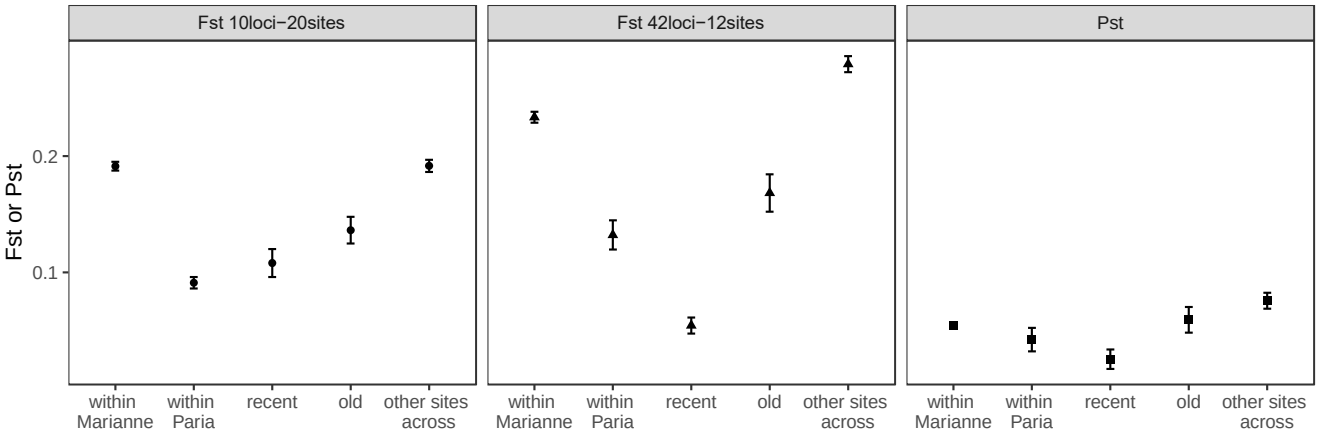


Figure 1.6 Comparison of the F_{ST} values for both genetic datasets and the P_{ST} values for the male color traits within sites located in the Marianne (all sites), within sites located in the Paria (all sites), between sites located in the upstream reached of the watersheds ("recent" gene flow event, between P7 and M3-M4), sites located in the downstream reaches of the watersheds ("old" gene flow event, between P18 and M9-M10), and finally all "other sites across" watersheds.

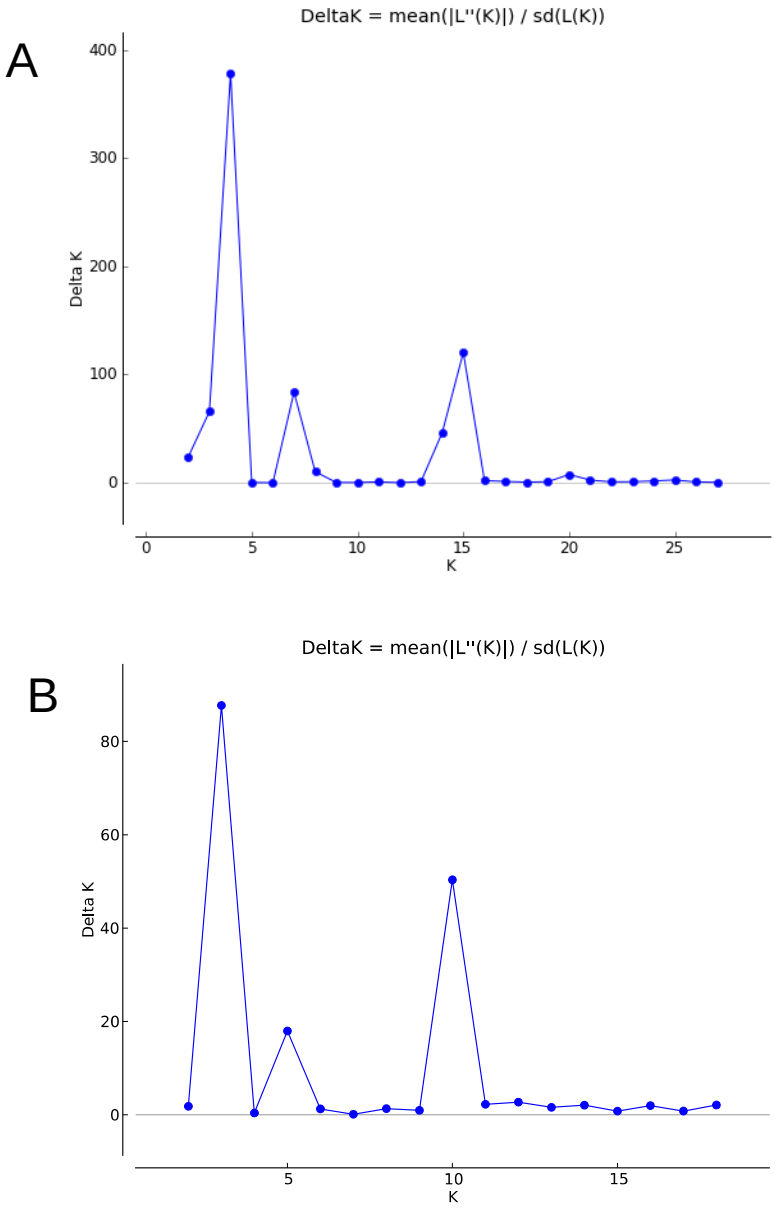


Figure S1.5 Delta K (difference in the log probability of data between successive K values) for (A) 10loci-20sites dataset and (B) 42loci-12sites dataset.

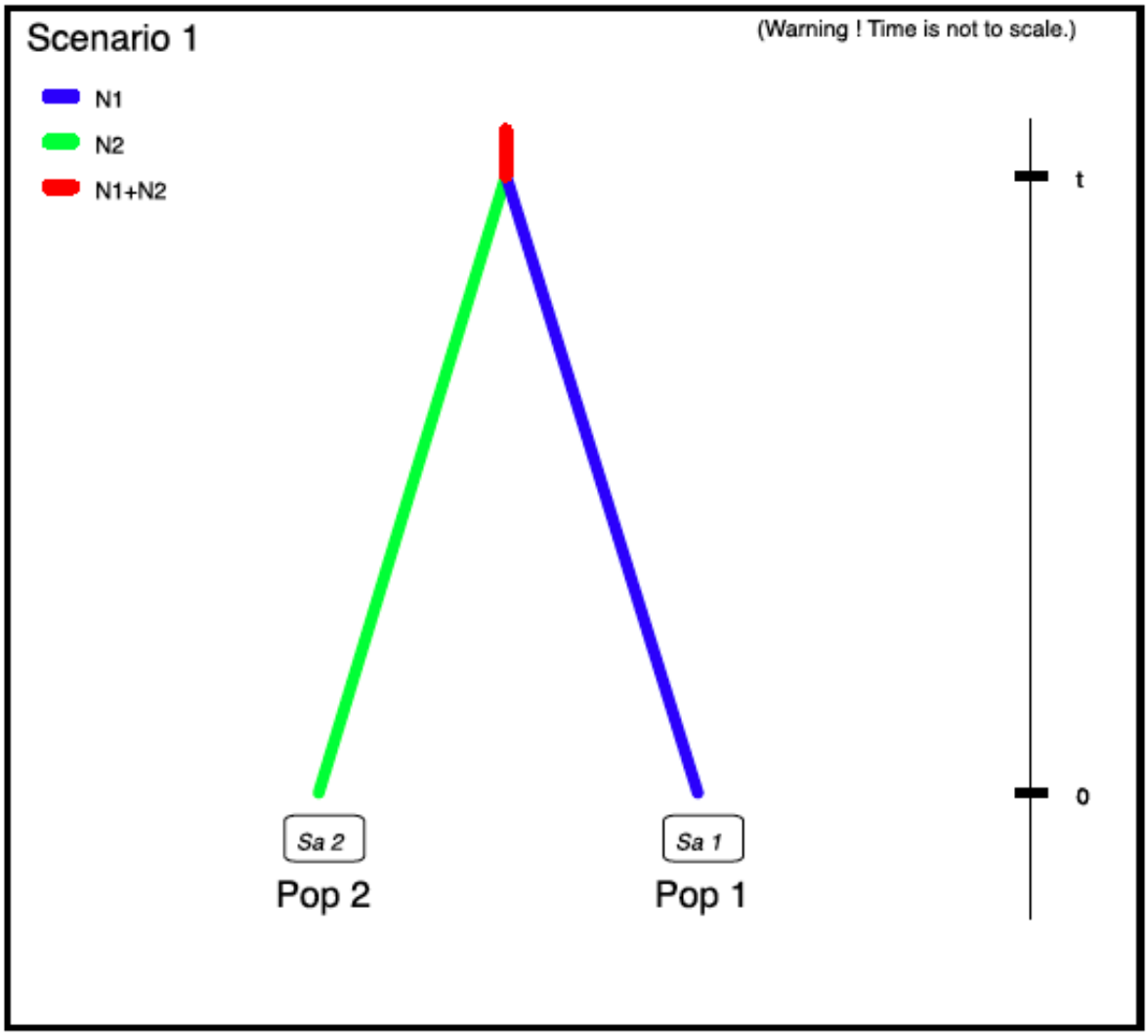


Figure S1.6 Historical model scenario used in DIYABC: two populations of size N1 & N2 have diverged t generations in the past from one population N1+N2. Sa designate samples.

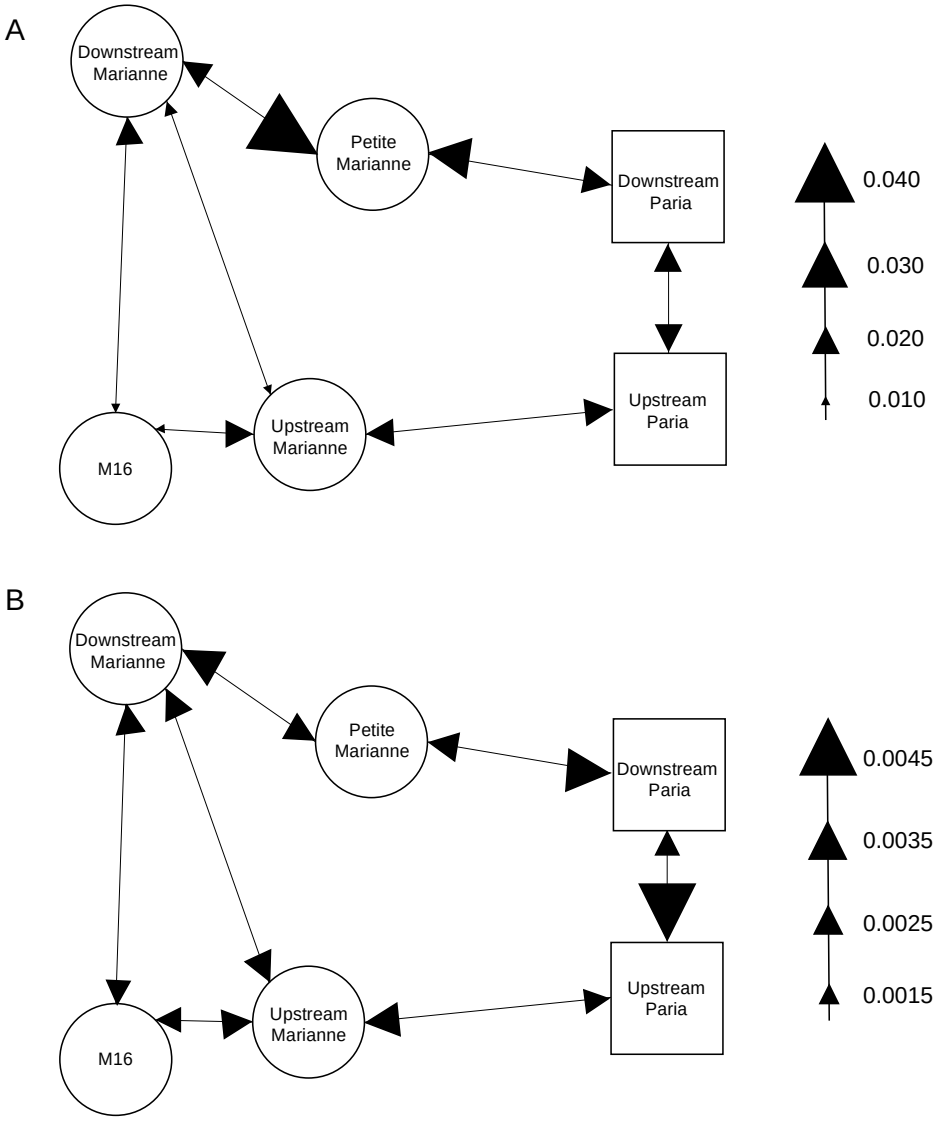


Figure S1.7 Migration rates (M = m/μ) among the different parts of the rivers, calculated using MIGRATE. Sizes of arrow heads represent the variation in the amount of migration; Circles represent sites in the Marianne River, squares represent sites in the Paria river. (A) 10loci-20sites dataset, (B) 42loci-12sites dataset. Arrows between all groups were modelled but are not represented here for clarity of the figure.

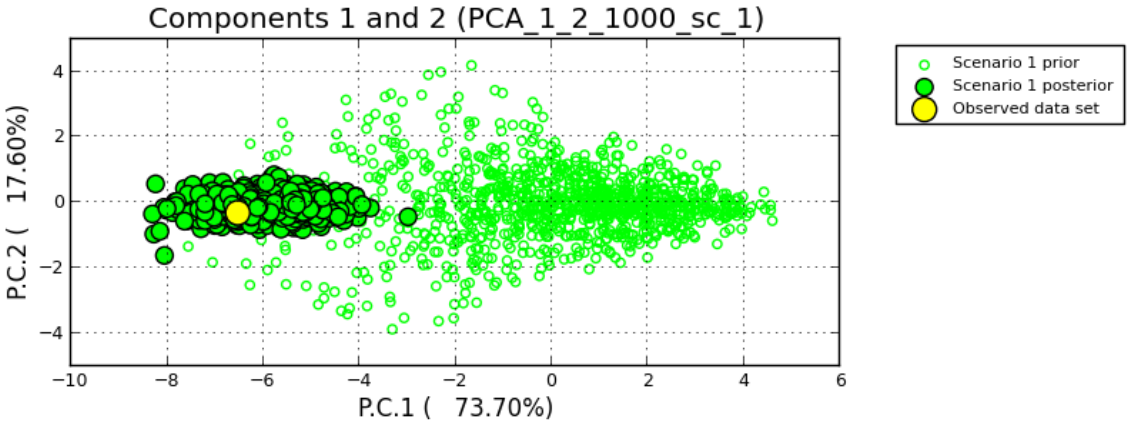


Figure S1.8 PCA plot indicating prior and posterior distributions for the first pairwise divergence estimate – between P7 and M3. The yellow dot among the bigger green dots indicate a good fit for the model

Table S1.1 List of individuals sampled per site and year in each dataset, as well as allele numbers and observed heterozygosity. All sites are low predation, except when notified (HP for high predation). When a site is present in both datasets, both values of observed heterozygosity and allele numbers are given (first 10loci-20sites, then 42loci-12sites).

Site – predation regime	10loci-20sites	42loci-12sites	Male color	Allele numbers	Observed Heterozygosity
P1 (2002)	38	38	20	93 – 134	0.632 – 0.354
P2 (2002)	-	-	20	-	-
P3 (2002)	41	-	20	106	0.667
P4 (2002)	-	-	20	-	-
P5 (2002)	-	-	20	-	-
P6 (2002)	-	-	18	-	-
P7 (2002)	40	-	20	87	0.702
(2010)	40	48	-	87 - 180	0.582 – 0.361
P8 (2002)	40	-	20	72	0.631
P9 (2002)	-	-	20	-	-
P10 (2002)	-	-	20	-	-
P11 (2002)	-	-	20	-	-
P12 (2002)	38	-	20	119	0.739
P13 (2002)	39	-	20	106	0.659
P14 (2002)	40	-	20	117	0.752
P15 (2004)	45	30	-	96 - 182	0.602 - 0.395
P16 (2004)	40	34	-	34 - 54	0.292 - 0.073
P17 (2004)	40	-	-	97	0.658
P18 (2002)	13	-	-	50	0.648
(2008)	40	25	-	78 - 221	0.685 – 0.480
M1 (2002)	40	-	-	84	0.643
M2 (2002) - HP	-	-	18	-	-
M3 (2002)	40	-	20	57	0.568
(2010)	38	50	-	52 – 140	0.430 – 0.302
(2013)	-	25	-	101	0.311
M4 (2002)	40	-	20	35	0.321
(2010)	32	43	-	38 - 99	0.325 – 0.274
M5 (2002)	-	-	20	-	-
M6 (2002)	-	-	20	-	-
M7 (2002) - HP	40	-	20	123	0.732
(2008) - HP	51	49	-	141 – 262	0.719 – 0.573
(2014) - HP	-	31	-	206	0.528
M8 (2014)	-	18	-	158	
M9 (2002)	40	-	20	89	0.564
(2013)	-	31	-	160	0.386
M10 (2002)	40	-	20	85	0.616
(2003)	-	36	-	183	0.382
(2010)	40	50	-	89 - 184	0.589 – 0.426
M11 (2006)	39	-	-	-	0.651
(*2002*)	-	-	19	88	-
M13 (2002) - HP	-	-	20	-	-
M14 (2002) - HP	-	-	20	-	-
M15 (2002) - HP	-	-	20	-	-
(2006) - HP	39	-	-	121	0.696
M16 (2002)	40	-	20	64	0.523
(2003)	-	34	-	130	0.315
(2006)	-	40	-	124	0.327
(2008)	40	40	-	59 - 122	0.476 – 0.283
(2010)	40	46	-	69 – 133	0.546 – 0.321
(2013)	-	31	-	122	0.338
M17 (2002) - HP	-	-	20	-	-
M20 (2002)	-	-	20	-	-

Table S1.2 Summary of mean and standard deviation for the color traits in each site.

	Total number of melanin spots		Total number of carotenoids spots		Total number of structural spots		Total relative area of carotenoids spots		Total relative area of structural spots		Total relative area of melanin spots	
Site	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
M2	3.33	1.53	2.33	0.77	3.56	1.46	0.10	0.03	0.15	0.07	0.14	0.09
M3	3.35	1.14	2.90	0.64	3.05	1.00	0.17	0.04	0.12	0.04	0.14	0.04
M4	2.30	1.49	2.35	0.99	3.40	0.88	0.11	0.04	0.13	0.03	0.09	0.05
M5	2.95	1.70	2.35	0.67	3.60	1.23	0.14	0.04	0.16	0.06	0.11	0.07
M6	3.35	1.42	2.70	0.86	2.90	0.79	0.14	0.05	0.12	0.04	0.13	0.04
M7	2.95	1.57	2.15	0.88	3.85	1.57	0.09	0.04	0.15	0.05	0.12	0.07
M9	2.70	1.08	2.80	1.06	3.55	1.57	0.14	0.04	0.11	0.05	0.19	0.09
M10	3.45	1.23	2.60	0.88	3.15	1.31	0.17	0.05	0.11	0.05	0.19	0.06
M11	2.68	1.45	2.26	0.99	2.84	1.17	0.13	0.04	0.11	0.08	0.20	0.10
M13	3.30	1.17	2.05	1.32	4.50	1.05	0.09	0.05	0.16	0.05	0.12	0.07
M14	3.25	1.37	2.10	1.29	4.15	1.35	0.08	0.05	0.15	0.04	0.18	0.07
M15	2.60	1.64	1.90	0.85	3.25	1.45	0.07	0.02	0.14	0.08	0.12	0.09
M16	4.05	1.19	3.00	1.59	3.70	1.42	0.11	0.05	0.17	0.07	0.15	0.06
M17	2.45	1.64	1.90	1.52	4.25	1.55	0.08	0.06	0.19	0.05	0.12	0.08
M20	3.90	1.86	3.65	1.27	3.10	1.48	0.11	0.05	0.15	0.09	0.14	0.07
P1	3.10	0.79	2.05	0.83	2.35	1.09	0.16	0.05	0.08	0.04	0.17	0.05
P2	2.80	1.01	2.50	0.83	2.75	1.07	0.15	0.04	0.13	0.05	0.18	0.08
P3	2.90	1.25	2.50	1.10	2.95	0.94	0.16	0.05	0.12	0.04	0.17	0.06
P4	2.95	0.89	2.40	0.94	3.10	1.02	0.18	0.04	0.14	0.03	0.13	0.06
P5	3.00	1.34	2.10	0.85	3.35	1.09	0.15	0.06	0.14	0.06	0.15	0.06
P6	3.33	0.84	2.50	0.71	2.11	0.90	0.19	0.05	0.07	0.04	0.19	0.06
P7	3.45	1.28	2.60	0.94	3.15	1.04	0.17	0.05	0.12	0.05	0.16	0.05
P8	2.95	1.23	2.30	0.86	2.90	0.64	0.16	0.04	0.12	0.04	0.12	0.05
P9	3.25	1.33	2.60	0.50	2.85	0.88	0.18	0.05	0.11	0.04	0.15	0.05
P10	2.45	1.39	2.30	1.03	3.35	0.99	0.13	0.05	0.13	0.05	0.12	0.06
P11	3.80	1.85	2.45	0.89	2.60	0.99	0.17	0.04	0.09	0.04	0.15	0.04
P12	2.75	0.85	2.50	1.10	2.60	1.14	0.17	0.06	0.10	0.05	0.15	0.07
P13	2.75	0.79	2.35	1.18	2.95	0.89	0.16	0.06	0.13	0.04	0.16	0.06
P14	2.90	1.07	2.15	0.67	2.30	0.80	0.21	0.04	0.09	0.03	0.19	0.08

Table S1.3 Heatmap of Pst values for each site in the male color dataset. Values in green represents low phenotypic differentiation, values in red represents higher phenotypic differentiation.

	M10	M11	M13	M14	M15	M16	M17	M2	M20	M3	M4	M5	M6	M7	M9
M11	0.064	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M13	0.009	0.091	-	-	-	-	-	-	-	-	-	-	-	-	-
M14	0.001	0.056	0.002	-	-	-	-	-	-	-	-	-	-	-	-
M15	0.066	0.001	0.093	0.061	-	-	-	-	-	-	-	-	-	-	-
M16	0.051	0.156	0.017	0.027	0.156	-	-	-	-	-	-	-	-	-	-
M17	0.011	0.012	0.029	0.014	0.015	0.076	-	-	-	-	-	-	-	-	-
M2	0.000	0.052	0.009	0.002	0.055	0.049	0.008	-	-	-	-	-	-	-	-
M20	0.046	0.152	0.014	0.023	0.152	0.000	0.071	0.044	-	-	-	-	-	-	-
M3	0.000	0.073	0.007	0.001	0.074	0.050	0.013	0.000	0.044	-	-	-	-	-	-
M4	0.065	0.001	0.094	0.055	0.003	0.164	0.009	0.052	0.160	0.077	-	-	-	-	-
M5	0.005	0.035	0.022	0.008	0.039	0.074	0.002	0.003	0.069	0.007	0.033	-	-	-	-
M6	0.004	0.037	0.021	0.007	0.041	0.071	0.003	0.002	0.066	0.006	0.035	0.000	-	-	-
M7	0.003	0.025	0.016	0.006	0.029	0.057	0.002	0.002	0.052	0.004	0.022	0.000	0.000	-	-
M9	0.001	0.034	0.011	0.003	0.038	0.048	0.005	0.000	0.043	0.001	0.031	0.001	0.001	0.001	-
P1	0.140	0.004	0.159	0.102	0.001	0.233	0.031	0.113	0.232	0.162	0.013	0.088	0.090	0.054	0.069
P10	0.044	0.002	0.072	0.042	0.004	0.135	0.005	0.036	0.131	0.052	0.000	0.021	0.023	0.015	0.022
P11	0.004	0.025	0.019	0.008	0.029	0.064	0.001	0.003	0.058	0.006	0.022	0.000	0.000	0.000	0.001
P12	0.065	0.000	0.093	0.057	0.001	0.160	0.011	0.053	0.157	0.075	0.000	0.035	0.037	0.024	0.034
P13	0.051	0.002	0.079	0.046	0.005	0.147	0.005	0.041	0.143	0.060	0.001	0.024	0.026	0.016	0.024
P14	0.120	0.005	0.144	0.095	0.002	0.214	0.031	0.099	0.213	0.135	0.013	0.077	0.079	0.052	0.065
P2	0.065	0.003	0.094	0.053	0.006	0.167	0.006	0.050	0.164	0.078	0.001	0.030	0.032	0.018	0.028
P3	0.023	0.009	0.048	0.025	0.012	0.106	0.001	0.018	0.101	0.028	0.006	0.008	0.009	0.006	0.010
P4	0.027	0.017	0.055	0.027	0.021	0.122	0.000	0.020	0.118	0.034	0.014	0.007	0.009	0.005	0.010
P5	0.023	0.014	0.049	0.024	0.018	0.111	0.000	0.017	0.106	0.028	0.011	0.007	0.008	0.004	0.009
P6	0.073	0.001	0.099	0.057	0.003	0.170	0.009	0.057	0.168	0.086	0.000	0.036	0.039	0.022	0.032
P7	0.000	0.068	0.010	0.002	0.070	0.056	0.011	0.000	0.051	0.000	0.072	0.005	0.004	0.003	0.001
P8	0.048	0.004	0.076	0.043	0.006	0.144	0.004	0.038	0.140	0.057	0.001	0.021	0.023	0.014	0.022
P9	0.015	0.033	0.040	0.017	0.037	0.105	0.001	0.010	0.100	0.020	0.032	0.002	0.002	0.001	0.004

	P1	P10	P11	P12	P13	P14	P2	P3	P4	P5	P6	P7	P8
M11	-	-	-	-	-	-	-	-	-	-	-	-	-
M13	-	-	-	-	-	-	-	-	-	-	-	-	-
M14	-	-	-	-	-	-	-	-	-	-	-	-	-
M15	-	-	-	-	-	-	-	-	-	-	-	-	-
M16	-	-	-	-	-	-	-	-	-	-	-	-	-
M17	-	-	-	-	-	-	-	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	-	-	-	-	-	-
M20	-	-	-	-	-	-	-	-	-	-	-	-	-
M3	-	-	-	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	-	-	-	-	-	-	-	-	-
M5	-	-	-	-	-	-	-	-	-	-	-	-	-
M6	-	-	-	-	-	-	-	-	-	-	-	-	-
M7	-	-	-	-	-	-	-	-	-	-	-	-	-
M9	-	-	-	-	-	-	-	-	-	-	-	-	-
P1	-	-	-	-	-	-	-	-	-	-	-	-	-
P10	0.013	-	-	-	-	-	-	-	-	-	-	-	-
P11	0.056	0.015	-	-	-	-	-	-	-	-	-	-	-
P12	0.006	0.001	0.024	-	-	-	-	-	-	-	-	-	-
P13	0.018	0.000	0.016	0.002	-	-	-	-	-	-	-	-	-
P14	0.000	0.014	0.053	0.007	0.018	-	-	-	-	-	-	-	-
P2	0.027	0.000	0.018	0.002	0.000	0.024	-	-	-	-	-	-	-
P3	0.031	0.003	0.005	0.008	0.003	0.030	0.004	-	-	-	-	-	-
P4	0.066	0.007	0.004	0.017	0.008	0.055	0.011	0.000	-	-	-	-	-
P5	0.047	0.005	0.004	0.013	0.006	0.043	0.008	0.000	0.000	-	-	-	-
P6	0.017	0.000	0.023	0.001	0.001	0.016	0.001	0.006	0.016	0.012	-	-	-
P7	0.159	0.048	0.004	0.070	0.056	0.131	0.073	0.025	0.030	0.025	0.082	-	-
P8	0.023	0.000	0.014	0.003	0.000	0.022	0.000	0.002	0.006	0.004	0.001	0.052	-
P9	0.108	0.018	0.001	0.033	0.021	0.085	0.030	0.004	0.004	0.003	0.038	0.016	0.018

Table S1.4 Heatmap of paired Fst values for each site in the 10loci-20sites dataset. Values in green represents low genetic differentiation, values in red represents higher genetic differentiation.

	P1-02	P3-02	P7-02	P7-10	P8-02	P12-02	P13-02	P14-02	P15-04	P16-04	P17-04	P18-02	P18-08	M1-02
P1-02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P3-02	0.015	-	-	-	-	-	-	-	-	-	-	-	-	-
P7-02	0.116	0.084	-	-	-	-	-	-	-	-	-	-	-	-
P7-10	0.118	0.090	0.027	-	-	-	-	-	-	-	-	-	-	-
P8-02	0.133	0.100	0.067	0.080	-	-	-	-	-	-	-	-	-	-
P12-02	0.044	0.028	0.081	0.086	0.093	-	-	-	-	-	-	-	-	-
P13-02	0.038	0.024	0.077	0.086	0.097	0.025	-	-	-	-	-	-	-	-
P14-02	0.038	0.024	0.074	0.080	0.087	0.014	0.020	-	-	-	-	-	-	-
P15-04	0.109	0.083	0.034	0.036	0.044	0.088	0.079	0.077	-	-	-	-	-	-
P16-04	0.236	0.211	0.229	0.202	0.245	0.235	0.238	0.221	0.182	-	-	-	-	-
P17-04	0.116	0.086	0.012	0.028	0.052	0.085	0.078	0.075	0.021	0.211	-	-	-	-
P18-02	0.093	0.074	0.082	0.086	0.088	0.048	0.065	0.052	0.089	0.209	0.092	-	-	-
P18-08	0.088	0.062	0.085	0.086	0.099	0.038	0.052	0.040	0.090	0.222	0.092	0.017	-	-
M1-02	0.173	0.153	0.184	0.173	0.201	0.138	0.156	0.144	0.167	0.343	0.168	0.130	0.138	-
M3-02	0.146	0.113	0.028	0.042	0.087	0.114	0.108	0.103	0.045	0.254	0.027	0.108	0.111	0.218
M3-10	0.187	0.153	0.100	0.075	0.157	0.159	0.160	0.144	0.094	0.307	0.095	0.149	0.159	0.262
M4-02	0.263	0.220	0.153	0.129	0.240	0.234	0.227	0.224	0.143	0.427	0.155	0.218	0.228	0.315
M4-10	0.260	0.219	0.174	0.119	0.248	0.233	0.231	0.218	0.149	0.426	0.167	0.247	0.237	0.310
M7-02	0.090	0.074	0.099	0.105	0.109	0.063	0.076	0.065	0.097	0.255	0.098	0.081	0.084	0.099
M7-08	0.068	0.058	0.078	0.084	0.092	0.052	0.057	0.053	0.075	0.211	0.076	0.069	0.074	0.088
M9-02	0.133	0.125	0.188	0.186	0.204	0.103	0.118	0.119	0.178	0.326	0.183	0.107	0.118	0.219
M10-02	0.109	0.103	0.164	0.162	0.180	0.085	0.098	0.096	0.156	0.307	0.160	0.096	0.103	0.200
M10-10	0.129	0.122	0.179	0.177	0.197	0.097	0.116	0.111	0.170	0.305	0.176	0.099	0.112	0.210
M11	0.098	0.096	0.136	0.139	0.153	0.085	0.086	0.091	0.133	0.276	0.135	0.111	0.112	0.183
M15	0.069	0.055	0.066	0.075	0.079	0.056	0.059	0.053	0.056	0.209	0.062	0.086	0.086	0.061
M16-02	0.242	0.219	0.243	0.233	0.269	0.200	0.223	0.209	0.218	0.409	0.225	0.166	0.185	0.096
M16-08	0.257	0.236	0.261	0.248	0.287	0.219	0.240	0.229	0.235	0.418	0.243	0.193	0.199	0.107
M16-10	0.224	0.202	0.223	0.213	0.249	0.185	0.206	0.196	0.199	0.372	0.209	0.160	0.172	0.081

	M3-02	M3-10	M4-02	M4-10	M7-02	M7-08	M9-02	M10-02	M10-10	M11	M15	M16-02	M16-08
P1-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P3-02	-	-		-	-	-	-	-	-	-	-	-	-
P7-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P7-10	-	-	-	-	-	-	-	-	-	-	-	-	-
P8-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P12-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P13-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P14-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P15-04	-	-	-	-	-	-	-	-	-	-	-	-	-
P16-04	-	-	-	-	-	-	-	-	-	-	-	-	-
P17-04	-	-	-	-	-	-	-	-	-	-	-	-	-
P18-02	-	-	-	-	-	-	-	-	-	-	-	-	-
P18-08	-	-	-	-	-	-	-	-	-	-	-	-	-
M1-02	-	-	-	-	-	-	-	-	-	-	-	-	-
M3-02	-	-	-	-	-	-	-	-	-	-	-	-	-
M3-10	0.088	-	-	-	-	-	-	-	-	-	-	-	-
M4-02	0.178	0.232	-	-	-	-	-	-	-	-	-	-	-
M4-10	0.195	0.175	0.113	-	-	-	-	-	-	-	-	-	-
M7-02	0.136	0.181	0.228	0.232	-	-	-	-	-	-	-	-	-
M7-08	0.105	0.142	0.188	0.189	0.021	-	-	-	-	-	-	-	-
M9-02	0.217	0.260	0.328	0.323	0.141	0.083	-	-	-	-	-	-	-
M10-02	0.192	0.236	0.303	0.298	0.117	0.066	0.007	-	-	-	-	-	-
M10-10	0.206	0.248	0.304	0.303	0.128	0.084	0.039	0.036	-	-	-	-	-
M11	0.156	0.201	0.257	0.259	0.111	0.066	0.008	0.008	0.042	-	-	-	-
M15	0.082	0.130	0.172	0.184	0.023	0.017	0.107	0.090	0.103	0.093	-	-	-
M16-02	0.280	0.326	0.393	0.391	0.168	0.138	0.268	0.246	0.256	0.226	0.098	-	-
M16-08	0.297	0.341	0.395	0.395	0.186	0.155	0.279	0.260	0.270	0.241	0.111	0.015	-
M16-10	0.257	0.303	0.348	0.352	0.154	0.127	0.250	0.229	0.239	0.212	0.090	0.019	0.016

Table S1.5 Heatmap of paired Fst values for each site in the 42loci-12sites dataset. Values in green represents low genetic differentiation, values in red represents higher genetic differentiation.

	P1-02	P7-10	P15-04	P16-04	P18-08	M3-10	M3-13	M4-10	M7-08	M7-14
P1-02	-	-	-	-	-	-	-	-	-	-
P7-10	0.184	-	-	-	-	-	-	-	-	-
P15-04	0.150	-0.001	-	-	-	-	-	-	-	-
P16-04	0.280	0.186	0.213	-	-	-	-	-	-	-
P18-08	0.004	0.143	0.117	0.160	-	-	-	-	-	-
M3-10	0.190	0.024	0.039	0.251	0.136	-	-	-	-	-
M3-13	0.171	0.036	0.054	0.285	0.167	0.031	-	-	-	-
M4-10	0.223	0.100	0.077	0.350	0.171	0.084	0.084	-	-	-
M7-08	0.136	0.202	0.187	0.271	0.081	0.217	0.189	0.255	-	-
M7-14	0.124	0.229	0.208	0.302	0.132	0.237	0.237	0.297	0.001	-
M8-14	0.163	0.233	0.222	0.378	0.161	0.227	0.280	0.312	0.035	0.091
M9-13	0.133	0.248	0.235	0.315	0.092	0.256	0.261	0.305	0.118	0.113
M10-03	0.113	0.260	0.244	0.293	0.152	0.263	0.262	0.330	0.107	0.133
M10-10	0.130	0.263	0.241	0.262	0.132	0.267	0.253	0.314	0.123	0.148
M16-03	0.300	0.348	0.322	0.498	0.264	0.360	0.369	0.408	0.180	0.230
M16-06	0.289	0.340	0.307	0.460	0.244	0.356	0.347	0.392	0.176	0.217
M16-08	0.308	0.356	0.327	0.490	0.264	0.370	0.366	0.412	0.195	0.240
M16-10	0.293	0.343	0.315	0.452	0.243	0.360	0.341	0.393	0.188	0.225
M16-13	0.283	0.336	0.310	0.488	0.264	0.343	0.366	0.392	0.178	0.228

	M8-14	M9-13	M10-03	M10-10	M16-03	M16-06	M16-08	M16-10	M16-13
P1-02	-	-	-	-	-	-	-	-	-
P7-10	-	-	-	-	-	-	-	-	-
P15-04	-	-	-	-	-	-	-	-	-
P16-04	-	-	-	-	-	-	-	-	-
P18-08	-	-	-	-	-	-	-	-	-
M3-10	-	-	-	-	-	-	-	-	-
M3-13	-	-	-	-	-	-	-	-	-
M4-10	-	-	-	-	-	-	-	-	-
M7-08	-	-	-	-	-	-	-	-	-
M7-14	-	-	-	-	-	-	-	-	-
M8-14	-	-	-	-	-	-	-	-	-
M9-13	0.155	-	-	-	-	-	-	-	-
M10-03	0.168	-0.019	-	-	-	-	-	-	-
M10-10	0.172	0.014	0.003	-	-	-	-	-	-
M16-03	0.278	0.308	0.332	0.324	-	-	-	-	-
M16-06	0.252	0.302	0.319	0.319	0.009	-	-	-	-
M16-08	0.275	0.305	0.338	0.334	0.014	0.015	-	-	-
M16-10	0.242	0.302	0.323	0.323	0.010	0.010	0.013	-	-
M16-13	0.283	0.296	0.320	0.311	0.022	0.024	0.012	0.015	-

Table S1.6 Estimated recent migration rates among sites in the Paria and the Marianne rivers, estimated with BAYESASS, from the 10loci-20sites dataset. Values underlined and in bold differ significantly from zero, based on 95% credible intervals. Values in diagonal represent the proportions of non-immigrant individuals at each location.

To/From	P1	P3	P7	P8	P12	P13	P14	P15	P16	P17	P18	M1	M3	M4	M7	M9	M10	M11	M15	M16
P1	**0.8854**	0.0058	0.0058	0.0057	0.0058	0.008	0.0057	0.0058	0.0058	0.0057	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0082	0.0059	0.0058	0.0058
P3	**0.1957**	**0.6723**	0.0056	0.0056	0.0058	0.0283	0.0057	0.0055	0.0056	0.0058	0.0128	0.0056	0.0055	0.0055	0.0056	0.0056	0.0065	0.0056	0.0058	0.0056
P7	0.0055	0.0057	**0.8477**	0.0067	0.0055	0.0055	0.0055	0.0065	0.0056	0.0054	0.0111	0.0055	0.0309	0.0193	0.0057	0.0056	0.0055	0.0055	0.0056	0.0055
P8	0.0057	0.0056	0.0079	**0.8842**	0.0055	0.0054	0.0056	0.013	0.0055	0.0053	0.0057	0.0055	0.0058	0.0057	0.0058	0.0056	0.0057	0.0056	0.0055	0.0053
P12	0.0108	0.0058	0.0056	0.0056	**0.6772**	**0.212**	0.006	0.0059	0.0056	0.0056	0.0076	0.0057	0.0058	0.0056	0.0057	0.0058	0.0063	0.0057	0.0058	0.0057
P13	0.0157	0.0055	0.0182	0.0059	0.0063	**0.8523**	0.0057	0.021	0.0055	0.0055	0.006	0.0057	0.0073	0.0056	0.0056	0.0058	0.0057	0.0055	0.0057	0.0056
P14	0.0063	0.0056	0.0056	0.0055	0.0135	**0.2128**	**0.6734**	0.0055	0.0056	0.0055	0.0098	0.0056	0.0056	0.0055	0.0056	0.0055	0.0058	0.0056	0.0063	0.0055
P15	0.0051	0.0052	0.0127	0.0155	0.0051	0.0055	0.005	**0.8723**	0.0103	0.0051	0.0052	0.0051	0.0082	0.0093	0.0051	0.0049	0.0051	0.0051	0.0052	0.0051
P16	0.0054	0.0056	0.0056	0.0054	0.0057	0.0056	0.0054	0.0057	**0.8946**	0.0055	0.0057	0.0058	0.0055	0.0055	0.0056	0.0053	0.0053	0.0056	0.0055	0.0057
P17	0.0057	0.0055	**0.1419**	0.0068	0.0055	0.0055	0.0056	**0.0874**	0.0055	**0.6723**	0.0054	0.0057	0.0079	0.0057	0.0056	0.0055	0.0056	0.0056	0.0057	0.0054
P18	0.0052	0.005	0.005	0.0051	0.0052	0.0148	0.0052	0.0048	0.005	0.005	**0.8949**	0.0048	0.0049	0.0049	0.005	0.005	0.005	0.005	0.0051	0.005
M1	0.0055	0.0056	0.0056	0.0054	0.0054	0.0058	0.0057	0.0056	0.0055	0.0056	0.0056	**0.8927**	0.0055	0.0055	0.0053	0.0058	0.0056	0.0054	0.0057	0.0073
M3	0.0056	0.0055	0.009	0.0061	0.0055	0.0055	0.0056	0.0061	0.0054	0.0057	0.0056	0.0056	**0.889**	0.0065	0.0055	0.0057	0.0055	0.0056	0.0055	0.0056
M4	0.0056	0.0055	0.0054	0.0057	0.0056	0.0054	0.0056	0.0055	0.0056	0.0055	0.0055	0.0056	0.0056	**0.8943**	0.0054	0.0057	0.0057	0.0056	0.0058	0.0054
M7	0.0057	0.0056	0.0057	0.0071	0.0056	0.0071	0.0055	0.0057	0.0055	0.0058	0.0056	0.0075	0.0056	0.0057	**0.8647**	0.0055	0.0227	0.0057	0.012	0.0058
M9	0.0055	0.0054	0.0054	0.0053	0.0054	0.0053	0.0054	0.0055	0.0054	0.0054	0.0054	0.0054	0.0055	0.0054	0.0053	**0.6721**	**0.2304**	0.0054	0.0055	0.0055
M10	0.0058	0.0055	0.0056	0.0055	0.0055	0.0056	0.0056	0.0056	0.0057	0.0056	0.0058	0.0056	0.0055	0.0054	0.0056	0.0057	**0.8936**	0.0055	0.0055	0.0055
M11	0.0057	0.0057	0.0057	0.0056	0.0058	0.0056	0.0057	0.0057	0.0062	0.0056	0.0059	0.0057	0.0057	0.0058	0.0057	0.0056	**0.2178**	**0.6723**	0.0125	0.0056
M15	0.0056	0.0059	0.0055	0.0056	0.0057	0.0057	0.0058	0.0056	0.0056	0.0055	0.0067	0.006	0.0057	0.0056	0.013	0.0056	0.0068	0.0057	**0.8829**	0.0057
M16	0.0056	0.0056	0.0054	0.0056	0.0056	0.0053	0.0056	0.0056	0.0056	0.0054	0.0054	0.006	0.0055	0.0057	0.0055	0.0057	0.0057	0.0057	0.0057	**0.8938**

Table S1.7 Estimated recent migration rates among sites in the Paria and the Marianne rivers, estimated with BAYESASS, from the 42loci-12sites dataset. Values underlined and in bold differ significantly from zero, based on 95% credible intervals. Values in diagonal represent the proportions of non-immigrant individuals at each location.

To/From	P1	P7	P15	P16	P18	M3	M4	M7	M8	M9	M10	M16
P1	**0.9239**	0.0079	0.0066	0.0066	0.0067	0.0067	0.0068	0.0068	0.0067	0.0065	0.0084	0.0065
P7	0.0055	**0.6722**	**0.2649**	0.0056	0.0055	0.0131	0.0058	0.0056	0.0055	0.0055	0.0055	0.0054
P15	0.0072	**0.2546**	**0.6738**	0.0072	0.0071	0.0072	0.0071	0.0072	0.0072	0.0072	0.0071	0.0071
P16	0.0085	**0.2408**	0.0083	**0.6751**	0.0084	0.0083	0.0086	0.0084	0.0084	0.0084	0.0084	0.0084
P18	**0.2064**	0.009	0.009	0.0089	**0.6756**	0.009	0.0089	0.0091	0.0103	**0.0359**	0.0089	0.0089
M3	0.0053	**0.2749**	0.0053	0.0053	0.0053	**0.672**	0.0053	0.0053	0.0054	0.0053	0.0053	0.0053
M4	0.0061	**0.2666**	0.006	0.0061	0.0062	0.006	**0.6728**	0.006	0.006	0.0061	0.0061	0.006
M7	0.0058	0.0056	0.0054	0.0055	**0.0392**	0.0054	0.0057	**0.8246**	**0.0299**	0.0054	**0.0618**	0.0057
M8	0.0107	0.0108	0.0108	0.0106	**0.0962**	0.0108	0.0108	**0.1295**	**0.6774**	0.0109	0.0107	0.0108
M9	0.0257	0.0257	0.0259	0.0252	0.0256	0.0257	0.0254	0.0256	0.0257	**0.6923**	0.0514	0.0258
M10	0.0056	0.0054	0.0051	0.0053	0.0183	0.0055	0.0054	0.0055	**0.0367**	0.0056	**0.8962**	0.0053
M16	0.0055	0.0058	0.0057	0.0059	0.0058	0.0058	0.0059	0.0059	0.0173	0.0057	0.0057	**0.9252**

Table S1.8 Mean divergence time estimates from pairwise comparison of locations across watersheds, calculated using DIYABC. 95% credible interval are given in parenthesis.

Pairwise comparison	Divergence time (years)
Upstream Marianne - Upstream Paria	
M3-P7	26.52 (6.28-74.4)
M4-P15	50.4 (8.36-172.8)
M4-P7	54 (8.64-194)
M3-P15	33.16 (6.76-94.8)
Upstream Marianne - Downstream Paria	
M3-P1	704 (79.6-3252)
M3-P16	303.6 (27.48-1732)
M3-P18	532 (59.2-2588)
M4-P1	748 (251.2-3644)
M4-P16	452 (40-2616)
M4-P18	460 (49.2-2404)
Petite Marianne - Downstream Paria	
M9-P1	265.6 (88.4-988)
M9-P16	912 (89.2-4480)
M9-P18	652 (94.4-2552)
M10-P1	255.6 (32.56-1024)
M10-P16	756 (72-3792)
M10-P18	624 (86.4-2516)
Petite Marianne - Upstream Paria	
M9-P15	1316 (170.8-5120)
M9-P7	1436 (183.6-5480)
M10-P15	988 (120.4-3988)
M10-P7	936 (102-4240)
Downstream Marianne - Upstream Paria	
M7-P1	572 (74.8-2480)
M7-P16	1092 (112.4-4880)
M7-P18	1296 (724-4200)
M8-P1	2296 (1284-6680)
M8-P16	2568 (408-7000)
M8-P18	1900 (344-5760)
Downstream Marianne - Upstream Paria	
M7-P15	1812 (266.4-5840)
M8-P15	2736 (472-6960)
M7-P7	1952 (277.6-6160)
M8-P7	2908 (488-7240)
Marianne M16 - All Paria	
M16-P1	3088 (572-7200)
M16-P15	2440 (359.6-6800)
M16-P16	2972 (504-7160)
M16-P18	3324 (652-7360)
M16-P7	2192 (317.2-6640)

Linking Statement to Chapter 2

In my first chapter, I described the population genetic structure of guppies located in two watersheds in northern Trinidad. I found two locations of either historical or contemporary gene flow accross the two watersheds. The datasets I used in this study sometimes contained multiple years for the same sites, and in addition to the main results discussed in Chapter 1, my analyses also revealed varying levels of admixture and gene flow among years. This temporal variation raised a question that is not often addressed in population genetic studies: Is gene flow stable over time and are genetic and phenotypic variation resistant and/or resilient to disturbances?

In my next chapter, I studied some of the same sites, and thanks to a long-term sampling conducted by lab members over more than 15 years, I genotyped guppies in four different years. These time points were chosen because they are immediately before and after two massive flood events that happened in March 2005 and December 2016. These intense disturbances gave me the opportunity to test the stability (i.e. the resistance and resilience) of the population genetic structure of our sampling sites through time.

CHAPTER 2: Resistance and resilience of guppy genetic and phenotypic diversity to "black swan" flood events.[§]

Abstract

Rare extreme "black swan" disturbances can impact ecosystems in many ways, such as destroying habitats, depleting resources, and causing high mortality. In rivers, for instance, exceptional floods that occur infrequently (e.g., so-called "50-year floods") can strongly impact the abundance of fishes and other aquatic organisms. Beyond such ecological effects, these floods could also impact intraspecific diversity by elevating genetic drift or dispersal and by imposing strong selection, which could then influence the population's ability to recover from disturbance. And yet, natural systems might be resistant (show little change) or resilient (show rapid recovery) even to rare extreme events – perhaps as a result of selection due to past events. We considered these possibilities in two rivers where native guppies experienced two extreme floods - one in 2005 and another in 2016. For each river, we selected four sites and used archived "historical" samples to compare levels of genetic and phenotypic diversity before versus after floods. Genetic diversity was represented by 33 neutral microsatellite markers, and phenotypic diversity was represented by body length and male melanic (black) color. We found that genetic diversity and population structure was mostly *resistant* to even these extreme floods; whereas the larger impacts on phenotypic diversity were short-lived, suggesting additional *resilience*. We discuss the determinants of these two outcomes for

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guppies facing floods, and then consider the general implications for the resistance and resilience of intraspecific variation to black swan disturbances.

Introduction

A classic approach in evolutionary biology is the interrogation of spatial patterns of genetic or phenotypic variation. The patterns documented through such surveys are frequently used to infer the role of various evolutionary forces, such as natural selection, sexual selection, gene flow, or drift (Endler, 1986; MacColl, 2011; Schluter, 2000). More recently, increasing effort has been directed toward the inclusion of a temporal dimension into spatial-based inferences – either by examining year-to-year changes (e.g.,Siepielski, Dibattista, and Carlson 2009; Gotanda and Hendry 2014) or decadal-scale patterns (e.g., Parmesan & Yohe, 2003; Hendry *et al.*, 2008; Leigh *et al.*, 2019; Millette *et al.*, 2020). This integration has generally revealed that temporal variation can be important, but that it usually does not severely dampen spatial variation. Yet we contend that a key feature of temporal variation deserves more focused consideration. In particular, work on temporal variation often focuses on rather gradual environmental changes, whereas rare but extreme environmental disturbances might permanently impact previous inferences.

Rare but strong disturbances – sometimes called "black swan events" in analogy with the extreme rarity but striking appearance of such birds – can dramatically influence ecosystems and populations (Anderson et al., 2017; Fey et al., 2015). Examples of black swan events can include natural disasters such as hurricanes, earthquakes, storms, or floods that cause mass mortality or emigration (Ameca y Juárez et al., 2012). Several studies have evaluated the impact of black swan events from an ecological perspective by considering how ecosystems cope with disturbances (H. Mooney & Godron, 1983) – through either "resistance" or "resilience" (Pimm, 1984). The resistance of a system can be thought of as its tendency to avoid displacement from its original undisturbed state,

such as when organismal responses buffer the impact of a disturbance. The resilience of a system is defined as its tendency to return to the original state following an initially strong displacement (Pimm, 1984), such as when large immediate changes in community structure later rebound to near the initial state. Of course, these properties can be highly non-linear, such as when a system resists change until a "tipping point" of disturbance is reached that causes a rapid shift between alternative states (Dakos et al., 2019). Further, the resilience or resistance of a system might change as black swan events increase in frequency, as has been suggested to be particularly likely in the context of climate change (Manyena, 2006).

Most of the existing work on resistance or resilience to black swan events has been ecological in emphasis; that is, it focuses on how natural disturbances influence populations, communities, or ecosystems (Matthews et al., 2014; Reusch et al., 2005). For example, a number of studies quantify the magnitude of mortality in groups of species, and how those species then later recolonize or repopulate the environment and thus re-assemble into "normal" communities (Meffe, 1984; Minckley & Meffre, 1987). In contrast to such studies of inter-specific diversity, we have little understanding of how intra-specific diversity is impacted and recovers from natural disasters (Banks et al., 2013). In particular, rare extreme disturbances could have dramatic effects on phenotypic and genetic diversity through changes in natural selection, gene flow, or genetic drift. Some recent examples include contemporary evolution in response to hurricanes (Donihue et al., 2018) and extreme cold or hot weather (Campbell-Staton et al., 2017). Another particularly likely effect of natural disasters on intra-specific diversity is

expected to be seen in the meta-population genetic structure of some widely distributed species.

Meta-population genetic structure strongly reflects long term processes that consistently structure the movement of organisms and gametes across the landscape (Orsini et al., 2008). For instance, genetic differences tend to increase with increasing geographical distance (E. Crispo & Hendry, 2005; Wright, 1943) and as a result of physical barriers to movement, such as mountain ranges, rivers, waterfalls, or areas of unsuitable habitat (Erika Crispo et al., 2006; Gascon et al., 2000; Mcrae et al., 2005). However, extreme conditions can occasionally bridge these barriers, creating rafts of vegetation (Waters & Craw, 2018) or nonbiodegradable objects (Carlton et al., 2017). The resulting sudden – even if only temporary – increases of gene flow could disrupt the stability of population structure and have long term effects on neutral or adaptive genetic and phenotypic variation.

The specific context for which we will examine the effects of extreme events on meta-population structure is the occurrence of relatively rare intense floods on fish populations in rivers. Floods generate very high flows that greatly disturb the system state by displacing organisms from their current positions, changing the physical arrangement of the riverbed, carrying mud and debris, and causing high mortality (Resh et al., 1988). Of course, rivers are naturally dynamic systems that experience regular high-flow periods (Lake, 2000; Resh et al., 1988), including in tropical rivers where annual rainfall can be greater than 700 mm/year (Latrubesse et al., 2005). As a result, many organisms in systems subject to periodic high floods show particular adaptations to flood regimes (Lytle and Poff 2004). Yet some floods are truly exceptional – becoming major black-

swan disturbances whose frequency and intensity deviate far from the normal. Examples include sudden flash floods that happen during a dry season (Weese et al., 2011) or exceptionally strong 100-year spring freshets in temperate systems (Matthews et al., 2014). Our study considers how such black swan temporal disturbances might influence typical spatial patterns that reflect longer-term processes.

The spatial meta-population structure of guppies (*Poecilia reticulata*) in the Northern Range mountains of Trinidad is strongly dictated by geographical distance and by waterfalls (Alexander et al., 2006; Barson et al., 2009; Carvalho et al., 1991; Erika Crispo et al., 2006; Fajen & Breden, 1992; Shaw et al., 1994; Willing et al., 2010). For instance, genetic diversity is lower in upstream areas, especially when upstream populations are separated from downstream populations by waterfalls. Similarly, genetic differences between guppy populations are greater when they are separated by greater distances and by waterfalls. In short, guppy population structure is strongly determined by abiotic physical features that limit gene flow in the upstream direction. At the same time, gene flow in the downstream direction is limited by biotic factors, especially the general tendency of guppies to show positive rheotaxis, where they orient and swim upstream in a current (Blondel et al., 2020; Mohammed et al., 2012). Yet this biotic resistance to the effects of water flow are sensitive to rare perturbations, such as stream capture events and human-mediated introductions (Becher & Magurran, 2000; Blondel et al., 2019). Another such perturbation could be extreme floods, which are rare but potentially catastrophic.

Guppies in the Northern Range mountains experience regular moderate flooding events during the wet season (Magurran, 2005). The system is clearly resistant to these normal events given the universal documentation of genetic differences associated with

distance and waterfalls (Barson et al., 2009; Crispo et al., 2006; Shaw et al., 1994) – but is the system also resistant – or resilient - to the much larger floods that occur at rare intervals? Over our continuous 20 years of work in Trinidad, our study sites have experienced two extreme floods that had devastating effects on stream ecosystems. Owing to the very local nature of the downpours that cause such floods, they are not always evident on national-level meteorological or hydrological records – but rather are recorded in local newspaper accounts. As an example, on December 2016, the *Trinidadian Guardian Newspaper* reported "torrential rains" that destroyed houses and caused several landslides. At the same time, the *Loop T&T* reported rivers having changed their courses and flowing through properties. To these attestations from the media, we can add our own personal records and photographs (Figure S1, Figure S2, Figure S3).

In our study system, we suggest that signatures of *resistance* would appear as only minor (if any) changes in genetic diversity and phenotypic diversity after major floods, combined with overall stability of meta-population structure from before to after such floods. (Throughout this manuscript, "diversity" is used generally to imply variation within or among sites – with distinctions between those levels being noted where relevant.) Finding these signatures could be consistent with either (1) the floods having only minor demographic effects (which we can rule out – see below), (2) the floods having large demographic effects but still not large enough (or selective enough) to have genetic or phenotypic consequences (Banks et al., 2013), or (3) existing adaptation to this kind of disturbance (Lytle & Poff, 2004). If, by contrast, major floods had notable genetic

or phenotypic effects (i.e., low *resistance*), signatures of *resilience* would then appear as a rapid return to pre-flood genetic and phenotypic patterns.

We tested for these signatures of resistance and resilience by studying genetic diversity, phenotypic traits, and population structure in eight guppy populations located in two watersheds – all experiencing intense floods in 2005 and again 2016. We leveraged spatial variation among these populations to search for the above signatures of resistance and resilience. On the one hand, high resistance to floods would predict the maintenance of typical patterns: that is, low genetic diversity in guppies at isolated sites (i.e., upstream sites and/or above waterfalls) and their strong differentiation from guppies at other sites. On the other hand, low resistance would predict admixture from upstream and isolated populations into downstream populations, which would modify levels and patterns of genetic and phenotypic diversity. In the case of such low resistance, resilience would then predict a return to original genetic and phenotypic diversity levels and patterns after the flood.

Methods

Sampling

The guppies that we genotyped were part of a long-term sampling effort that started in 2002, during which guppies were caught in the same sites each year in February or March. The guppies were captured with butterfly nets, transported to our laboratory in Trinidad, euthanized with MS222, photographed (details below), and preserved in 95% ethanol. For the present study, we genotyped fish from our archived ("historical") samples preserved in 2004, 2005, 2016, and 2017 from each of eight sites in the

Marianne and Paria Rivers on the north slope of the Northern Mountain Range (Figure 1). We chose these specific years so as to bracket the intensity of two extreme floods – one that took place between January and March 2005 (Weese et al., 2011) and one that took place in December 2016 (pictures of before vs. after floods in Supplementary Materials). Although we did not directly measure population size before versus after floods, the black swan nature of these two events was clearly evident. For instance, Weese et al. (2011) reported that the 2005 flood reduced population sizes by "several orders of magnitude", and our catch-per-unit-effort (personal observations) decreased dramatically after the 2016 flood. Further, localized floods in other locations have been shown to have substantial effects on guppy demography (Grether et al., 2001). In each of the two rivers, we chose one site located in the upstream reaches of the main stem, one site located in the downstream reaches of the main stem, one site isolated above a waterfall, and one site in a tributary but not above a waterfall (Figure 2.1).

Genotypes

All genotyping was conducted at Dalhousie University. We extracted DNA using a modified glassmilk protocol (Elphinstone et al., 2003) from fin clips of 1221 ethanol-preserved guppies (see Table S2.1 for sample sizes). We genotyped 43 microsatellite loci following the procedures described in Zhan et al. (2017). In brief, we performed two PCRs: 43 loci were amplified in a first multiplex PCR and then we added the indexing sequences to the PCR products in a second PCR. Finally, we sequenced pooled microsatellite amplicons with Illumina MiSeq. Genotypes were scored using the software MEGASAT (Zhan et al., 2017).

All statistical analyses were performed in R using the R studio environment (R Core Team, 2018; RStudio Team, 2016), unless otherwise specified. We tested for departures from Hardy-Weinberg equilibrium in each site in each year at each locus using the *pegas* package (Paradis, 2010), with a sequential Bonferroni correction. We did not test for linkage disequilibrium as these particular loci have been specifically developed to be distributed across the guppy genome (NCBI BioProject PRJNA238429). Gene diversities (Nei, 1973) were calculated using the package *poppr* (Kamvar et al., 2014) for each sampling site in each year. This measure is similar to expected heterozygosity and represents a quantitative estimate of the genetic diversity within a population. We then used the function Hs.test from the package *adegenet* (Jombart, 2008) to test for differences in gene diversity before versus after each flood event for each sampling site. For this test, "individuals are randomly permuted between groups to obtain a reference distribution of the test statistics" (Jombart, 2008), and we did so with 999 permutations for each test followed by sequential Bonferroni correction. Allelic richness in each sampling site in each year was calculated using the allelic.richness function in the *Hierfstat* package (Goudet, 2005). We then built a linear model with allelic richness as the response variable and site (8 levels), flood (2 levels: before and after), and their interaction as explanatory variables. All pairwise F_{ST} comparisons were made using the pairwise.ft function in the *Hierfstat* package (Goudet, 2005). The inbreeding coefficient (F_{IS}) for each individual at each sampling site was calculated using the inbreeding function from the *adegenet* package (Jombart, 2008). We then built a linear model with F_{IS} as the response variable and the interaction between site and flood as explanatory variables.

We used Bayesian clustering in STRUCTURE (version 2.3.4; Pritchard *et al.*, 2000) to analyze genetic population structure. This method inferred the most probable number of clusters (*K*) that describe the genotypic distribution. We ran three iterations for each value of *K*, from 1 to 8 (the total number of sites). Burn-in length and run length were each set at 100,000 using the admixture model and the correlated alleles model. We then used the method of Evanno *et al.* (2005) to find the best *K*. We extracted the individual admixture data from the STRUCTURE results to compare admixture levels before versus after the floods. Here we used a linear model with admixture as the response variable and site, flood, and their interaction as explanatory variables. A log transformation was used to meet the assumption of homoscedasticity. We carried out post-hoc analysis on the levels of significant terms using the pairwise.t.test function in the *stats* package, with a Bonferroni correction.

Phenotypes

Reflecting improvements made to our methods over the 13 years of sampling, photographs of the fish were taken using different techniques in different years. In 2004, the fish were placed in groups of four or five on neutral-gray graph paper and photographed using a Nikon D100. In 2005, pictures were taken with the same camera or with a Nikon Coolpix 5400 but each fish was placed individually on a white background next to a color standard. For the 2016 and 2017 pictures, we used the same technique as in 2005 but with a Nikon D800 and D300 respectively.

For all fish in all years, we used the software Fiji (Schindelin et al., 2012) to measure body length as the distance between the anterior end of the mouth and the

anterior end of the caudal fin. Due to some missing pictures, these measurements excluded females from above the waterfall in the Marianne River in 2004, and males from above the waterfall in the Paria River in 2005. We then analyzed the variation in body length before and after floods using a general linear model. Body length was set as the response variable, and flood, site and sex were set as explanatory variables, as well as their interaction. A log transformation was used to meet the assumption of homoscedasticity.

To quantify male color, we used pictures from 2016 and 2017 only, due to lower photograph quality in 2004 and 2005. A color standard was used to standardize white balance across photos. For this color analysis, we used the package *patternize* (Van Belleghem et al., 2018) to quantify melanic color patterns for males: we analyzed melanic colors only because they were the mostly clearly and unambiguously identified by *patternize* and because previous work has shown melanic colors vary among our study sites (Millar et al., 2006). Each fish was landmarked in the program Fiji (Schindelin et al., 2012) using nine landmarks. Then, for each site, we selected black color and analyzed it using an RGB threshold. Briefly, the *patternize* package defines homology between pattern positions across specimens and categorize the distribution of colors using an RGB threshold (Van Belleghem et al., 2018). We then used the pixel coordinates of black color in a PCA to visualize changes in melanic color patterns before and after the December 2016 flood. We carried out post-hoc analysis on the levels of significant terms using the pairwise.t.test function in the *stats* package, with a Bonferroni correction.

Results

Of the 43 microsatellites loci that we genotyped, three were discarded owing to amplification/sequencing errors. We also removed seven loci with 100% missing data in one or more samples. Departures from Hardy-Weinberg equilibrium are visualized as a heatmap (Figure S2.4) and were randomly distributed among samples and loci – leading to no further exclusions. Thus, the final dataset for analysis used 33 loci. Total number of alleles per locus ranged from 3 to 23.

All analyses revealed high resistance of genetic population structure to extreme floods. First, of the 16 before-versus-after flood comparisons, only one indicated a significant shift in gene diversity. This lone exception was that of the downstream main stem of the Paria River, where gene diversity decreased after the 2016 flood (Table 2.1, Table 2.2). Second, no instances did we document a significant before versus after flood change in pairwise F_{ST} or F_{IS} (Table 2.2). Not surprisingly then, gene diversity differed 3 times more between sites in a given year than between years at a given site (Figure 2.2, Figure S2.5). Third, STRUCTURE analysis suggested an optimal number of clusters of K = 3 (Figure 2.3) both before and after floods – with assignment to a given cluster staying the same in all years.

Yet this general resistance to floods does not indicate a complete absence of effects. In particular, STRUCTURE revealed some changes in admixture after the floods at some of the sites (Figure 2.3). Two noteworthy – and opposing – effects are of particular interest. After the first flood, admixture increased in the site located in the downstream main stem of the Marianne River. Admixture was back to previous levels before the second flood,

suggesting the resilience of this population. After the second flood, admixture decreased in the site located in the downstream main stem of the Paria.

In contrast to genetic population structure, analyses of phenotypes showed more obvious immediate effects of floods, as well as some resilience from one flood to the second. In particular, body length systematically increased after floods in the site located upstream in the main stem of the Paria, for both males and females. (Table 2.3; Figure 2.4, Figure S2.6). Overall, size increases after a flood were evident at six of the eight sites for at least one of the sexes for at least one of the events (Figure 2.4; Figure S2.6). These increases – at least in the Paria – were tied to decreases in gene diversity (Figure 2.4). In the seven instances that size increased after the first flood (in the Marianne, males from the downstream main stem, downstream tributary and above waterfalls; in the Paria, males and females from the downstream tributary and downstream main stem; Figure S6), size went back to previous levels before the second flood. In contrast, body length decreased after the 2016 flood for males and females in the downstream main stem of the Paria, and for males in the downstream tributary of the Paria. Male melanic color patterns also showed some interesting shifts due to floods (Figure 2.5). In the Paria, for instance, melanic colors decreased after the second flood in the site located downstream in the main stem (Figure S2.7).

Discussion

Rare catastrophic “black swan” events can cause massive mortality and threaten population persistence (Anderson et al., 2017; Mangel & Tier, 1994). For example, major floods can disturb the physical habitats of rivers (Resh et al., 1988), reduce fish biomass

(Grether et al., 2001; Meffe, 1984), and dramatically perturb their population genetic structure (Apodaca et al., 2013; Erika Crispo & Chapman, 2009). For two major local flooding events in Trinidad, we found that the genetic and phenotypic diversity in guppies were mostly resistant, with minimal changes to pre-flood patterns (Table 2.1, Table 2.2; Figure 2.2, Figure 2.3). Some upstream-to-downstream dispersal was evident, but its effects were minor (resistance) and temporary (resilience). By contrast, phenotypic diversity was more heavily impacted in the short term but then mostly recovered – thus showing more resilience than resistance to these events. (Again, "diversity" is used in a general sense to be inclusive of within and among population variation and patterns.)

Resistance

After the two floods, patterns of genetic variation stayed relatively stable (Figure 2.3). That is, we did not observe major changes in genetic diversity or allelic richness (Table 2.1) and the increase in gene flow was minimal (Table 2.2). These results support previous findings in some other fishes that observe little change between pre- and post-flood values of genetic diversity, and minor to no downstream displacement after severe floods (Franssen et al., 2006; Plath et al., 2010; Pujolar et al., 2011). Several hypotheses can explain the strong resistance of these populations to such black swan events.

First, guppies and other stream fishes have evolved adaptations to cope with the effects of periodic high-water velocities. Behavioral mechanisms include hiding, maintaining their position in the stream, or orientating quickly and efficiently against the flow as evidenced in guppies (Blondel et al., 2020) and other fishes (David & Closs, 2002; Meffe, 1984). Adaptations can also be evident in life history traits, where

organisms synchronize reproduction events in season with low flow probability (Lytle & Poff, 2004). Although guppies reproduce all year long, Reznick (1989) found that females had fewer offspring during the wet season – the season with the highest probability of floods.

Second, it is possible that high levels of dispersal occurred but had little effect on genetic variation owing to high mortality overall – and especially for migrants. Indeed, strong adaptive divergence between upstream (often low-predation) and downstream (often high-predation) guppy populations (D. Reznick et al., 2001) suggests that migrants from the former to the latter would have minimal effects on the downstream populations. Indeed, this mechanism is supported by an experimental study of some of the same sites, which focused on the natural and sexual effects of selection against migrants after the first flood (Weese et al., 2011). In particular, in high predation environments, low-predation guppies had lower survival probability than high predation ones, and low predation males sired fewer offspring than high-predation ones.

Third, another potential explanation for the resistance we observed is that the floods were not strong enough – that is, they were more "normal" than at the "black swan" level. It is hard to be definitive in this regard owing to the lack of detailed hydrological monitoring. For example, we could not directly measure water discharge or flow velocity in each site, and the effects appear to be very localized. Yet, we don't think this is true considering the extent of the two floods that stood out in our 20 years of sampling these sites (Figures S2.1, S2.2, S2.3), and the large demographic effects that we observed. It does remain possible that the demographic effects were large but still not sufficient to have appreciable effects on diversity. For example, genetic effects might only be evident

when population sizes get below some extreme threshold (Peakall & Lindenmayer, 2006) and, even then, it might take some time for those effects to be manifest (Leigh et al., 2019; Millette et al., 2020; Stoffel et al., 2018). At the phenotypic level, the lack of observable effects might simply mean that the flood effects were non-selective, although that seems unlikely in guppies (Fitzpatrick et al., 2014; van Oosterhout et al., 2007), and for organisms that experience extreme climatic events in general (Coleman & Wernberg, 2020).

Although we generally observed strong signatures of resistance to floods in most of the guppy populations, one site – located downstream in the main stem of the Paria – was noticeably impacted. In this site, admixture decreased after both flood events (Figure 2.3). For instance, after the second flood, gene diversity decreased by 12.5% (Table 1), body length for males and females decreased by 25% (Figure 2.4) and melanic color patterns shifted towards less melanics (Figure 2.5, Figure S2.7). We hypothesize that, because this site is located at the junction of the main stem and of two tributaries, it usually (under non-flood conditions) represents a mix of local fish and immigrants from the upstream tributaries. In the case of a catastrophic flood, guppies from all locations are displaced and genetic diversity decreases – hence, contrary to the initially expected scenario, a major flood may have "purged" this site of the immigrants it normally harbors under more benign normal conditions. Major predators are lacking throughout the Paria River and so the above mentioned "selection against immigrants" might be minimal at this site in the absence of a flood.

Resilience

Although the guppy system seems mostly *resistant* to the floods, we could see some signatures of resilience emerging from temporal changes at some of our study sites. First, increased levels of admixture in the downstream main stem site in the Marianne after the first flood went down to pre-flood levels eleven years later (Figure 2.3). We explain this increase after the flood by elevated gene flow to this downstream site, likely receiving migrants from more upstream sites, thereby increasing the genetic signature of fish from another genetic cluster (Figure 2.3). However, this genetic signature didn't last, and admixture went back to pre-flood levels, which might be explained by selection against upstream migrants into downstream sites (Weese et al., 2011).

Body length showed low resistance to the two floods. In 6 out of the 8 sites, we found that, after floods, body size increased for either males, females or both (Figure 2.4; Figure S2.6). This result suggests that floods are selecting against small guppies. Several hypotheses could explain this finding. First, small size guppies could be less efficient at swimming against the flow, and thus more likely to be swept downstream. Second, small size guppies could be more sensitive to the effect of the floods and suffer from higher mortality than bigger guppies. Whenever these changes in body length happened during the first flood, guppy body length was back to pre-flood size before the second flood suggesting resilience of this phenotypic trait.

Resistance versus resilience

We found that most aspects of guppy population genetic structure were highly resistant to floods. This resistance might be only "apparent," such as if the disturbance

was not actually that severe (see above), although this seems unlikely because of the observed dramatic demographic effects (Weese *et al.*, 2011; personal observation). Hence, resistance was more likely due to mechanisms guppies have evolved that resist displacement from their home sites, including the avoidance of high current and the evolution of positive rheotaxis (Blondel et al., 2020). By contrast, *phenotypic* variation was not as resistant as genetic variation – which might be due to several factors. In particular, the genetic markers we used to infer population structure are neutral loci, which can be subject to a time lag between disturbance and effect (Epps & Keyghobadi, 2015). On the contrary, phenotypic traits are a product of the interaction between genes and the environment and might be more likely to reflect any immediate disturbance, either because of differential selection or phenotypic plasticity (Labonne & Hendry, 2010).

Generally, we suggest that biological systems are likely to converge on either strong resistance with little resilience or vice versa. The reason is that, if a system is highly resistant, then little opportunity exists to manifest resilience: that is, if no change occurs due to disturbance (thus high resistance) then recovery from disturbance (high resilience) is moot. Importantly, this argument applies at the whole-system level. Individuals, by contrast, still could be experience natural selection for traits that promote resilience and, thus, a displaced individual could still manifest behaviors that enhance resilience. In cases where a system is not very resistant (a disturbance causes large genetic or phenotypic change), those individual behaviors that enhance resilience (e.g., return to home site) then could be important in generating resilience – as could ongoing selection after the disturbance. Given this expected "trade-off" *at the system level* (either

resistance or resilience but probably not both), we might ask what sorts of systems will converge on resistance or resilience? One possibility favoring resistance might be constraints (such as waterfalls) that limit the potential for resilience. That is, if a guppy is washed over a waterfall, it can't very well just swim back. One possibility favoring resistance might be the rapidity of potential recovery response – as argued above for phenotypic traits as opposed to neutral markers. We urge further work on the balance between resistance and resilience of genetic and phenotypic variation in responses to such determinants.

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We thank all field assistants and students for guppy collection over the years, especially Sandra Klemet-N'Guessan, Adam Reddon and Vincent Galloy who hiked through landslides to sample sites after the December 2016 flood. We thank three anonymous reviewers for their comments, which improved the manuscript. Permits for collection and exportation were obtained from the Ministry of Agriculture, Land and Fisheries of Trinidad. Fish handling was in compliance with a McGill Animal Use Protocol. This study was funded by FRQNT TEAM grants to Marilyn Scott, Gregor Fussmann and Andrew Hendry; as well as NSERC Discovery grants to Andrew Hendry and Paul Bentzen. Léa Blondel was funded by the BESS NSERC CREATE grant and by a Delise Alison Award from the Redpath Museum at McGill University.

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Table 2.1 Measures of Nei's gene diversity (1973) and allelic richness for each sampling site in each year. Significant differences after a sequential Bonferroni correction, before versus after a flood (2004 versus 2005 or 2016 versus 2017), are displayed in bold.

River	Category	Gene diversity				Allelic richness			
		2004	**2005**	**2016**	**2017**	**2004**	**2005**	**2016**	**2017**
Marianne	Downstream main stem	0.51	0.52	0.50	0.53	3.25	3.25	3.09	3.39
	Downstream tributary	0.41	0.43	0.44	0.46	2.87	2.97	2.95	3.08
	Above Waterfall	0.39	0.33	0.32	0.32	2.77	2.62	2.45	2.47
	Upstream main stem	0.16	0.17	0.17	0.19	1.56	1.60	1.62	1.70
Paria	Downstream main stem	0.40	0.38	**0.40**	**0.35**	2.76	2.56	2.69	2.39
	Downstream tributary	0.40	0.38	0.37	0.38	2.66	2.56	2.56	2.56
	Above Waterfall	0.22	0.21	0.24	0.24	1.77	1.73	1.78	1.77
	Upstream main stem	0.29	0.29	0.30	0.29	2.13	2.17	2.26	2.16

Table 2.2 Output of the linear models between the several genetic diversity measures and flood, site, as well as their interaction as explanatory variables. Significant terms are displayed in bold.

Explanatory variable	Df	*F*	*P*
F_{IS}			
Flood	1	0.08	0.774
Site	7	1.75	0.094
Site x Flood	7	0.60	0.757
F_{ST}			
Flood	1	2.23	0.138
Pair of sites	55	24.02	**<0.001**
Flood x Pair of sites	55	1.25	0.157
Allelic Richness			
Flood	1	0.05	0.829
Site	7	12.12	<**0.001**
Site x Flood	7	0.27	0.967
Genetic Admixture			
Flood	1	1.00	0.318
Site	7	50.15	<**0.001**
Site x Flood	7	6.79	<**0.001**

Table 2.3 Output of the linear model for body length, with site, flood, sex, and their interaction as explanatory variables. Significant terms are displayed in bold.

Explanatory variable	**Df**	***F***	***P***
Site	7	48.22	**<0.001**
Flood	1	0.00	0.991
Sex	1	65.66	**<0.001**
Site x Flood	7	18.84	**<0.001**
Site x Sex	7	6.50	**<0.001**
Flood x Sex	1	0.64	0.424
Site x Flood x Sex	7	5.09	**<0.001**

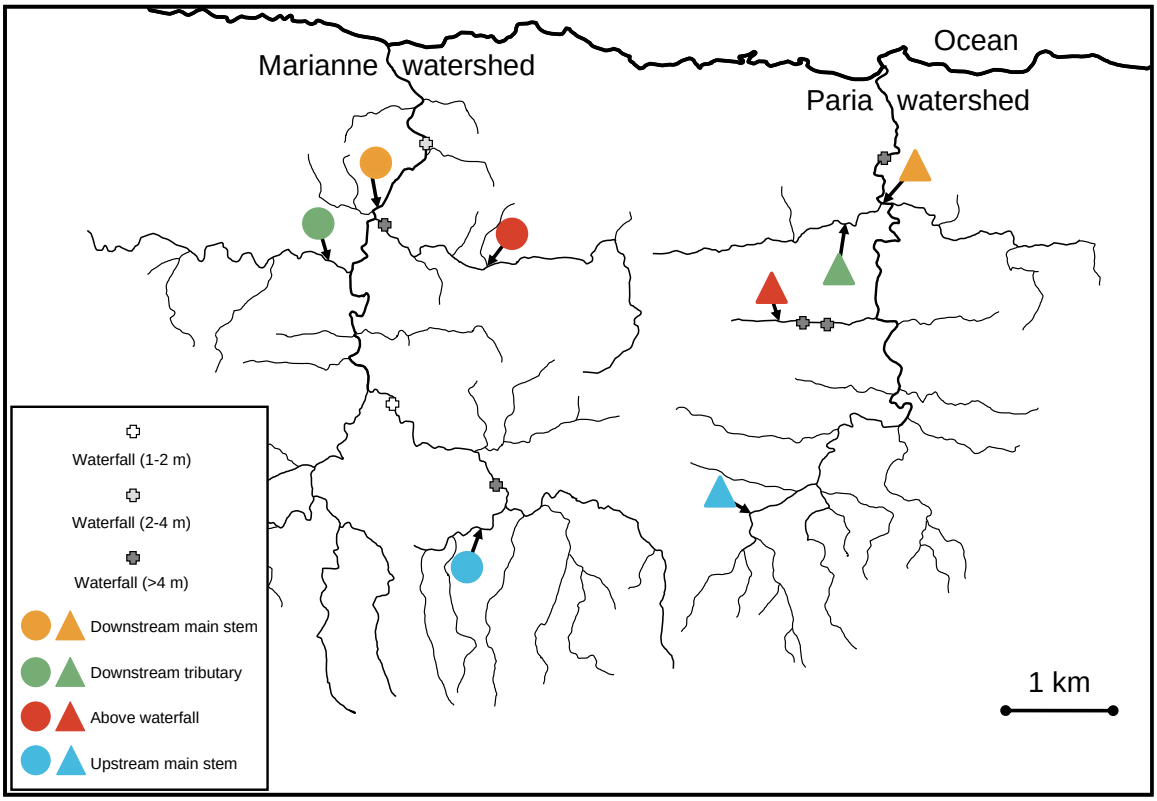


Figure 2.1 Location of sampling sites in the Marianne River (circles) and the Paria River (triangles). In each river, we sampled a site located in the downstream main stem (yellow), a downstream tributary (green), above a waterfall (red), and in the upstream main stem (blue).

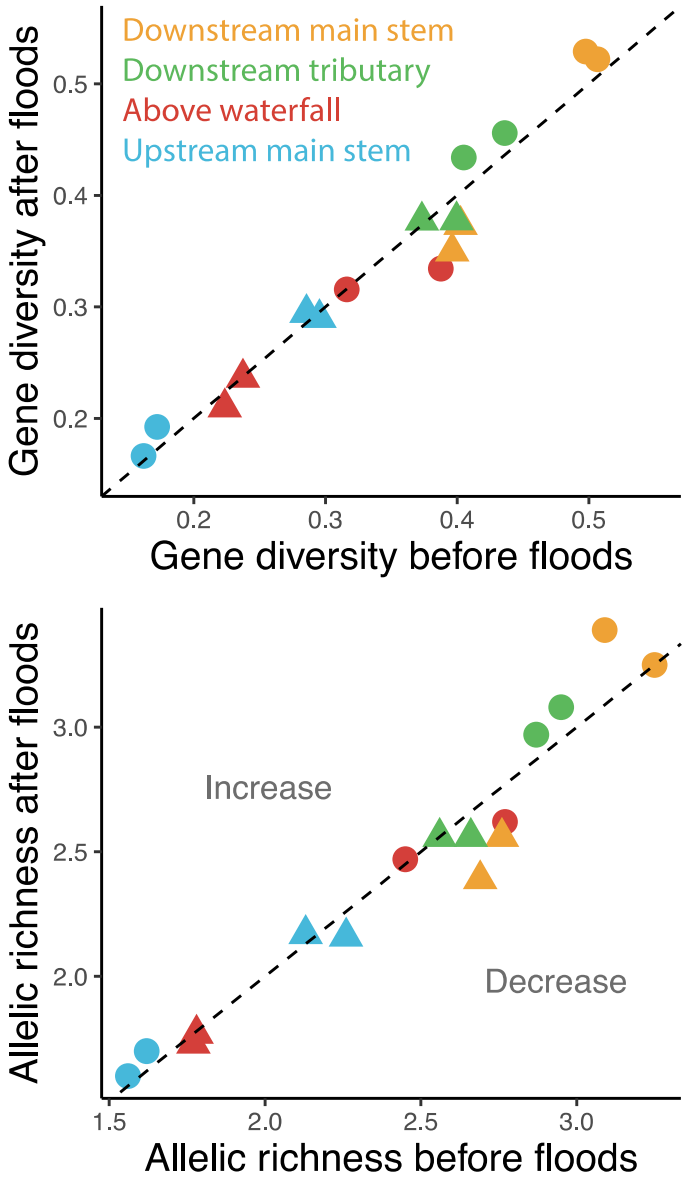


Figure 2.2 Nei's gene diversity (upper panel) and allelic richness (lower panel) before versus after floods for each site category in the Marianne River (circles), and in the Paria River (triangles). Each data point corresponds to either the 2005 flood or the 2016 flood. The one-to-one line represents the case of no change due to a flood. Points above the line represent an increase after a flood and points below the line represent a decrease after a flood.

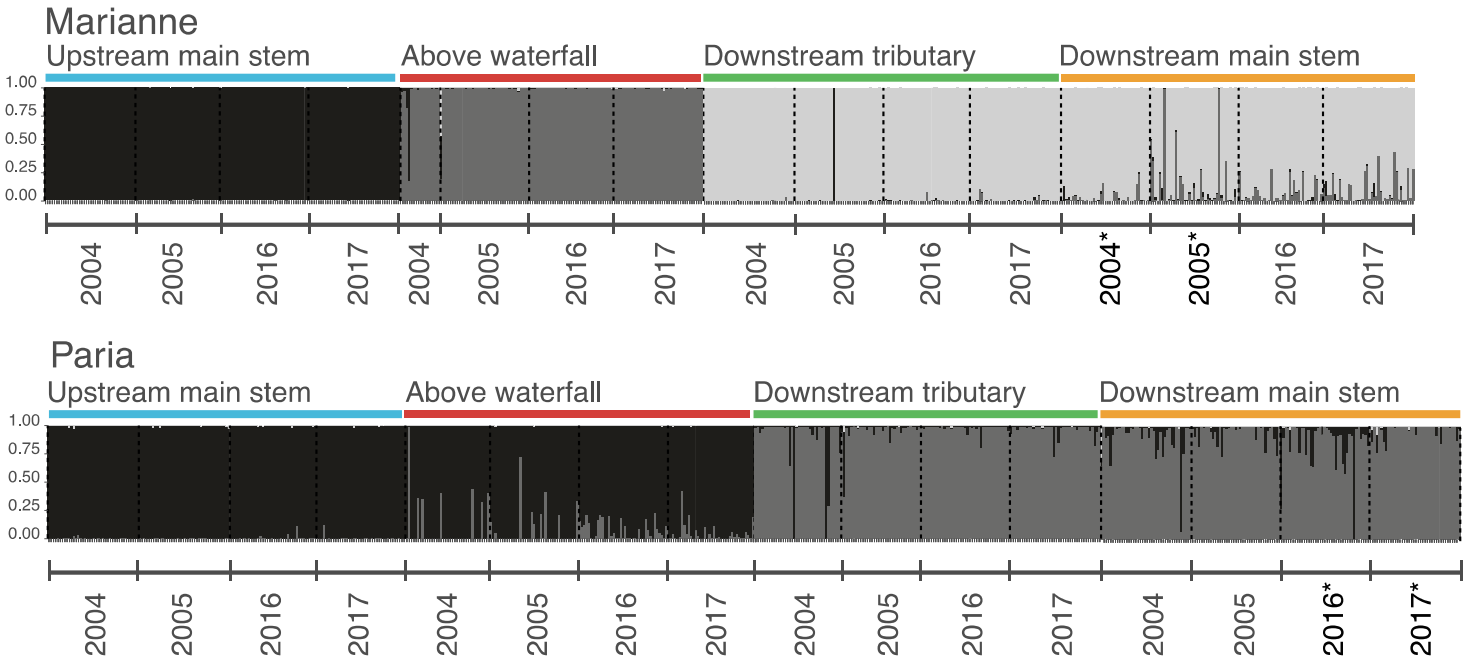


Figure 2.3 Results of the STRUCTURE analysis. Every vertical line represents an individual and is shaded according to its cluster assignment. The optimal number of clusters was $K = 3$. When an individual bar shows multiple shades, it reflects individual admixture among clusters. Significant increases or decreases in admixture between years are indicated by an asterisk following the year.

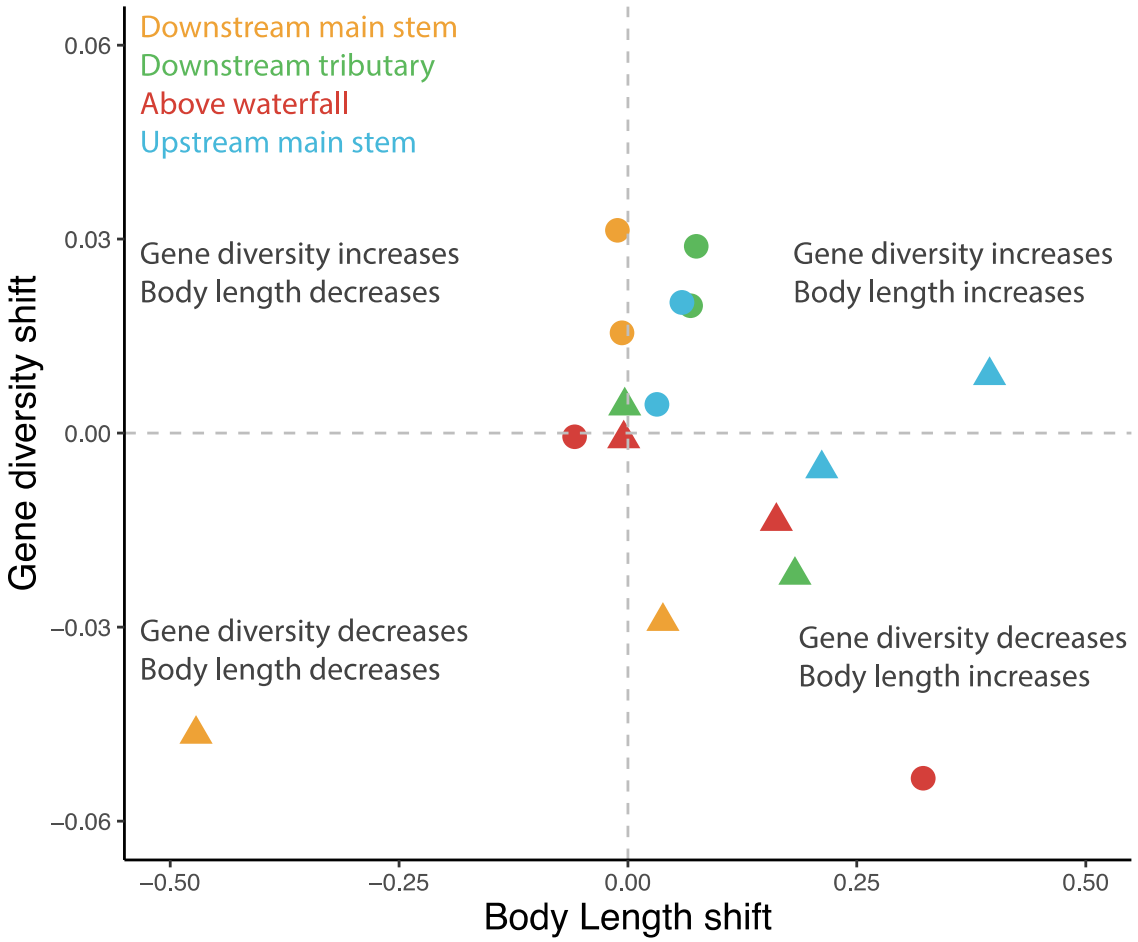


Figure 2.4 Joint shifts in body length and gene diversity after floods. Circles represent sites in the Paria River whereas triangles represent sites in the Marianne River – each having one point for each flood.

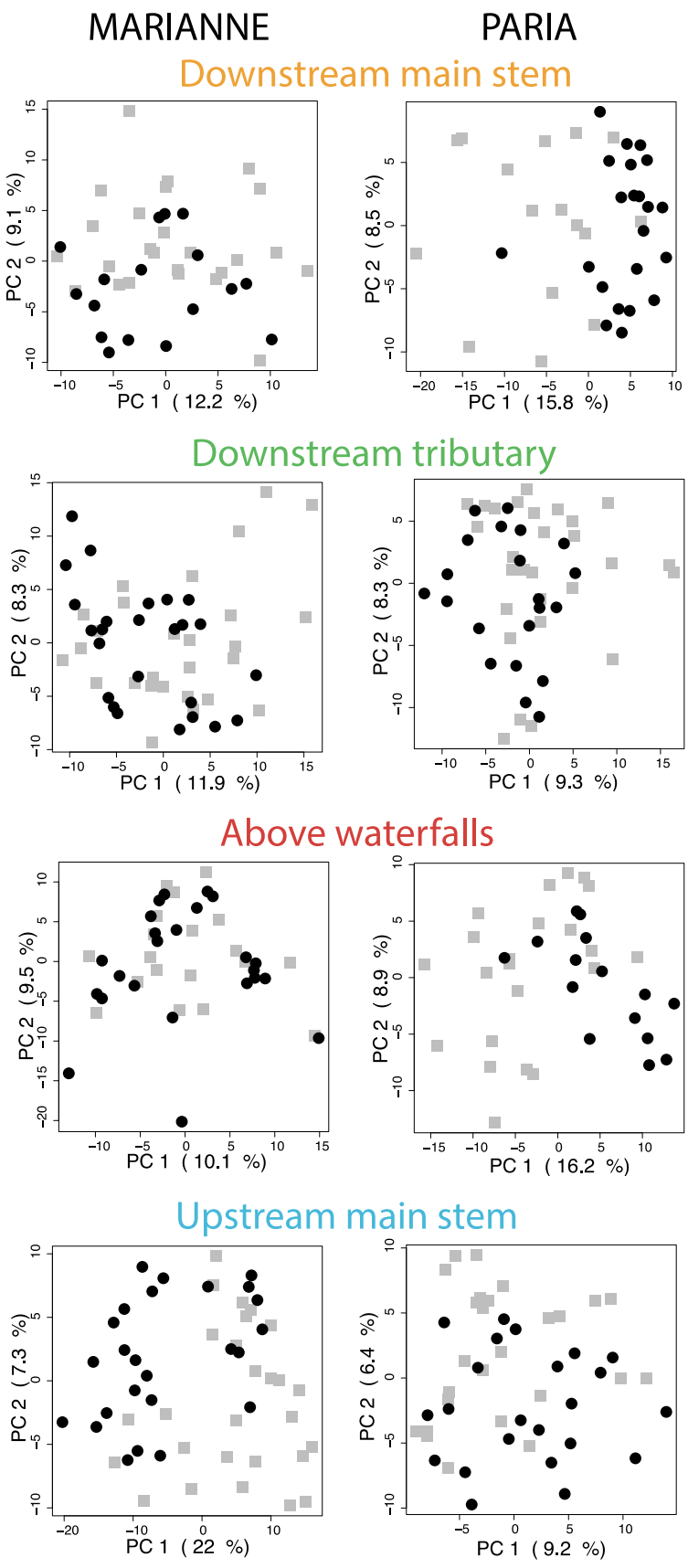
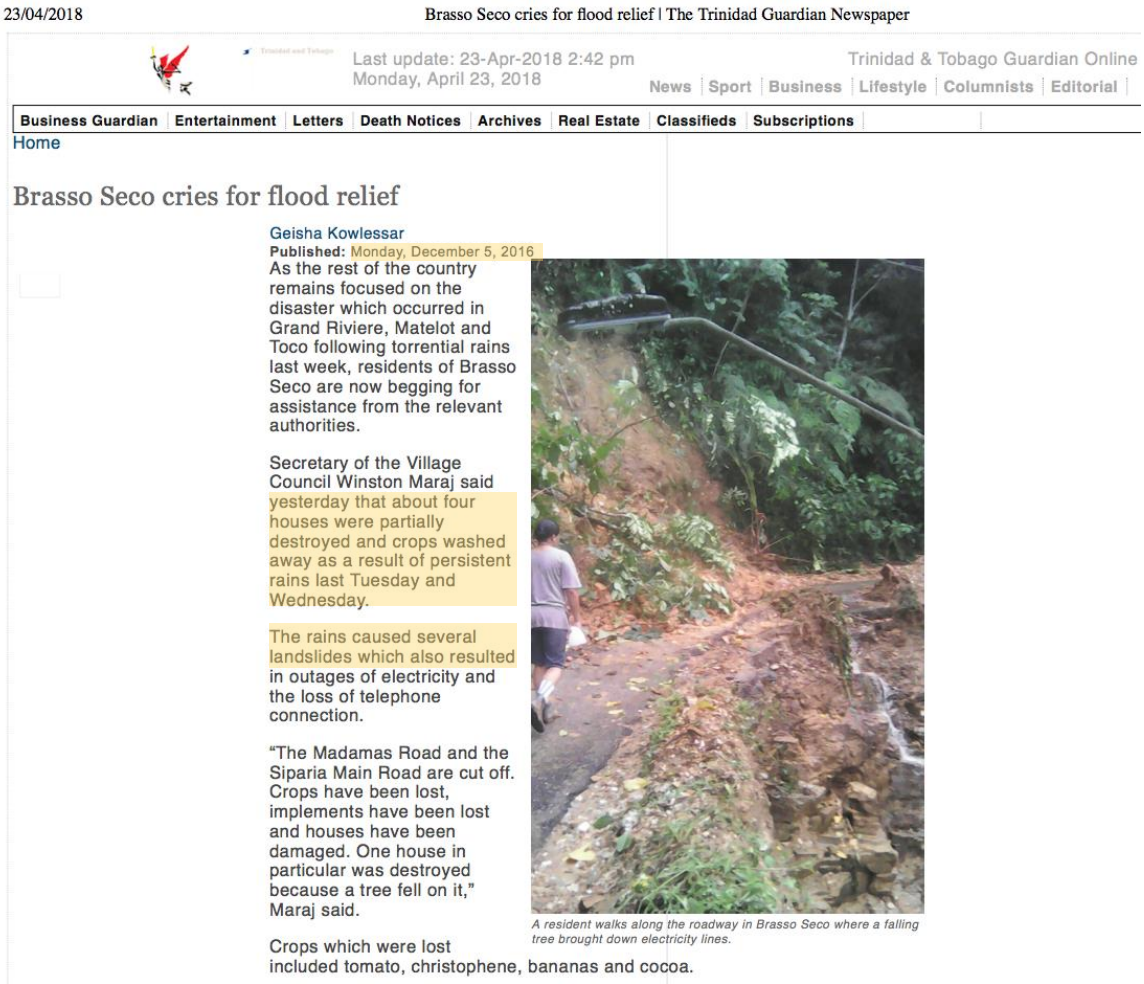

MARIANNE
PARIA
Downstream main stem
PC 2 (9.1 %)
PC 1 (12.2 %)
PC 2 (8.5 %)
PC 1 (15.8 %)
Downstream tributary
PC 2 (8.3 %)
PC 1 (11.9 %)
PC 2 (8.3 %)
PC 1 (9.3 %)
Above waterfalls
PC 2 (9.5 %)
PC 1 (10.1 %)
PC 2 (8.9 %)
PC 1 (16.2 %)
Upstream main stem
PC 2 (7.3 %)
PC 1 (22 %)
PC 2 (6.4 %)
PC 1 (9.2 %)

Figure 2.5 PCA plots of the male melanic color pattern for each site in each watershed. Gray squares are individuals from 2016, black points are individuals from 2017. PC axis are unique to each site and represent the coordinates of the black pixels in our pictures.



23/04/2018 Brasso Seco cries for flood relief | The Trinidad Guardian Newspaper

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Brasso Seco cries for flood relief

Geisha Kowlessar
Published: Monday, December 5, 2016

As the rest of the country remains focused on the disaster which occurred in Grand Riviere, Matelot and Toco following torrential rains last week, residents of Brasso Seco are now begging for assistance from the relevant authorities.

Secretary of the Village Council Winston Maraj said yesterday that about four houses were partially destroyed and crops washed away as a result of persistent rains last Tuesday and Wednesday.

The rains caused several landslides which also resulted in outages of electricity and the loss of telephone connection.

"The Madamas Road and the Siparia Main Road are cut off. Crops have been lost, implements have been lost and houses have been damaged. One house in particular was destroyed because a tree fell on it," Maraj said.

Crops which were lost included tomato, christophene, bananas and cocoa.

A resident walks along the roadway in Brasso Seco where a falling tree brought down electricity lines.

Figure S2.1 Extract from the article published in The Trinidadian Guardian Newspaper about the December 2016 flood. Full article available online at http://www.guardian.co.tt/news/2016-12-04/brasso-seco-cries-flood-relief

Minister assures relief coming to Brasso Seco

LOOP NEWS CREATED : 4 DECEMBER 2016 T&T NEWS

With respect to the Madamas Road in Brasso Seco, the Minister stated that teams were sent in to clear the landslips there.

Residents indicated that some parts of the road are completely gone, with rivers having changed their courses flowing through estates and the main road.

Those in neighbouring Madamas Village are marooned as the roads remain inaccessible.

Sinanan is currently visiting Matelot and surrounding areas to get a firsthand view of the damage and progress made in relief and restorative efforts.

Brasso Seco residents say they are grateful for the quick response of government workers in clearing all the landslides on the main roads. They also thanked T&TEC, which has been working feverishly to get electricity restored.

The residents are appealing to hiking clubs and anyone willing to lend a helping hand in whichever way possible to join them in assisting the residents of the Madamas area.

Figure S2.2 Extract from the article published in Loop T&T about the December 2016 flood. Full article available online at http://www.looptt.com/content/minister-assures-relief-coming-brasso-seco

Figure S2.3 Pictures of before versus after the 2016 flood of our camp site located downstream in the main stem of the Paria River. Pictures taken by Andrew Hendry (left) and Léa Blondel (right).

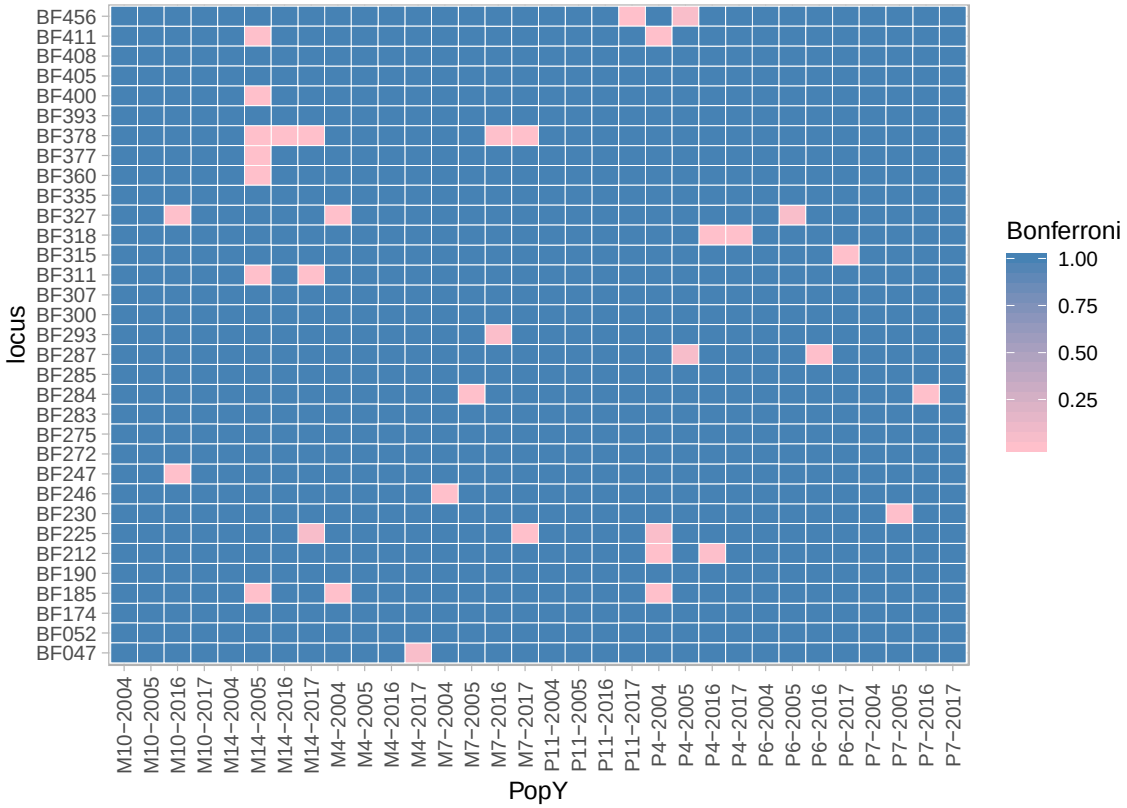

Figure S2.4 Heatmap of the departures from Hardy-Weinberg equilibrium for the 32 samples at the 33 loci.

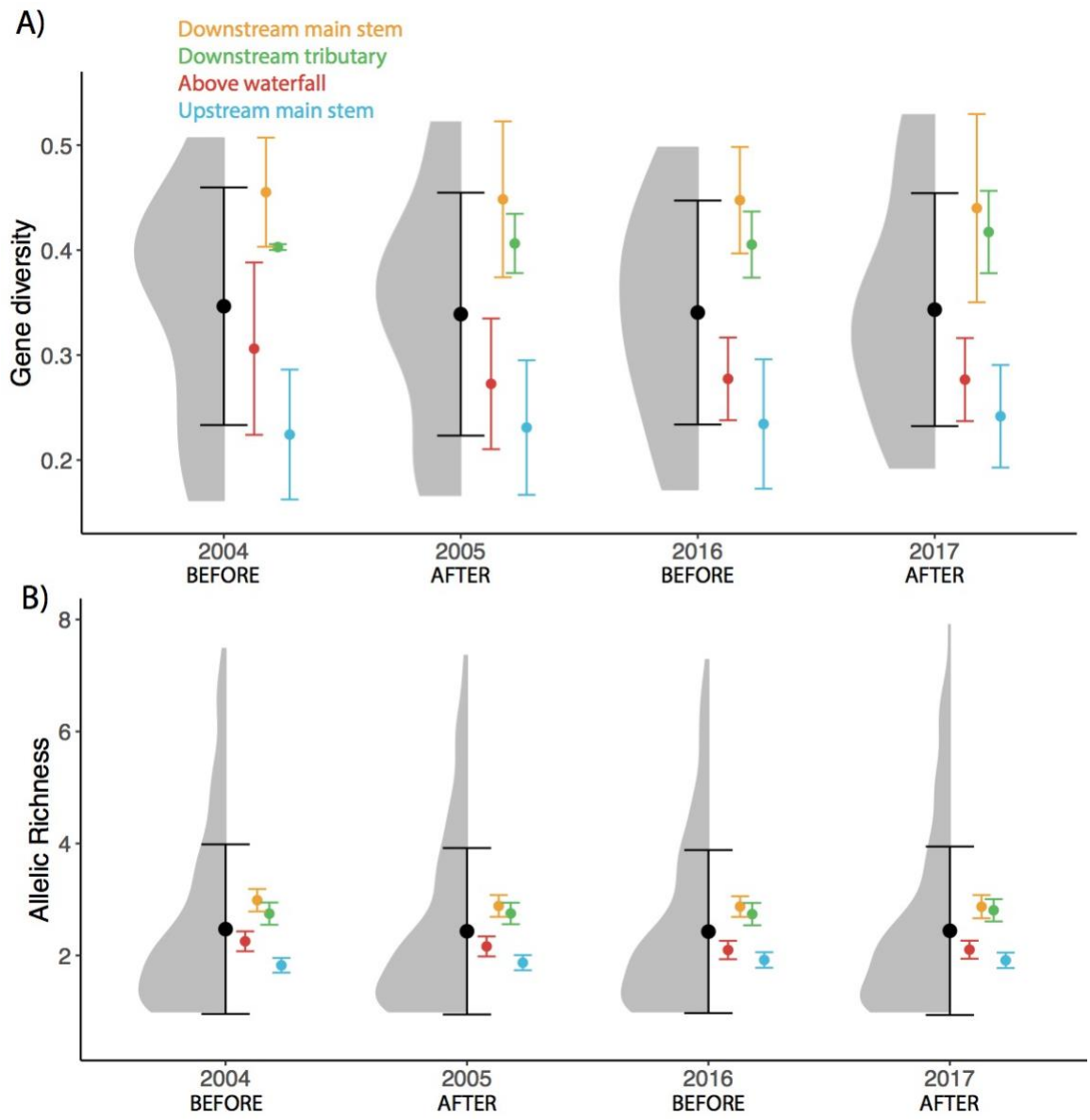

Figure S2.5 A) Nei's gene diversity in each year (before or after a massive flood). B) Allelic richness in each year (before or after a massive flood). Black circles represent the overall mean and standard error for all sites in each year. To their left, Grey violin plots represent the distribution of the raw data. To their right, mean and standard error for each site category is represented in color.

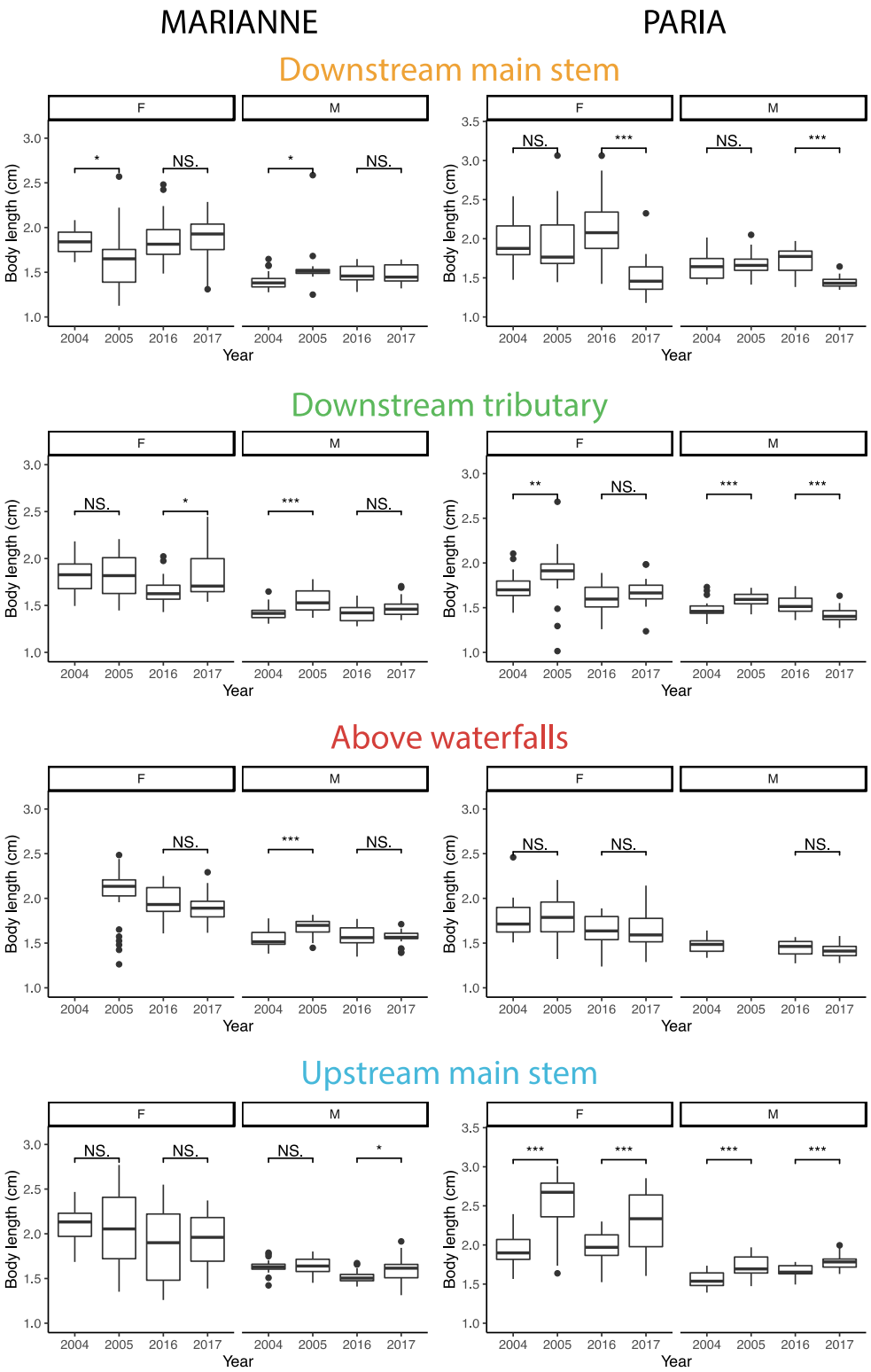

Figure S2.6 Mean body length for all sites among years. Females and males are displayed separately. Significance was calculated as pairwise t tests with a Bonferroni correction: NS. = not significant; * = <0.05; ** = <0.01; *** = <0.001

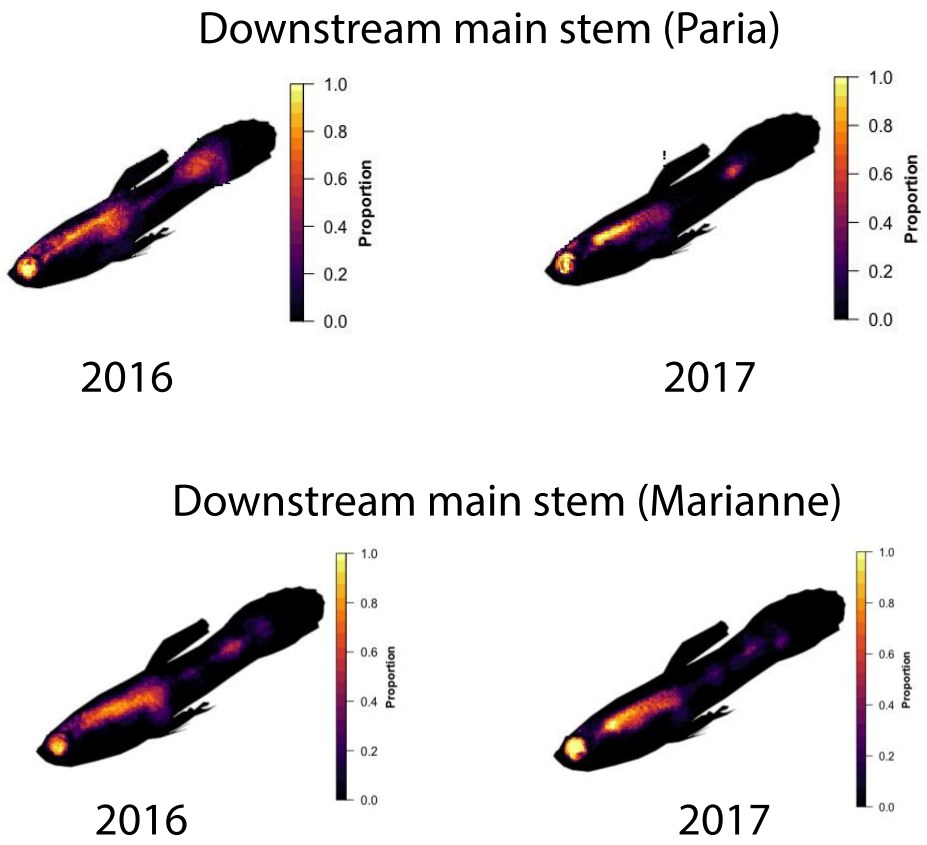

Figure S2.7 Extracted melanic patterns visualized as heatmaps for sites located downstream in the main stem for the Paria (above panel) and the Marianne (bottom panel) Rivers. Males from this site in the Paria River display less melanic colors after the flood (2017) than before (2016). We don't see the same shift in the Marianne River.

Linking statement to Chapter 3

In chapter 2, I investigated the resistance and resilience of guppy genetic and phenotypic variation to extreme flood events. I found that guppy population genetic structure was mostly resistant to floods, whereas phenotypic variation showed immediate impact, but resilience. The results from this chapter highlights the importance of adaptations from guppy populations to this kind of disturbance. One of the mechanisms by which guppy resist water flow is rheotaxis – a behavior that enable aquatic organisms to align themselves against or with the flow, and to maintain their position in a current.

The results from chapter 2 raises several questions: Do upstream guppies show high levels of positive rheotaxis? If they do, do they display the same behavior for the same performance? If there is a strong selection pressure to stay above waterfalls, does positive rheotaxis decrease along the river gradient and with the presence of barrier waterfalls? In my next chapter, I thus tested the rheotactic behavior of two guppy populations that evolved in upper reaches of their streams.

CHAPTER 3: Asymmetric Isolation and the Evolution of Behaviors Influencing Dispersal: Rheotaxis of Guppies above Waterfalls**

Abstract

Populations that are asymmetrically isolated, such as above waterfalls, can sometimes export emigrants in a direction from which they do not receive immigrants, and thus provide an excellent opportunity to study the evolution of dispersal traits. We investigated the rheotaxis of guppies above barrier waterfalls in the Aripo and Turure rivers in Trinidad—the later having been introduced in 1957 from a below-waterfall population in another drainage. We predicted that, as a result of strong selection against downstream emigration, both of these above-waterfall populations should show strong positive rheotaxis. Matching these expectations, both populations expressed high levels of positive rheotaxis, possibly reflecting contemporary (rapid) evolution in the introduced Turure population. However, the two populations used different behaviors to achieve the same performance of strong positive rheotaxis, as has been predicted in the case of multiple potential evolutionary solutions to the same functional challenge (i.e., "many-to-one mapping"). By contrast, we did not find any difference in rheotactic behavior above versus below waterfalls on a small scale within either river, suggesting constraints on adaptive divergence on such scales.

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Introduction

Populations that become newly established in isolated places, such as on islands or above waterfalls or dams, provide excellent opportunities to study evolutionary processes (Mayr, 1967; Hendry & Kinnison, 1999; Reznick & Ghalambor, 2001). The establishment of these populations can reflect natural processes or human activities. Relevant natural processes include rare climatological events (e.g., storms or floods) or geological events (e.g., earthquakes or volcanoes) that spread organisms to new places (Lescak *et al.*, 2015). Relevant human actions can reflect unintentional introductions (e.g., rats on ships or zooplankton in ballast water) or intentional releases (e.g., biocontrol or "assisted migration"; (Minteer & Collins, 2010)). Human-driven establishment can also happen when scientists introduce organisms into new environments to test ecological or evolutionary theories (Carvalho *et al.*, 1996; Losos *et al.*, 1997; Huey *et al.*, 2005; Ghalambor *et al.*, 2015). Especially in these latter cases, a particular emphasis is placed on maintaining strong isolation of the new populations, such that evolutionary and ecological dynamics can unfold without the complicating influence of unplanned inputs from external sources (Endler, 1980b; Huey *et al.*, 2005; Reznick & Ghalambor, 2005).

In a number of these establishments, regardless of their cause, the resulting isolation of new populations is asymmetrical: that is, they do not receive gene flow from other populations whereas they can still export genes to at least some other populations. For instance, fish establishing in headwater lakes or above waterfalls are unlikely to receive many immigrants from further downstream, yet they commonly export emigrants into downstream populations (Krueger & May, 1991; Adams *et al.*, 2001; Bolnick *et al.*, 2008). This unidirectional export of genetic material from asymmetrically isolated

populations into non-isolated populations can have considerable neutral (altered genetic diversity) or adaptive (altered adaptive potential) consequences (Fitzpatrick *et al.*, 2015). In particular, the phenotypic traits of newly established populations might be quite different from nearby non-isolated populations into which they export alleles. Accordingly, some studies have shown that introduced populations established high in stream watersheds can have massive genetic consequences for natural populations further downstream (Krueger & May, 1991; Carvalho *et al.*, 1996; Fitzpatrick *et al.*, 2017). However, if the tendency for emigration has a (at least partial) genetic basis, alleles that increase emigration will be lost from the populations and—owing to asymmetric isolation—will not return (Weese *et al.*, 2011). Hence, asymmetrically isolated populations should evolve traits that reduce emigration and, consequently, their effects on downstream populations should decrease through time.

In riverine fishes, one way to avoid downstream emigration is to increase the tendency to maintain position in the stream and swim against the current, referred to as positive rheotaxis (Arnold, 1974; Montgomery *et al.*, 1997; Northcutt, 1997). Rheotactic behavior is genetically based in many fishes (Northcote, 1981, 2010; Jonsson, 1982b), and so we hypothesize that asymmetrically isolated riverine fish populations (those above waterfalls) should evolve positive rheotaxis. Consistent with this hypothesis, several studies have shown that fish populations above waterfalls have higher positive rheotaxis than those below waterfalls (Jonsson, 1982b; Morita & Yamamoto, 2001; Northcote, 2010). Few studies, however, have asked how repeatable this evolution might be, which is an inference that requires assaying multiple populations of a focal species that independently established above waterfalls. Therefore, **our first objective** was to test

whether two different above-waterfall populations of Trinidadian guppies (*Poecilia reticulata*) show positive rheotaxis.

Positive rheotaxis could be attained in several different ways. For instance, fish could avoid high current, could increase their upstream orientation, or could swim faster or longer in the upstream direction. Positive rheotaxis is therefore a "performance" metric that could be achieved through multiple different behavioral, physiological, or morphological solutions. Thus, the problem of evolving positive rheotaxis in above-waterfall populations is another example of many-to-one mapping (Thompson *et al.*, 2017). In such cases, a key question becomes whether parallel evolution of performance (positive rheotaxis) is the result of non-parallel solutions. Therefore, **our second objective** was to ask whether the two above-waterfall guppy populations evolved positive rheotaxis through the same or different behavioral solutions.

We also wanted to consider the possibility of fine-scale variation in rheotaxis. In particular, we hypothesized that increasing degrees of asymmetric isolation should lead to increasing selection against behaviors favoring downstream emigration. For example, the fish above a series of barrier waterfalls might be expected to show stronger positive rheotaxis than the fish between those waterfalls, which might be expected to show stronger positive rheotaxis than the fish below those waterfalls. Thus, **our final objective** was to evaluate these hypotheses by testing the rheotaxis of guppies from above, below, and between two barrier waterfalls in each river.

To our knowledge, the rate at which rheotaxis evolves has never be considered. Given that contemporary (or "rapid") evolution has been documented in many organisms experiencing strong selection (Thompson, 1998; Hendry & Kinnison, 1999; Hairston *et*

al., 2005), especially guppies (Reznick, 1997), we suggest that positive rheotaxis will evolve quickly in guppies introduced above waterfalls. One of our two study populations was introduced by scientists, which has given us the irresistible temptation to speculate on the potential rapidity of rheotaxis evolution. (We acknowledge here that this speculation was discouraged by anonymous reviewers.) However, given the impossibility of evaluating initial rheotaxis in the introduced population, and given ambiguity as to the precise ancestral source (details below), our inferences on this point will remain speculative.

Materials and Methods

History of an Introduction and Its Effects

In 1957, guppies were introduced by Caryl Haskins from below all barrier waterfalls in the Guanapo River (Caroni drainage) into a previously guppy-free site above a series of barrier waterfalls in the Turure River (Oropuche drainage) (Magurran, 2005). Uncertainty exists as to the exact source population for this introduction, with suggestions including the Arima River (Shaw *et al.*, 1991, 1992; Magurran *et al.*, 1992; Carvalho *et al.*, 1996), the Guanapo River (Magurran, 2005; Fitzpatrick *et al.*, 2015), or the Aripo River (Becher & Magurran, 2000). This introduction was unknown to scientists until Shaw et al. (1991) found a puzzling signature of Caroni genotypes in the lower Turure River of the Oropuche drainage. They formulated an hypothesis that Caroni drainage guppies had been introduced into the Turure River, which was later confirmed by personal communication from Caryl Haskins (Shaw *et al.*, 1991). Guppies in the Caroni and Oropuche drainages had otherwise been isolated from each other for

approximately 0.6-1.2 million years, thus generating dramatic genetic differences (Carvalho *et al.*, 1991; Shaw *et al.*, 1991; Fajen & Breden, 1992). After the introduction, however, a strong genetic signature in both nuclear and mitochondrial genes of Guanapo fish was detected well downstream of the initial asymmetrically isolated introduction site in the Turure River (Shaw *et al.*, 1992; Becher & Magurran, 2000; Fitzpatrick *et al.*, 2015). For instance, Shaw et al. (1992) investigated six enzyme-coding loci and found that Turure fish located 1 km downstream of the introduction site had alleles normally found only in the Caroni drainage (Shaw *et al.*, 1992). Becher et al. (2000) investigated mitochondrial DNA and found that only 12% of genotypes in the downstream sites corresponded to the native Oropuche population. Finally, Fitzpatrick et al. (2015) investigated microsatellite markers and found that sites located 1 km downstream of the introduction primarily clustered with introduced fish and not with the native population. In short, the fish that Haskins first introduced initially had very strong downstream genetic effects reflecting substantial emigration and, presumably, the absence of strong positive rheotaxis.

Fish Sampling

We used butterfly nets to collect guppies during the dry season (March 2015) from two rivers: the Aripo River in the Caroni drainage and the Turure River in the Oropuche drainage (Figure 3.1). The collection sites in both rivers are "Low Predation" (LP) owing to the absence of predatory fish other than *Rivulus hartii* (Reznick *et al.*, 2001). Established guppy populations were present downstream of the collection area in both rivers and were found upstream of the collection area in the Aripo but not the Turure.

With the guppies being asymmetrically isolated in both sampling locations by downstream waterfalls, it allowed us to test Objective 1 (both populations should show positive rheotaxis) and Objective 2 (whether positive rheotaxis was achieved through similar behavioral strategies in both populations). In each of the two rivers, collections took place from three pool types: one pool above an upstream waterfall ("Above"), one pool below a downstream waterfall ("Below"), and one pool between the two waterfalls ("Between") (Figure 1). This sampling allowed us to test Objective 3: whether rheotaxis showed fine-scale variation depending on their location relative to multiple barrier waterfalls. Collected fish were immediately transferred to a field lab in Trinidad, where they were kept in separate tanks according to their pool type/river for one week prior to transport to Montréal (Québec, Canada) by air cargo.

Fish were then kept in the laboratory after an 8 month quarantine and lab acclimation period. They were separated in river and pool type specific tanks in still water with an air pump and were fed daily with brine shrimp. The experiments used a combination of the wild-caught females (F0) and their female offspring (F1), which were raised in common-garden conditions. The experiments used females rather than males because female guppies generally express high site fidelity, contrary to male guppies that show more movement between pools (Croft *et al.*, 2003a). Hence, wild-caught males might have been less representative of the local population, perhaps having recently arrived from elsewhere.

Rheotaxis Apparatus

We tested the rheotactic behavior of Turure and Aripo guppies (number of tested females in Table 3.1) in a circular-flow tank similar to the one described in Jiang et al. (2015), where guppies could swim freely in either direction: against (upstream) or with (downstream) the current (Figure 3.2). Two pumps were used to generate flow and were located outside the testing tank to prevent fish from hiding behind the pump. The tank was placed in a room without windows and with a fixed source of light directly above the tank.

Rheotaxis Trials

Each fish was individually tested in the circular-flow tank. After an acclimation period of 15 min, the pumps were turned on for 5 min to create a continuous flow. During these 5 min, the fish movement was videotaped with a webcam (Logitech C270) for later analyses. At the end of the experiment, the pumps were turned off and the fish was allowed to recover for 5 min before being anesthetized for the measurement of length (cm $\pm$ 0.001) and mass (g $\pm$ 0.0001). Even though female guppies showed no attraction to conspecific cues in an experimental flow chamber in a previous study (Archard *et al.*, 2008), we ran a carbon filter to filter chemicals for 5 min between trials. We also recorded water temperature for each trial.

Video Analysis

Each individual video trial was converted to 450 images (1.5 frames per second) using the software Adapter version 2.1.6. We then used ImageJ (Rasband, 2012) with the

MtrackJ plugin (Meijering, 2006) to track the anterior and posterior ends of each fish. The anterior and posterior end were then translated into x and y coordinates for each frame. From these coordinates, we quantified net displacement, cumulative upstream movement, flow regime experienced, and upstream orientation (Jiang *et al.*, 2015).

Net displacement was calculated as the total distance traveled during the trial: from a fish starting point, any movement in the upstream direction (against the flow) was summed up, and any movement in the downstream direction (with the flow) was subtracted, until the end point. This distance can be positive (the fish swam mostly in the upstream direction) or negative (the fish swam mostly in the downstream direction). To facilitate this inference, we also estimated the distance covered in the downstream direction in the absence of positive rheotaxis. This estimate was made using an inanimate prop (a miniature spoon with tape wrapped around the anterior tip of it) having the same mass as a guppy (0.40 g). Net downstream displacement for this mimic of a non-swimming guppy was -4146 ± 2361 cm, depending on the flow regime occupied. Thus, net displacement of a guppy less than this distance would reflect positive rheotaxis. Cumulative upstream movement was calculated as the total distance that the fish swam in the upstream direction (against the current). For these two variables, higher values indicate stronger positive rheotactic behavior. Importantly, however, a fish can show net displacement downstream despite strong positive rheotaxis. That is, in a strong current, a fish could be displaced downstream despite furiously and continuously swimming against the current.

The flow regime experienced by the fish was determined in each frame as the presence of the fish in one of the four flow zones (Figure 3.2) as 0, 1, 2, or 3 for the

minimal (4.2 cm/s), low (6.9 cm/s), medium (8.8 cm/s), and high (17.1 cm/s) flow zones respectively. Flow was measured by dropping food coloring in water and recording its diffusion in subsequent time frames. The 450 flow regime measurements per trial per fish were then averaged for each fish to obtain the mean flow regime score over the entire trial. A fish could either decide to avoid the flow by staying in the minimal flow zone or decide to actively swim in higher flow zones.

For upstream orientation, we determined in each of the 450 frames to what extent each fish was facing upstream by measuring the angle between the fish and the tangent of the flow. We then calculated the proportion of time the fish was aligned upstream within ±45° of the flow during the entire trial. High values for upstream orientation indicate more positive rheotactic behavior.

Statistical Analyses

All analyses were performed using the R language (RStudio Team, 2016; R Core Team, 2018). Significance was set at $\alpha = 0.05$ and means ± standard deviations are reported throughout, unless otherwise specified. We excluded two outliers from the analysis: both from the Aripo River, one from the Below pool type and one from the Between pool type. Both fish displayed values for net displacement that were either 4 times or 15 times higher than the mean. For each of the four response variables (net displacement, cumulative upstream movement, flow regime, and upstream orientation) we used a separate linear model. We set as fixed effects, the pool type from which the fish (for F0s) or its parents (for F1s) had been collected (three levels: “Above”, “Between”, “Below”), the river (two levels: “Aripo” and “Turure”), and the interaction between pool type and

river. We set as covariates, the fish mass, the mean temperature during the trial, and the fish generation (F0 or F1). For every response variable, we built a full model, and then ran a model selection procedure using Akaike Information Criterion (AIC; see supporting information for the full model selection), dropping effects that did not improve the model in the following order: mass, generation and mean temperature.

The final model for net displacement was Y ~ MeanTemperature + pool + river + pool x river. The final model for cumulative upstream movement was Y ~ mass + MeanTemperature + generation + pool + river + pool x river. The final model for flow regime was Y ~ generation + MeanTemperature + pool + river + pool x river. The final model for upstream orientation was Y ~ pool + river + pool x river. We then checked for independence and homogeneity of the residuals and transformed the response variable if needed. Flow regime and cumulative upstream movement were log transformed to meet normality of the residuals and homoscedasticity of the variance. When pool type, or the interaction between pool type and river was significant, we explored two a priori planned contrasts (Chatham, 1999): Above pool type versus the combination of Between/Below (upstream vs. downstream; contrast 1); and Between versus Below (contrast 2; Figure S3.1). These contrasts were set up to test if there was a difference between upstream and downstream populations, but also to compare fine scale variation. Fish collected from Between pools could express high positive rheotaxis because they were located above a waterfall, but they could also express low positive rheotaxis because they were located downstream of Above fish.

Finally, we used a generalized additive mixed model (GAMM) in the R package gamm4 (Wood & Scheipl, 2017) to analyze the temporal fish response relative to the

flow (flow regime and upstream alignment) during the trial. Time was entered as frame number. We included River and Pool type as linear factors and time-by-river as a non-linear factor-smooth interaction, with the smoothing parameter estimation "REML". Fish ID was entered as a random factor, with a random slope and a random intercept. We used a quasipoisson distribution for flow regime, and a binomial distribution for alignment (0 is not aligned, 1 is aligned). Neither GAMM model provided a good fit to the raw data. Therefore, we used generalized additive models (GAMs) in the R package mgcv (Wood, 2011) on the average flow regime or the average alignment per pool type and per river using a gaussian family. We recognize that, in doing so, we lost the ability to take into account individual level variation by using the mean in our model, but this was our only option. We corrected for autocorrelation of the temporal data points in our models using the corARMA() function in the R package nlme (Pinheiro *et al.*, 2017).

Results

We first summarize our main findings. (i) Fish in both rivers showed strong positive rheotaxis. (ii) Turure and Aripo fish achieved this positive rheotaxis in different ways: fish from Turure first occupied low-flow areas, and then moved to high-flow areas, whereas fish from Aripo occupied intermediate-flow areas throughout the trial. (iii) Rheotactic behavior within each river did not differ between fish from above the upstream waterfall and those from below the downstream waterfall.

Summary Statistics

Average mass of the fish used in the experiment was 0.30 ± 0.11 g, with no differences between rivers ($F_{1,63}$ = 0.999, P = 0.321), nor among the three pool types ($F_{3,63}$ = 0.192, P = 0.901). However, F0 fish were heavier than F1 fish (0.34 ± 0.08 g vs. 0.15 ± 0.07 g; $F_{1,56}$ = 60.6, P < 0.001). Average temperature during the trials was 23 ± 1 °C, with no difference for fish from different rivers ($F_{3,64}$ = 0.5, P = 0.477) or pool types ($F_{3,64}$ = 0.1, P = 0.973). Overall, fish expressed strong positive rheotactic behavior, given that net downstream displacement ranged from -1268 to 559 cm, whereas downstream displacement in the absence of swimming ranged from −6236 to −1472 cm. Fish spent 70 ± 11% of their time aligned against the flow, although this was often in low-flow zones (almost 75% of the alignment against the flow occurred in the minimal or low-flow zones).

Linear Models for Rheotactic Behavior

We did not detect any difference between the two rivers for the four measured variables (net displacement, cumulative upstream movement, flow regime, and upstream orientation; Table 3.2; Figure 3.3), thus indicating similar overall rheotaxis between the Turure fish and the Aripo fish. Fish from different pool types along the river gradient also expressed similar rheotactic behaviors as no differences were found among Above, Between or Below fish (Table 3.2; Figure 3.3). However, although non-significant, net displacement and cumulative upstream movement decreased from the Above to Below pool types for the Turure fish (Figure 3.3).

Generalized Additive Model for Temporal Patterns of Flow Regime and Alignment

Fish from the two rivers showed complex and distinct patterns of movement over the course of the trial. The Turure fish immediately positioned themselves in the low-flow zone at the beginning of the trial before swimming in higher flow zones, whereas Aripo fish maintained their position in an intermediate-flow regime during the entire trial (Figure 3.4; Figure S3.2). This difference was significant when river was included as a fixed factor in the GAM ($F = 110.3$, $P < 2e{-}16$).

Fish from the two rivers aligned themselves against the flow as soon as the pumps were turned on (Figure 3.5; Figure S3.3), but upstream alignment for guppies from Aripo decreased with time, whereas upstream alignment for guppies from Turure increased with time. Again, this difference was significant ($F = 4.933$, $P = 0.026$).

Discussion

Objective 1. Rheotaxis in Two Asymmetrically Isolated Guppy Populations

Guppies in both populations exhibited strong positive rheotaxis: they all aligned against the flow during the duration of the trial and they all swam in the upstream direction during most of the trial. The overall average tendency was still toward some net downstream displacement; yet based on comparisons with an inanimate prop, this displacement was on average 41 times less than expected if the guppies had not actively aligned against the current. Hence, our results confirm that guppies located in the upstream reaches of rivers express strong positive rheotaxis: that is, a strong tendency to swim against the flow (Mohammed *et al.*, 2012).

Of additional interest, strong positive rheotaxis in the Turure population might reflect contemporary adaptive evolution following their introduction. We feel this argument is justified given several previous observations: rheotaxis has a strong genetic basis in various fishes (Raleigh, 1967; Jiang *et al.*, 2017) and many Turure guppies were clearly displaced downstream soon after their introduction (Shaw *et al.*, 1992; Carvalho *et al.*, 1996; Becher & Magurran, 2000; Fitzpatrick *et al.*, 2015). This second assertion is supported by the fact that the genotypic signature of introduced guppies replaced many native genotypes up to at least 1 km downstream of the introduction site in the Turure (Shaw *et al.*, 1992; Carvalho *et al.*, 1996; Becher & Magurran, 2000; Fitzpatrick *et al.*, 2015). Given that the introduced Turure population initially must have shown substantial downstream gene flow, strong positive rheotaxis in the present-day population would suggest an evolutionary change following introduction. However, ambiguity remains for this inference of contemporary (rapid) evolution. One reason is that the source population for the Turure introduction is not certain and so we could not quantify rheotaxis in the specific ancestral population. We therefore instead focused on exploring whether the introduced guppies now showed similar positive rheotaxis to another upstream population. As a result, we cannot be certain how much, and in which direction, the Turure population has changed in their rheotactic behavior. Yet we do know that the source population was at least not from an isolated location above barrier waterfalls (Magurran, 2005). Thus, as seen in numerous other fishes (review in (Northcote, 2010)), the downstream origin of the Turure fish was likely to dictate initially weak positive rheotaxis, a supposition supported by the dramatic effect their downstream movement had on native populations (Shaw *et al.*, 1992; Becher & Magurran, 2000; Fitzpatrick *et*

al., 2015)**.** Further support comes from Mohammed et al. (2012), who found that guppies located in lower reaches of a stream were more likely to be swept downstream than guppies located further upstream (Mohammed *et al.*, 2012). It therefore seems likely that the current strong positive rheotaxis in the Turure River reflects at least some (and perhaps substantial) contemporary evolution following their introduction more than 60 years ago.

Objective 2. Multiple Solutions to the Same Problem.

Guppies in the two populations achieved the same overall performance (positive rheotaxis) using two different behavioral strategies, most notably in their temporal patterns of occupying flow zones (Figure 3.4). Aripo guppies generally occupied an intermediate-flow zone throughout the trials. Turure guppies, by contrast, initially all occupied a lower flow zone than all Aripo fish. After about two minutes (200 time frames), Turure guppies nearly all occupied a higher flow zone than nearly all Aripo fish. Only after around three and a half minutes (320 time frames) did Turure guppies converge on a similar flow regime to Aripo guppies. These dramatically different temporal responses of guppies from the two populations suggests the evolution of very different strategies for coping with water flow: that is, multiple behavioral solutions have been used to the same evolutionary problem—as has been suggested for such many-to-one trait-to-performance mapping situations. Overall, this evidence of populations evolving different solutions to a similar overall selective problem is consistent with recent work on other traits in guppies (Schwartz & Hendry, 2007; Millar & Hendry,

2012; Fitzpatrick *et al.*, 2014), other fishes (e.g., (Thompson *et al.*, 2017)), and other organisms in general (Bolnick *et al.*, 2018).

We do not know the specific reason why the two populations now show such different behavioral solutions (temporal differences in flow zone choice and in upstream orientation) to achieve roughly the same performance (positive rheotaxis and similar net displacement). Nevertheless, we here speculate in hopes of generating hypotheses that might inspire future hypothesis testing. Some reasons might reflect contingencies associated with the specific pool of fish introduced, such as the river topology of the source or other environmental particularities. Alternatively, the differences between populations could be explained by recent selective forces; that is, site-specific contemporary evolution of the Turure fish following their introduction. The portion of the Turure River where guppies were introduced in 1957 is locally called the "Turure water steps" and consists of high limestone waterfalls each over 7 m in height. This considerable drop of water creates refuge zones at the bottom of the waterfall. We speculate that, in case of a flood or other increase in flow, guppies are probably able to use these low-flow zones to hide. These refuge zones are not as obvious in the Aripo population, where the waterfalls are much smaller. Perhaps this difference shapes the differential behavior solutions of the two populations. Alternatively, perhaps the young Turure population will eventually converge on the solution achieved by the much older Aripo population.

Objective 3. Rheotaxis along A River Gradient

Guppies sampled in the different pool types along each river expressed similar overall rheotactic behavior. This similarity among pools located on either side of waterfalls could be due to several reasons. First, guppies (and especially females) express site fidelity and do not migrate seasonally for reproduction or for food. By contrast, the majority of studies on rheotaxis have focused on salmonids, which are typically migratory below (but not above) waterfalls, potentially favoring greater divergence in rheotaxis across such barriers than would be the case for non-migratory guppies. Second, the scale of our study might have been too small to detect any difference in rheotactic behavior: the sampled pools were only separated by 75 m for Aripo and 85 m for Turure, which is a scale on which downstream gene flow can be very high (Becher & Magurran, 2000; Crispo *et al.*, 2006; Blondel *et al.*, 2019), potentially homogenizing adaptive variation. Furthermore, even though guppies sampled in the Turure were the most upstream site, this was not the case for the Aripo, which could also receive migrants from upstream. Third, waterfalls in the Turure and Aripo might not have been high enough to prevent upstream migration, instead allowing mixing of the upstream and downstream populations under at least some conditions. Although this last explanation is perhaps unlikely, it would be more likely for the Aripo River where the waterfalls are much smaller than in the Turure. Another potential explanation for the lack of small-scale differences is that guppies from these two rivers were all sampled from pools located in low predation areas, meaning that any emigrating fish from above the waterfall to the below pool would not be as disadvantaged as if they were emigrating into a high predation environment (see Weese *et. al*, 2011).

But Is It Evolution?

Our experiments used a mixture of wild-captured and first-generation lab reared guppies, which might therefore have retained plastic or maternal contributions to rheotactic behavior. Thus, technically, we cannot state with absolute confidence the genetic contribution to the patterns and differences observed. Nevertheless, the lab-reared guppies never experienced a water current before the experiment, and the wild-caught guppies had not experienced any current for 8 months prior to testing. Combined with the previously-noted genetic basis for rheotaxis differences observed in other fishes (Raleigh, 1967; Jiang *et al.*, 2017), we therefore suspect that at least some of the differences in temporal patterns do reflect genetic differences. Moreover, we know from previous studies that rheotaxis is a behavior mediated by the lateral line in fish (Montgomery *et al.*, 1995, 1997), and that variation in lateral line morphology has been found to be associated with genetic differences in several guppy populations (Fischer *et al.*, 2013). Formal studies investigating the genetic basis versus phenotypic plasticity of rheotaxis would be required to answer this question.

Conclusion

We provided evidence that upstream Turure and Aripo guppies demonstrate similar strong positive rheotaxis. That is, all of the guppies aligned against the flow for the majority of the trial, and they all swam in the upstream direction much more than expected had they exhibited passive responses. However, we also found a striking behavioral difference between the populations in how they achieved this level of positive rheotaxis, suggesting alternative behavioral solutions to the same functional challenge. At

a smaller scale, rheotaxis across waterfalls within a river was similar, suggesting that selection for positive rheotaxis was not strong enough at that scale. Overall, our findings imply that upstream guppies have evolved different behavioral mechanisms—perhaps rapidly—to maintain populations over barrier waterfalls despite asymmetric isolation.

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Table 3.1 Sample sizes (numbers of fish analyzed for rheotaxis) in the Aripo and Turure rivers. The unbalanced design reflects lab mortality.

Site Locations Relative to Waterfalls	**Aripo**		**Turure**	
	F0	**F1**	**F0**	**F1**
Above	11	2	14	2
Between	5	5	6	3
Below	6	0	5	2
Total	**22**	**7**	**25**	**7**

Table 3.2 Output of the linear models for each of the four response variables.

Response Variable	Adj. R^2	*F*	*P*
Net displacement	0.00		
Mean temperature		0.771	0.384
River		0.882	0.352
Pool		0.152	0.860
River x Pool		1.214	0.305
Log of cumulative upstream movement	0.28		
Mass		0.992	0.324
Mean temperature		2.028	0.160
Generation		6.004	**0.018**
River		1.885	0.176
Pool		0.728	0.488
River x Pool		1.268	0.290
Log of flow regime	0.54		
Mean temperature		0.926	0.340
Generation		32.020	**6.29e-07**
River		1.250	0.269
Pool		0.849	0.433
River x Pool		1.364	0.265
Upstream orientation	-0.07		
River		0.017	0.900
Pool		0.293	0.748
River x Pool		0.169	0.845

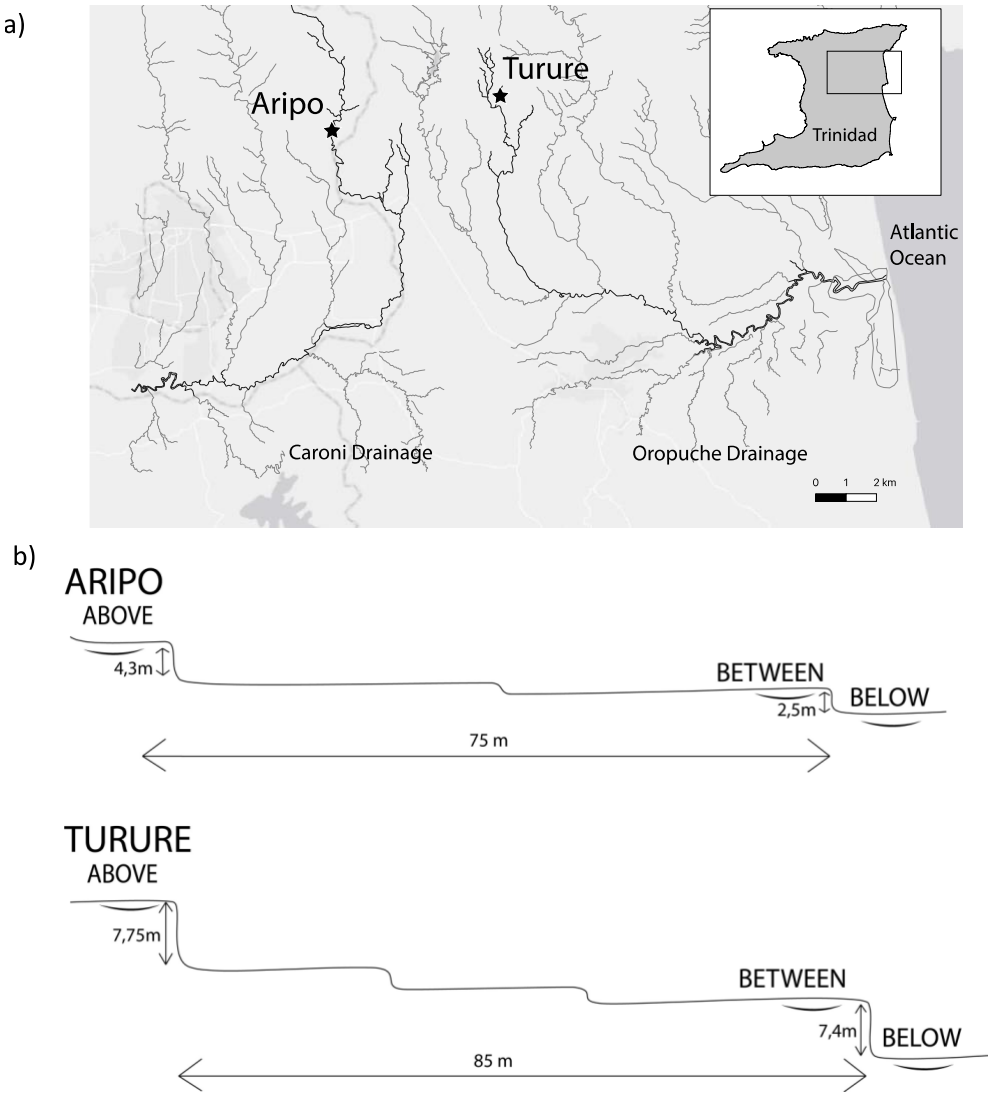

Figure 3.1 (a) Location of collection sites in the two rivers in the Northern Mountain range in Trinidad. (b) Profile for the two rivers showing the relative location and height of waterfalls in relation to the distance between them. Rivers flow from the left (upstream) to the right (downstream). Above, Between and Below represent the three collection sites per stream.

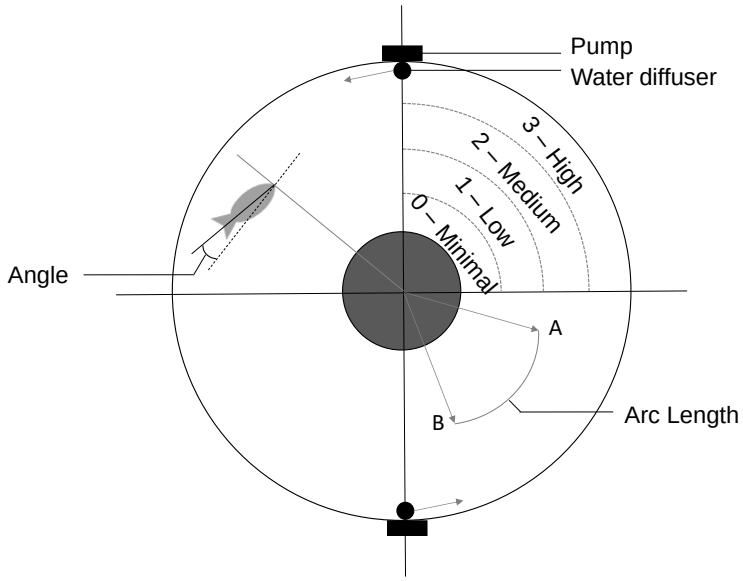

Figure 3.2 The experimental circular-flow tank. Dotted lines delineate different approximate flow regimes (High, Medium, Low and Minimal: upper right quadrant). Distance was measured as the arc length distance between two points (lower right quadrant). Angle was measured as the alignment of the fish against the flow (upper left quadrant). Direction of the water is given by arrows from the water diffusers. The tank dimensions were: testing chamber diameter = 65 cm; inside diameter = 25 cm, and water depth = 16 cm.

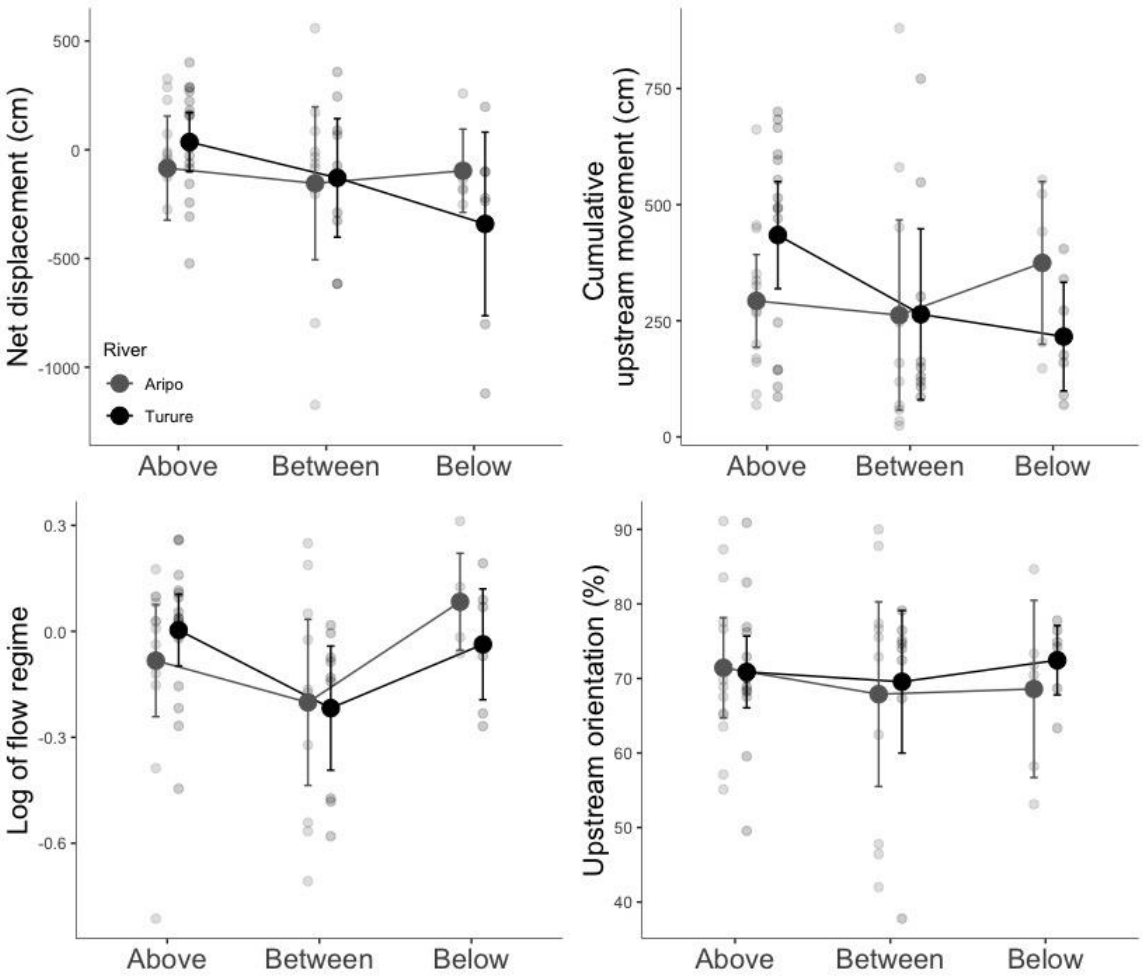

Figure 3.3 Means and 95% confidence intervals for net displacement, log of flow regime, cumulative upstream movement and upstream orientation, for each pool type in each river. The raw data points are presented in grey.

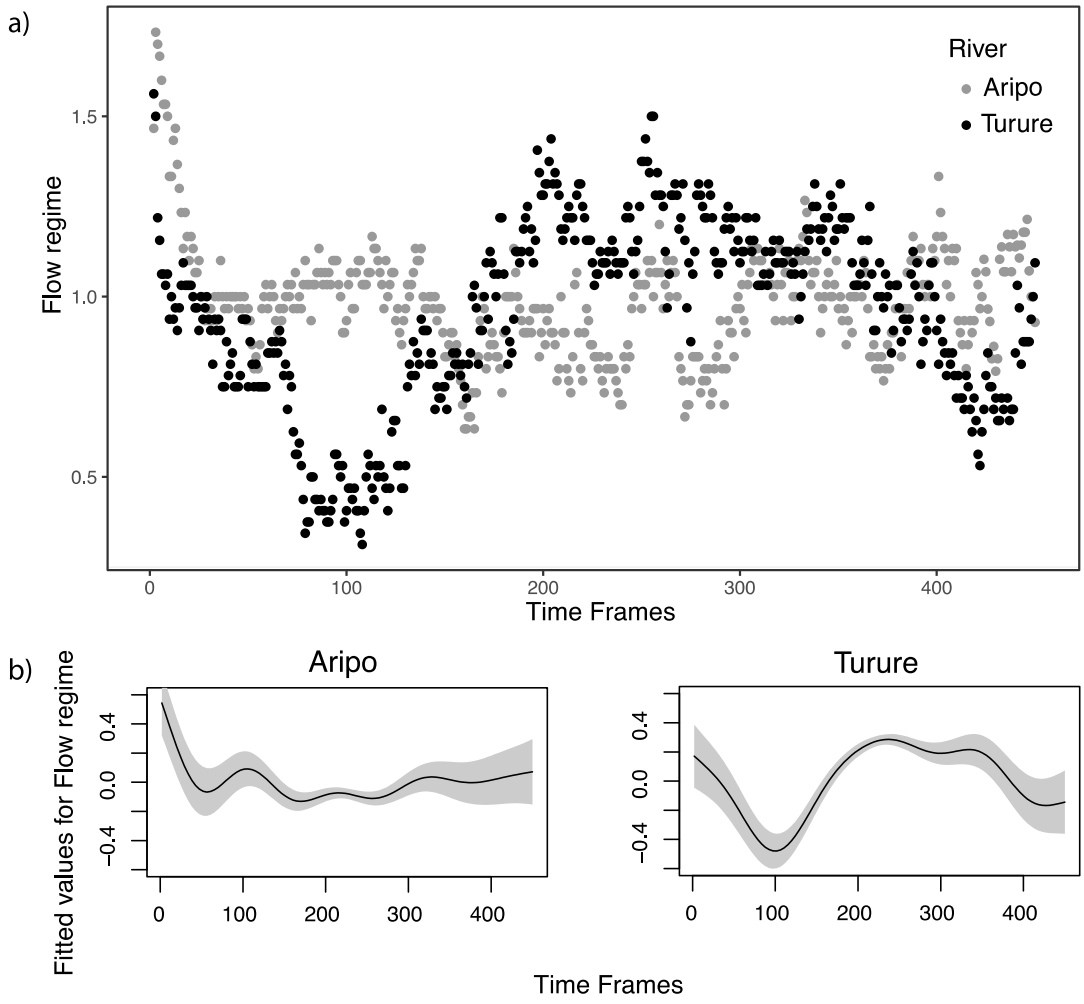

Figure 3.4 Values for flow regime during the entire duration of the trial (450 time frames over 5 min). (a) Individual values averaged at each time frame for each river across all pool types show a different behavioral pattern. High values mean that fish are swimming in higher flow zones. (b) Fitted values with GAM for the two rivers, the grey areas represent 95% confidence bands. Values are centered around 0.

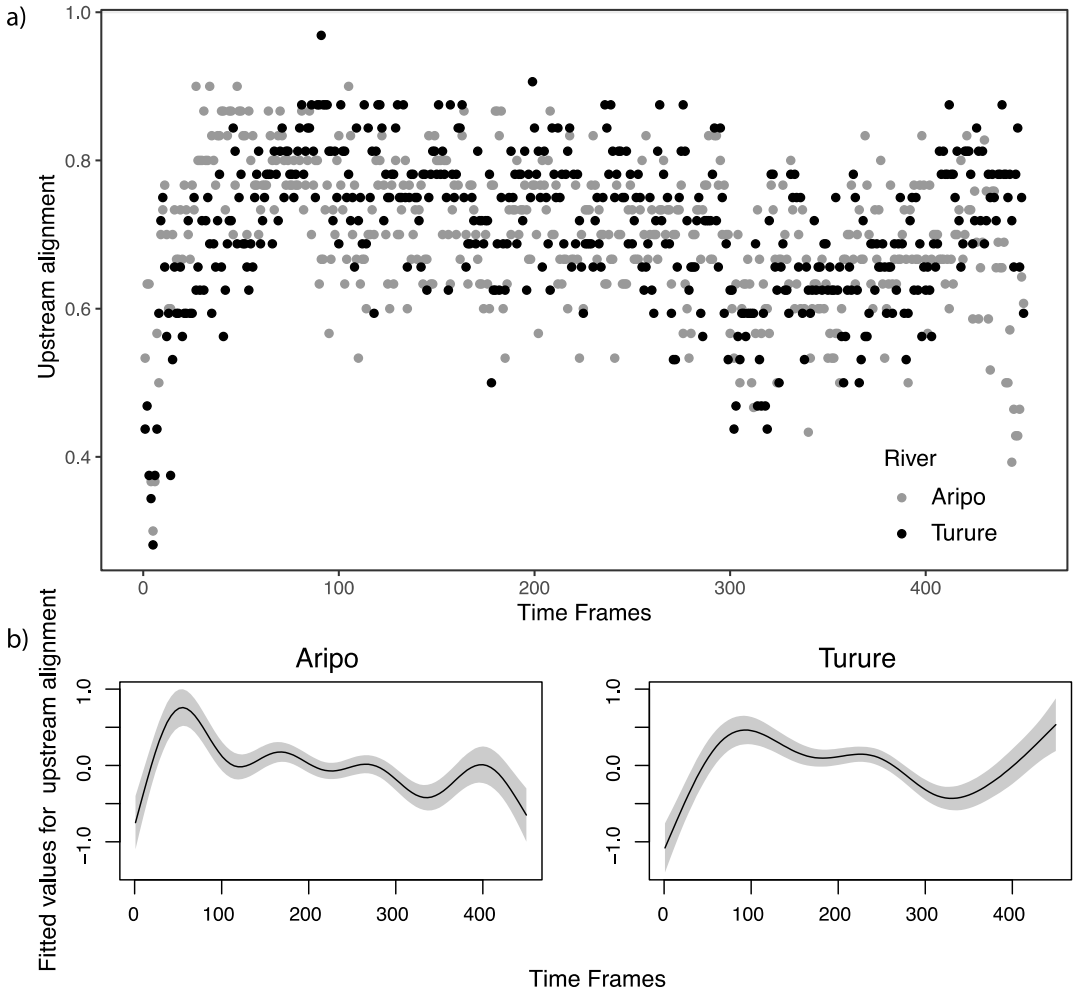

Figure 3.5 Values for alignment of the fish against the flow during the entire duration of the trial (450 time frames = 5 min). (a) Raw values for the two rivers. Maximum value (1) means that all fish were aligned with the flow, minimum value (0) means that none of the fish were aligned with the flow for that time frame. (b) Fitted values with GAM for the two rivers, the grey areas represent 95% confidence bands. Values are centered around 0.

Linking Statement to Chapter 4

The next chapter of this thesis continues with the exploration of variation in rheotactic behavior. In Chapter 3, I found that guppy populations evolved different mechanisms to achieve the same level of positive rheotaxis. In these two populations, the likely factors influencing the difference in behavior were the river physical environment and the speed of water flow. However, individual fish condition should also explain variation in rheotactic behavior.

In Chapter 4, I address how interspecific interactions with parasites can influence movement. In this case, I hypothesize that ectoparasites will negatively impact rheotaxis and thus make them more likely to be displaced downstream in case of strong water currents. I specifically ask: Do parasites affect the rheotactic behavior of guppies from an upstream population? If so, is rheotaxis a function of parasite presence or parasite load?

CHAPTER 4: Parasite load rather than parasite presence decreases upstream movement in Trinidadian guppies. [††]

Abstract

Several factors can influence whether an organism remains in the environment to which it is adapted. For example, the impact of parasites on host behavior, physiology, and morphology, can in turn influence host movement. In rivers, fish have to swim efficiently against the current to maintain their position in the water to avoid being displaced downstream. This behavior is referred to as positive rheotaxis. We hypothesized that both the presence and number of ectoparasites on a host would affect the ability of fish to avoid being swept downstream and thus allow them to remain in their habitat. We used the guppy-*Gyrodactylus* host-ectoparasite model to test whether parasite presence and parasite load had an effect on fish rheotaxis. We quantified rheotaxis of uninfected and infected fish in a circular flow tank in the laboratory at two trials (pre-infection and post-infection). We show that both infected and uninfected individuals expressed similar levels of positive rheotaxis both prior to infection and five to six days after parasite or sham infection. On the contrary, with increasing parasite load guppies covered less distance in the upstream direction and spent more time in slower flow zones. We also found that male guppies spent less time aligned against the flow than females. Our results indicate some of the mechanisms by which parasites impact guppy movement in the wild

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and emphasize their important selection pressure that acts both directly and indirectly on guppies.

Introduction

In spatially variable habitats, individuals that are adapted to their local biotic and abiotic conditions have higher fitness (Kawecki & Ebert, 2004). However, there are a number of factors that can displace an organism away from the habitat to which it is adapted. First, the environment itself can dictate an organisms' movement. External and abiotic factors include wind, water flow, or any physical force that can move an individual away from its preferred habitat (Jonsson, 1991; Vanschoenwinkel *et al.*, 2008). Second, interspecific interactions with predators, competitors, or parasites can also drive an organism to leave its environment (Weisser *et al.*, 2001). For example, poorer competitors will be more likely to emigrate if they cannot access resources and mates (Serrano *et al.*, 2003), and in insects, the presence of predators can induce the production of dispersal-morphs that leave their natal area (Poethke *et al.*, 2010). Interspecific interactions of parasites with their host can also influence an animal to move away from an optimal habitat (Binning *et al.*, 2017). Parasites can alter the behavior of a host by forcing it to move to more predator-exposed areas to increase host transmission (Thomas *et al.*, 2010). Another example of behavioral change is when infected host move into warmer habitat to combat infection (Mohammed *et al.*, 2016; Wisenden *et al.*, 2019).

Guppies, *Poecilia reticulata,* are endemic to Trinidad and live in tropical rivers in the Northern Range that typically receive high rains during the wet season. In this dynamic system with large waterfalls, fish need to be able to swim efficiently against the flow and maintain their position, a behavior referred to as positive rheotaxis (Arnold,

1974; Montgomery *et al.*, 1997). Rheotaxis behavior has been found to vary among guppy populations as guppies located in upper reaches of streams showed more pronounced positive rheotaxis compared with those from lower reaches (Mohammed *et al.*, 2012). In this system, there is a strong selection pressure for upstream guppies to stay above barrier waterfalls and to avoid costs associated with downstream emigration, and guppies located above waterfall show strong positive rheotaxis to counter the water flow (Blondel *et al.*, 2020).

Wild Trinidadian guppies are infected with several species of ectoparasitic monogenean *Gyrodactylus* that attaches to their skin (Xavier *et al.*, 2015). Ectoparasites are external parasites that reduce host fitness by impacting their physiology and morphology (Lehmann, 1993). *Gyrodactylus* is transmitted among fish by direct contact and it has an unusual hyperviviparous reproductive strategy where the uterus of one worm contains a fully-grown embryo that itself contains a developing embryo. These two features enable the worm to quickly and successfully colonize a host. The resulting exponential growth in parasite numbers poses a very significant threat for host fitness and movement (Bakke *et al.*, 2007). *Gyrodactylus* prevalence (percent of infected hosts) and load (number of parasites per host) differ among guppy populations. Parasite number per guppy ranges from one to a dozen (Harris & Lyles, 1992; van Oosterhout *et al.*, 2007; Fraser & Neff, 2010), with outliers that can sometimes reach more than twice the population average (Martin & Johnsen, 2007) and even a hundred (Blondel, pers. observation). The number of ectoparasites on a host can not only cause physical injury but can also create a physical drag, impair movement, and interfere with hydrodynamics directly at the interface between the host and water (Östlund-Nilsson *et al.*, 2005;

Binning *et al.*, 2013, 2014). Among the few studies that have examined the relationship between *Gyrodactylus* infection and host movement, Hockley *et al.* (2014) examined flow preference in the lab and found that infected guppies spent more time in moderate flow and avoided turbulence. Thus, infection with *Gyrodactylus* could influence guppy rheotaxis and whether heavily infected guppies are swept downstream over waterfalls. In a field mark-recapture experiment, van Oosterhout *et al.* (2007) found a decrease of recapture rate by 19% percent with every additional parasite suggesting a negative influence of the number of *Gyrodactylus* on guppy survival and downstream movement in the wild. However, it has not been experimentally tested whether any impact on host movement is related to the simple presence of infection or is a function of the number of parasites on the surface of the fish.

The objective of this study was to investigate the relationship between parasite infection and host movement, using the ectoparasite, *Gyrodactylus turnbulli*, in wild-derived guppies *Poecilia reticulata.* Using a circular-flow tank containing individual guppies, we determined whether infected guppies demonstrated less positive rheotaxis than uninfected guppies, and whether positive rheotaxis changed as a function of parasite load per fish.

Methods

Guppy population

We used second generation lab-born male and female guppies from parents that had been originally collected in the upper Turure River (GPS coordinates: 10.6903, -61.1638) and transported in March 2015 to our laboratory in Montréal, Canada. These fish come from a low-predation environment with only one species of predator, *Rivulus hartii*. One

week before the start of the experiment, guppies were haphazardly selected and assigned to two tanks of 10 fish each for acclimation. The fish in these tanks were fed daily with brine shrimp. One fish in each group died before the experiment, leaving us with 9 fish per group. Each fish was weighed after the first and the second rheotaxis trial. Just after the first rheotaxis trial and for the duration of the experiment, each individual was isolated and maintained in a 1.8L tank within a flow-through system (Aquaneering Inc., San Diego, CA, USA). All fish protocols were approved by the McGill Animal Care Committee.

Gyrodactylus population and infection protocol

A laboratory isogenic strain of *Gyrodacytlus turnbulli* that had been maintained on guppies in our lab since 2013 (Tadiri *et al.*, 2013, 2016) was used as the source of infection. We used an infected female guppy from the parasite-infected guppy population as the donor for experimental infections. The infection procedure involved experimental transfer of one or two haphazardly selected individual parasites from the donor that was anaesthetized in in a solution of 0.02% Tricaine methanesulfonate (MS-222) to an anaesthetized recipient. To record infection dynamics, we anesthetized individual fish in MS-222 every second day and used a dissecting microscope to count the number of *Gyrodactylus* parasites. All control fish were also anesthetized every two days so that they went through the same procedures as the infected fish (sham infection).

Rheotaxis trials

An initial rheotaxis trial was done on all 18 fish and was repeated on each fish, five or six days after sham or parasite infection. The trials were conducted in a circular flow tank

originally designed and used by Blondel et al. (2020) (Figure 4.1). Briefly, we built the tank as a circular chamber, with two pumps located on the either side of the tank to generate a gradient of flow (high flow on the outside, minimal flow on the inside). Prior to each trial, water temperature in the circular flow tank was recorded with two thermometers. The test fish was first acclimated in the tank for 15 min. As soon as the water pumps were turned on, we video-recorded the chamber for 5 minutes with a Logitech C270 webcam located above the tank. At the end of the trial, the pumps were turned off and the fish was allowed to rest for five minutes before being transferred back to its tank. After each trial, we placed a carbon filter for five minutes in the water to filter organic chemicals. We also ran three trials with a small float having the same mass than a guppy (0.40g) to generate reference values that might approximate those of a non-swimming, passive fish. The values that we obtained with this small float represent a guppy that would only be displaced downstream, unable to swim counter-current.

Video analysis

Each 5-minute video was cut into 450 frames using the software Adapter (version 2.1.6) and each frame was analyzed using the software ImageJ (Rasband, 2012) with the plugin MtrackJ (Meijering, 2006). In each frame, the coordinates of the head and the tail were used to determine the position of the fish and to calculate four dependent variables: net displacement, cumulative upstream movement, upstream orientation, and flow regime. Net displacement was defined as the total distance covered by a fish, either in the upstream or downstream direction and was calculated by adding or subtracting the arc length between the fish position in each frame. Cumulative upstream movement was defined as the total distance covered in the upstream direction only, also using the arc

length between two positions in each frame. Upstream orientation was calculated as the percentage of time the fish was aligned within a 45° angle against the flow. Finally, flow regime was calculated as the flow score in each frame (0 for minimal, 1 for low, 2 for medium and 3 for the highest flow zone) averaged over the entire trial.

Statistics

All statistical analyses were conducted using the R language in R studio (RStudio Team, 2016; R Core Team, 2018). We used linear models to test whether rheotaxis performance measured by each of our four dependent variables (net displacement, cumulative upstream movement, upstream orientation and flow regime) was influenced by trial (before vs. after infection), treatment (sham vs. infected), and the trial-by-treatment interaction. We also included sex as a fixed effect, and mean temperature during the trial and fish mass as covariates. When mean temperature and mass were not significant, we dropped them from the final model. We used a log transformation of the data when assumptions of normality and homoscedasticity were not met.

We also used linear models on the subset of our data collected from the infected group during the post-infection trial, to test if parasite load was associated with any of the four dependent variables. As previously, we entered sex as a fixed effect, and mean temperature during the trial and fish mass as covariates. Partial R^2 values were calculated for each fixed effect in the final model using the etasq() function in the *heplots* package (Friendly, 2010).

Results

Summary statistics and infection

Before the start of the experiment, average mass of the fish was 0.08 ± 0.03 g and no difference was detected between those assigned to the control and infected groups. Average mass stayed the same at the end of the experiment, and specifically, infected fish did not lose weight during infection ($t_{(1,16)}$=0.276, P=0.786). All fish that were experimentally infected had parasites when examined 5 or 6 days after infection. The number of parasites ranged from 11 to 100, with an average of 45.8 parasites for females and 56 parasites for males. Overall, all guppies expressed strong positive rheotactic behavior, with a net displacement ranging from -1,170 cm to 424 cm upstream, whereas displacement of the inanimate prop (passive displacement) ranged from -6,236 to -1,472 cm. Results from the pre- and post-infection trials by sex and by parasite load is shown in Figure 4.2.

Linear models for parasite presence

Neither presence of infection, nor pre- vs post trial, nor their interaction had any effect on any of the four rheotaxis measures (Table 4.1). However, infected guppies tended to spend less time aligned against the flow (upstream orientation) in the second trial, whereas controls tended to spend more time aligned against the flow. Of additional interest, regardless of infection or trial, females spent almost 9% more time aligned against the flow than males (Table 4.1, Figure 4.3).

Linear models for parasite load

Within the infected group of fish, parasite load was not associated with net displacement nor with upstream orientation during the second trial (Table 4.2). However, fish with higher parasite load had lower cumulative upstream movement and spent more time in slowest flow regions of the tank (Figure 4.4). That is, with every additional 10 parasites, guppies covered 8% less distance in the upstream direction ($t_{(1,7)}$= -3.560, P=0.016) and obtained a flow zone score that was 10% less ($t_{(1,7)}$= -2.828, P=0.037).

Discussion

Our results indicate that guppy rheotaxis responds to parasite load but not the simple presence/absence of infection. Parasite presence did not have a detectable effect on any of the four rheotaxis measures, although we observed a decrease in upstream orientation between the first and second trial for infected fish. Finally, high loads of parasites negatively influenced distance covered in the upstream direction (cumulative upstream movement) and flow regime score.

Infected and uninfected guppies had the same levels of positive rheotaxis, indicating that we were not able to detect an effect of parasite presence on guppy rheotaxis. This could partly be explained because we found more variability in the outcome measures among infected fish than in the control fish. This variability could be attributed to several factors. One example is size, which has been found to explain variation in swimming behavior relative to velocity and turbulence, with larger guppies spending more time in high velocity regions (Hockley *et al.*, 2014). However, in our study, guppy mass (highly correlated with body length) did not influence rheotaxis or flow use in the circular-flow tank. Another factor influencing variation in rheotaxis could be the difference in

morphology between males and females, because males have longer tails and display poorer swimming performance (Karino *et al.*, 2006). Here we found that males and females covered similar distances in the upstream direction, and used on average the same flow zones, but that males orientated themselves 9% less of the time against the flow than females (Table 1, Figure 3). In addition to morphological differences, male and female guppies often differ in their movement behavior as females have a tendency to shoal more (Croft *et al.*, 2003b) whereas males spend more time moving between pools searching for mates (Croft *et al.*, 2003a). In our experimental setup, males could also travel more often among different areas of the tank, resulting in less time aligned against the flow.

Although the presence of *Gyrodactylus turnbulli* did not significantly affect guppy rheotaxis, parasite load of infected individuals did. The negative association between parasite load and cumulative upstream movement and flow regime suggests that *Gyrodactylus* affects both guppies behavior and performance. This result confirms previous findings by Hockley *et al.* (2014) who found that small guppies with higher parasite burden spend more time in moderate velocity regions, and by van Oosterhout *et al.* (2007) who found that in nature, males with higher parasites load were less likely to be recovered in a mark-recapture experiment. Several mechanisms could explain the lower cumulative upstream movement and flow regime score in the more heavily infected guppies. First, high *Gyrodactylus* load can cause fin clamping and unusual swimming behavior (Cable *et al.*, 2002), although we did not observe clamping of our infected fish and did not observe erratic behavior before the rheotaxis trials. Second, the number of parasites could directly increase the friction drag when the fish is swimming, thereby

making upstream swimming harder for the fish. Third, infected guppies typically show an increase in mucus production (Cone & Odense, 1984), as well as a thickening of the epidermis (Gheorghiu *et al.,* 2012). This accumulation of mucous on fish skin as a result of infection could also increase the friction drag. Finally, it is possible that as a behavioral response, heavily infected fish spent more time in low flow zones to reallocate the energy that they dedicated to swimming toward fighting infection instead.

The consequences of having reduced ability to swim upstream are multiple. Guppies that swim less efficiently in the upstream direction are more likely to be swept downstream over any barrier waterfall and thus unable to migrate back upstream. This downstream emigration has negative consequences as upstream guppies have lower fitness in more downstream parts of the river, because of sexual selection against immigrants (Weese *et al.*, 2011) and also because upstream guppies lack adaptations to predators that are present below waterfalls (Reznick & Endler, 1982). Furthermore, if the fish that are swept downstream are the more heavily infected, parasite transmission in downstream population might go up. This result fits with the more general hypothesis that fish in lower watercourses have more parasites because of the unidirectional river flow (Blasco-Costa *et al.*, 2013). Our study confirms part of the mechanisms by which infected guppies with high parasite load are displaced and highlight the strong selection pressure exerted by ectoparasites on fish movement.

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Table 4.1 Output of the linear models on the full dataset. For each measure, we tested the effect of sex, treatment (infected vs. sham), trial (pre- vs. post-infection) and the trial-by-treatment interaction. Significant *P*-values are in bold.

	df	***F***	***P***
Net displacement			
Sex	1	0.167	0.686
Treatment	1	0.168	0.684
Trial	1	0.947	0.338
Trial x Treatment	1	0.107	0.746
Log cumulative upstream movement			
Sex	1	1.890	0.179
Treatment	1	1.078	0.307
Trial	1	0.511	0.480
Trial x Treatment	1	1.465	0.235
Upstream orientation			
Sex	1	6.072	**0.019**
Treatment	1	2.734	0.108
Trial	1	0.438	0.513
Trial x Treatment	1	1.015	0.322
Flow regime			
Sex	1	0.001	0.976
Treatment	1	2.229	0.146
Trial	1	0.548	0.465
Trial x Treatment	1	1.350	0.254

Table 4.2 Output of the linear models on the subset of infected individuals. For each measure, we tested the effect of parasite load and sex. We also included mean temperature and mass as covariates. When mean temperature and mass were significant (or close to significance), they were kept in the model. Significant *P*-values are in bold.

	Estimate	***t***	***P***
Net displacement			
Parasite load	4.002	0.882	0.412
Sex	130.408	0.496	0.637
Log cumulative upstream movement			
Parasite load	-0.008	-3.560	**0.016**
Sex	0.050	0.310	0.769
Mean temperature	0.196	2.213	0.078
Upstream orientation			
Parasite load	-0.027	-0.393	0.708
Sex	-1.600	-0.394	0.707
Flow regime			
Parasite load	-0.010	-2.828	**0.037**
Sex	0.189	0.757	0.483
Mean temperature	0.345	2.518	0.053

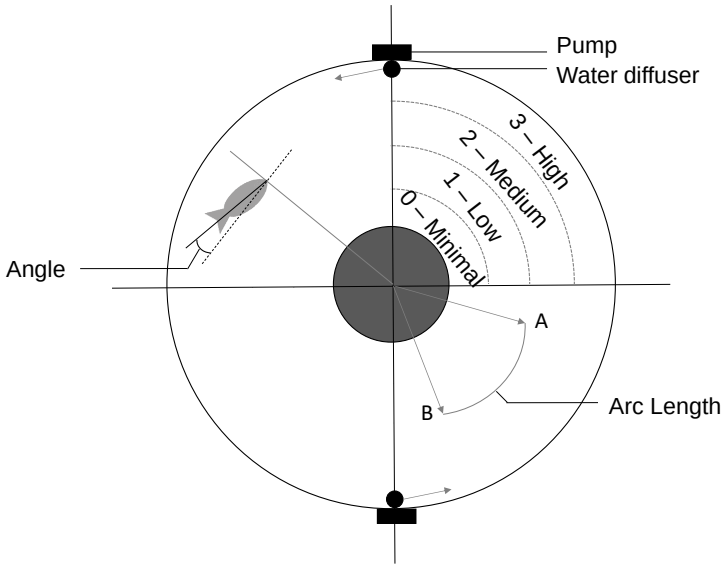

Figure 4.1 Overview schematic of the circular-flow tank. Tank dimensions: testing chamber diameter = 65 cm; inside diameter = 25 cm, and water depth = 16 cm. In lower right quadrant is represented the arc length measure used to calculate net displacement and cumulative upstream movement. In upper right quadrant is represented the four flow zones. In upper left quadrant is represented the angle against the flow measured to calculate upstream orientation. Reproduced with permission from Blondel et al. (2020).

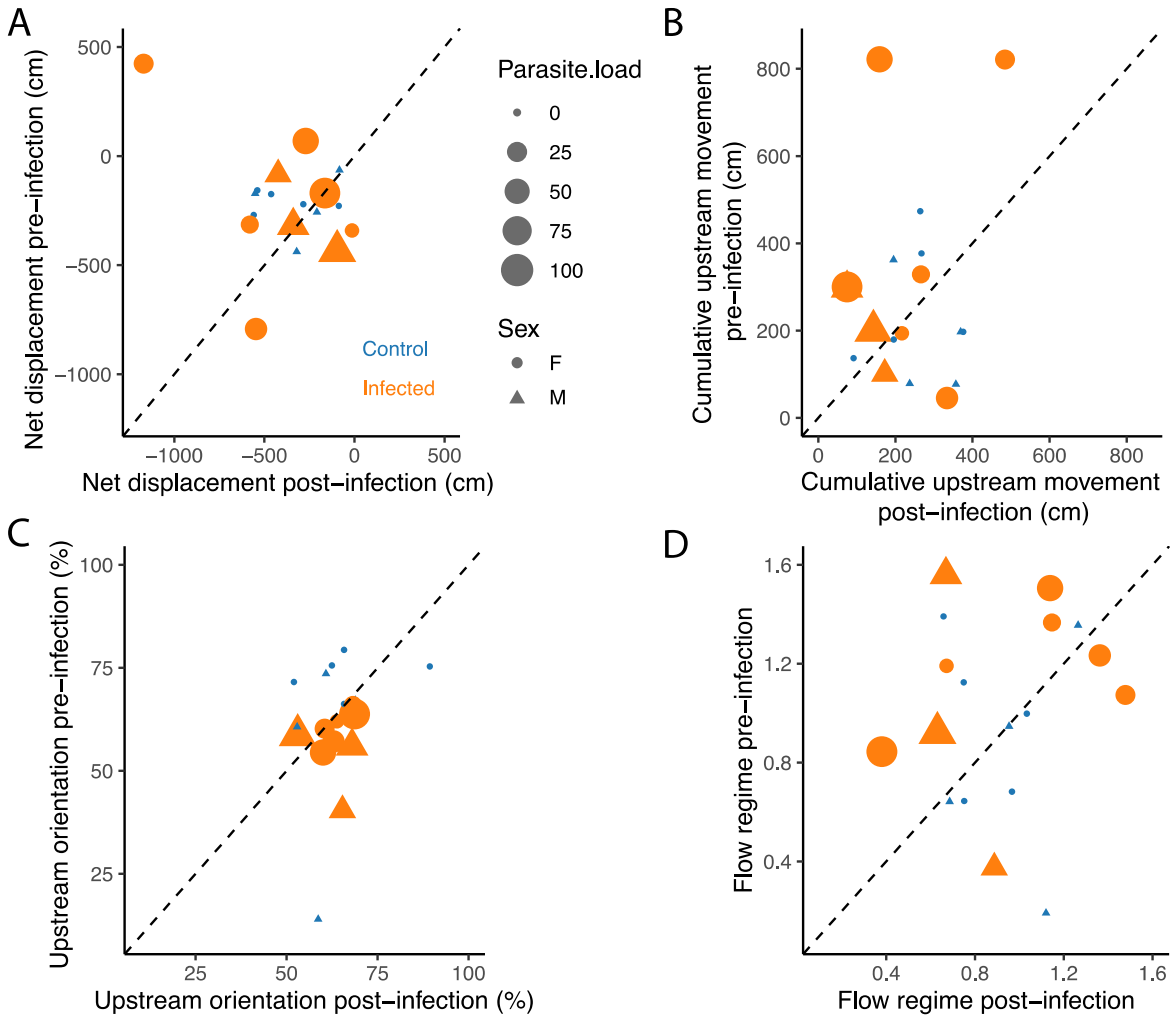

Figure 4.2 Individual values for net displacement (cm), cumulative upstream movement (cm), upstream orientation (%) and flow regime between control and infected fish, before and after infection. Points above the dotted one:one line represent an increase post-infection, points below represent a decrease. Size of points represent the parasite load when infected fish were tested post-infection. Shape of the points represent females (circles) and males (triangles).

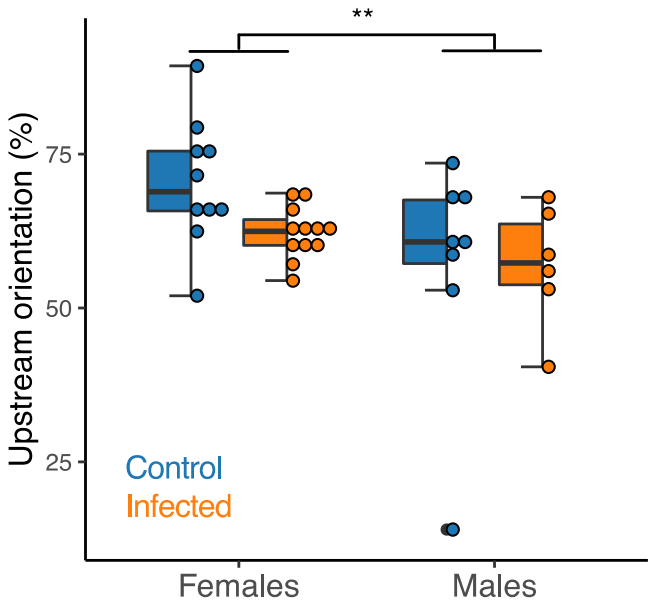

Figure 4.3 Upstream orientation by sex and by treatment for both trials combined. Half box plot (left) represents the distribution of the data (the median, first and third quartile, and the minimum and maximum). Half dot plot (right) shows another representation of the data, whereby each dot represent one observation.

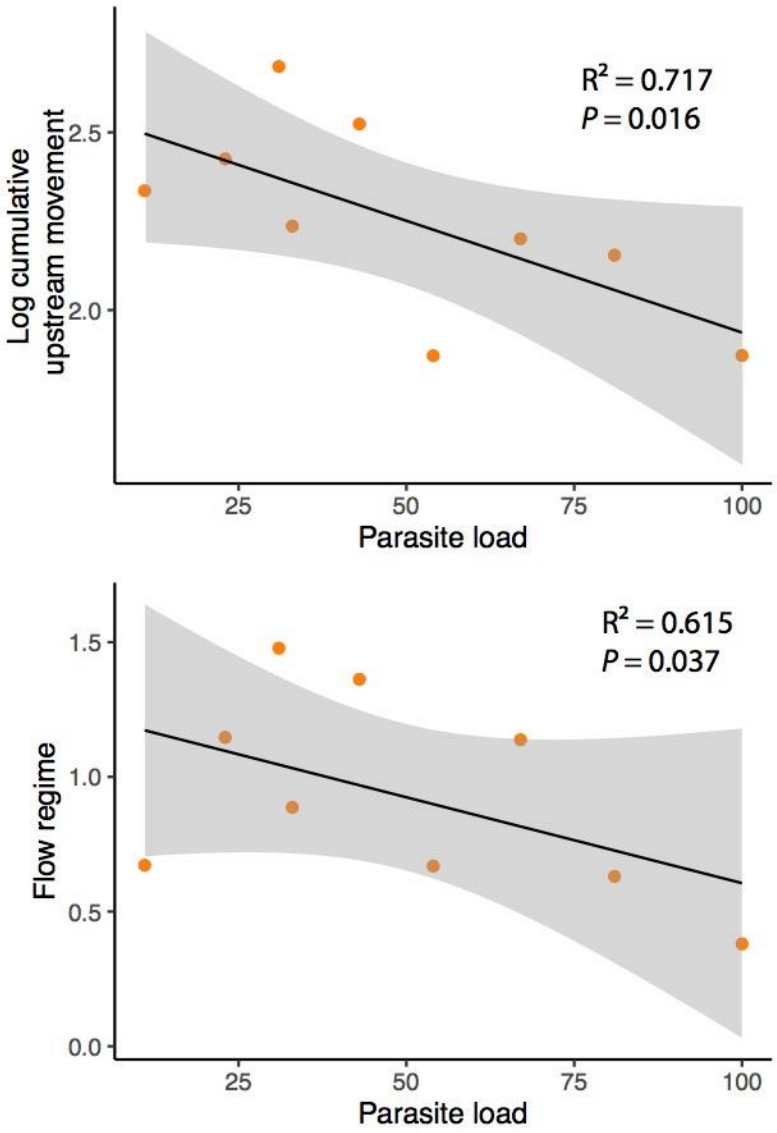

Figure 4.4 Linear model controlling for sex and mean temperature between parasite load and cumulative upstream movement (upper panel), and flow regime (lower panel) based on the subset of infected individuals. Partial R^2 and *P*-values come from the linear models.

General conclusion

"In nature nothing exists alone."
– Rachel Carson

In my thesis, I integrated several key processes about animal movement by characterizing the movement of genes inferred through population genetic structure, the movement of individual fish by looking at behaviors that enable them to stay in their population, and the impact of disturbance on movement, whether it is a massive flood that reshapes the river landscape, or a smaller and inconspicuous disturbance in the form of ectoparasites. In this general conclusion, I briefly summarize the main findings of each chapter and the consequences for guppies in particular and for movement in general. I also consider the questions that were raised by each chapter and discuss how they may be addressed in future work.

How does gene flow vary spatially and temporally?

In my first chapter, I described the population genetic structure of guppy populations in two watersheds and looked for correlations with spatial patterns of male color variation. I confirmed previous findings that most gene flow was in the downstream direction, and that genetic diversity was lower in upper than in lower reaches of the streams (Shaw *et al.*, 1991; Carvalho, 1993; Crispo *et al.*, 2006; Barson *et al.*, 2009; Willing *et al.*, 2010). I documented two regions where guppy neutral genetic variation was similar across watersheds. These two regions represent locations where gene flow events were either contemporary or more ancient. My results help to disentangle the role of contemporary and historical gene flow in

shaping population genetic structure and pave the way for more studies on the effect of gene flow on adaptation in wild populations experiencing high levels of selection. For example, it would be useful to investigate other traits under selection in the populations where we identified gene flow. In guppies, sexual and natural selection are both acting on many traits such as courtship behavior, coloration, infection status or morphology (de Lira *et al.*, 2018). Identifying the regions of the genome that are under selection, and determining if these regions are parallel (or not) would provide evidence for local adaptation (or for multiple evolutionary solutions to similar selection pressures) (Bolnick *et al.*, 2018).

Often, gene flow will be measured at one time among a few populations. However, as the first chapter shows, there is variation in population admixture at the same sites among years. Given these results, I examined the role of massive disturbance in the stability of meta-population structure and phenotypic variation in the same two watersheds. My results reveal that genetic diversity is mostly resistant to disturbance events. On the contrary, phenotypic diversity is less resistant, but shows resilience after a few years. Specifically, I observed that after the floods, guppy body length decreased in most of the sites. A next logical step into the investigation of the effect of the flooding would be to look for signatures of selection in the guppy genome, especially in the site where guppies were the most impacted (downstream main stem of the Paria). Quantifying selection would help to understand the biological effects that increasing natural disasters can have on the survival of species around the world. For example, green anole lizards experienced an extreme cold winter event in 2013-2014 (Campbell-Staton *et al.*, 2017). This intense weather event targeted regions of the genome that were also involved in local adaptation. Measuring the intensity of selection has important implications on our understanding of how species can be resistant or resilient to such events.

How is rheotaxis linked to fish dispersal?

The important conclusions that we can draw from my dissertation regarding rheotaxis are that upstream guppies have behavioral adaptations to water flow, which enable them to stay above waterfalls. In chapter 3, I demonstrated that guppies actively and effectively swim against the flow and use slower flow zones when first confronted with a sudden increase of water current. Interestingly, I also found that the two populations studied in this chapter achieved the same performance of positive rheotaxis but through different behaviors. Following these results, I investigated in chapter 4 a potential negative impact of ectoparasites on rheotaxis, which would have consequences on the above mechanisms to avoid downstream displacement. I performed experimental infections in the lab and did not detect an effect of infection presence only, but that parasite load negatively influenced positive rheotaxis.

These new findings also raise new questions: Is rheotaxis consistent over time? Is it heritable? Our results provide hints of answers to these two questions. First, about repeatability: in chapter 4, we measured individual rheotaxis twice, once before infection and once after. There was no difference between the two trials, suggesting that guppy individual rheotaxis is repeatable. Following this finding, another interesting topic to explore would be to determine whether rheotaxis is correlated with personality, or behavioral syndromes. Behavioral syndromes are a suite of correlated behavior across situations (Sih *et al.*, 2004). In the context of rheotaxis, one could hypothesize that bold individuals spend more time in higher flow zones, in contrast to shy individuals that stay in lower flow zones. One way to address this would be to conduct several experiments using individual fish and determine if rheotaxis is correlated with other tests of

boldness/shyness. Second, about heritability: our results hint to the genetic basis of rheotaxis. In chapter 3, we measured rheotaxis of wild guppies and their first generation (F1) offspring. We found that both generations displayed strong positive rheotaxis but that F1 fish spent more time in slower flow zones. If rheotaxis was entirely plastic in guppies, F1 fish would not have shown such strong rheotaxis after being maintained from birth in a flowless tank environment. A formal analysis of rheotaxis performance through the study of the second generation (F2) would also help resolve the effects of maternal effects on the response to water currents.

Implications

My dissertation helps to deepen our understanding of fish movement in a dendritic river network and how this movement is directly related to gene flow, local adaptation and metapopulation structure. While positive rheotaxis is playing a key role in population persistence over waterfalls, disturbances like massive floods or inconspicuous ectoparasites can impair movement and lead to passive dispersal in downstream environments. This dispersal has consequences for the fish itself, whether the emigrant is going to be able to survive and reproduce, but also for the downstream population, which can receive not only an influx of genes, but also of parasites. As species are facing more threats through climate change, anthropogenic disturbances or fragmentation, it is crucial to understand how movement in the form of gene flow also affect adaptive divergence. The results of my thesis increase our knowledge on behavior, evolutionary ecology and population genetics.

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