Genetic Population Structure of the Trinidadian Guppy (*Poecilia reticulata)* across Trinidad and Tobago

by

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Abstract

The Trinidadian guppy, *Poecilia reticulata*, is a tropical freshwater fish with a long history as a model species for the study of evolution and adaptation to changing environments. The guppy is widespread in Trinidad, and many rivers on the island are host to multiple populations subject to varying levels of predation. Population structure in the guppy is influenced by several factors, including colonization history, presence or absence of barrier waterfalls within rivers, and both documented and accidental human-mediated introduction events.

This study used genetic data from both microsatellite markers and mtDNA to investigate guppy population structure in 25 rivers and lakes across Trinidad and Tobago, with particular focus on the north shore Marianne and Paria Rivers. Most sites were located in the Northern Range Mountains of northern Trinidad, where rivers are divided into three major aquatic areas – the Caroni drainage, the Oropouche drainage, and the north shore. Results show a deep genetic divide between populations in the west-flowing Caroni drainage and those in the east-flowing Oropouche drainage, likely due to the colonization of these two drainages from two separate branches of the Orinoco, a large river located on the South American mainland. On Trinidad's north shore, guppies collected in rivers on the western side of the island appeared to be genetically related to Caroni drainage guppies, while those in rivers on the eastern side of the north shore were predominantly related to Oropouche drainage guppies but showed evidence of admixture from the Caroni. Detailed study of Marianne and Paria River guppy populations showed downstream-biased gene flow in both rivers, with waterfalls in the Marianne limiting the movement of guppies in that river. Evidence of migration between the Marianne and Paria River watersheds was also found at two separate locations.

List of Abbreviations Used

A	Number of alleles per locus
A_E	Allelic richness
AMOVA	Analysis of molecular variance
Bp	Base pairs
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
F	Frequency of the most common allele
H_E	Expected level of heterozygosity
H_o	Observed level of heterozygosity
HPD	Highest posterior density
HWE	Hardy-Weinberg equilibrium
MCMC	Markov chain Monte Carlo
mtDNA	Mitochondrial DNA
N	Number of individuals sampled
PCoA	Principle coordinates analysis
PCR	Polymerase chain reaction
PHWE	Probability of Hardy-Weinberg equilibrium
R	Allelic size range (measured in bp)
SDS	Sodium dodecyl sulphate
SNP	Single nucleotide polymorphism
UPGMA	Unweighted pair group method with arithmetic mean

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This thesis represents the culmination of three long years of hard work and the assistance of numerous people, such that it is nearly impossible to thank everyone appropriately. Nonetheless, I would like to extend my thanks first and foremost to my supervisor Paul Bentzen, for his guidance, patience, and willingness to share his knowledge. I would also like to thank all the members of my supervisory committee – Daniel Ruzzante, Jeff Hutchings, and Andrew Hendry – for taking the time to consider my project and to offer helpful feedback and suggestions for improvement.

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Chapter 1: Introduction

1.1 The Trinidadian Guppy

The Trinidadian guppy, *Poecilia reticulata*, is a small freshwater fish native to the coastal rivers and streams of Venezuela, Guyana, Suriname, and Trinidad and Tobago (Magurran, 2005). *P. reticulata* is indigenous to north-eastern South America, but can now be found worldwide in both freshwater streams and ponds and in aquaria. No doubt due to its hardy nature and high rate of evolution the guppy has been a popular aquarium fish for more than a century, and enthusiasts in North America and Europe have developed many domesticated forms of the species through extensive breeding. Aquarium guppies are generally bred to display bright colours, elaborate marking patterns, and huge ornate tailfins, often resulting in strains that scarcely resemble their wild relatives (Magurran, 2005). Over the same time period guppies have also been transported to streams, lakes, ponds, and wells around the world to help prevent the spread of malaria by controlling mosquito populations. The use of insectivorous fish for this purpose predates the development of modern pesticides and is currently employed in order to combat pesticide-resistant insects and to avoid the damaging environmental effects associated with chemicals such as DDT (Ghosh & Dash, 2007).

In addition to its popularity as an aquarium fish and usefulness in controlling insects, *P. reticulata* has an extensive history as a model species in the scientific study of population structure and variation, having been used to investigate morphological, behavioural, and life history trait changes in response

to environment and predation (Endler, 1980; Magurran & Seghers, 1990; Reznick & Endler, 1982). The appeal of the guppy as a model organism is largely due to its high rate of evolution and ability to rapidly adapt to environmental changes. One experiment, in which guppies were transplanted from a high predation environment to one with fewer predators, resulted in visible alterations to pattern and colouration in males that could be observed after the passage of only two years (Endler, 1980). Life history traits can also evolve rapidly – a recent experiment found that juvenile guppies from an established transplanted population had a higher survival rate in their new habitat when directly compared to juveniles from the original ancestral population after a period of just eight years, or about 13-26 generations (Gordon *et al*., 2009). The island of Trinidad has long provided an ideal location for this type of experimentation due to the wide variety of easily accessible guppy habitats found within the relatively small, contained geographic area of the island (Magurran, 2005).

1.2 Distribution of Guppies on Trinidad

On Trinidad, populations of guppies are found in almost every river, stream, and pond, regardless of size, turbidity, or water pollution level (Magurran & Phillip, 2001). These fish are often extremely abundant and are locally known as ‘millions fish’ due to their near-ubiquitous presence. Nonetheless, there are some remote rivers on the island completely devoid of *P. reticulata*, most likely because opportunities to colonize these areas have been limited (Magurran, 2005). Still, most rivers on the island are host to multiple distinct guppy populations, often separated either by distance or by large natural barriers such

as waterfalls. The structure of these populations tends to be relatively stable even over long periods of time, although gene flow, particularly in the downstream direction, is also apparent among many of them (Haskins *et al*., 1961). Often the presence of one or more physical barriers in the river renders upstream migration essentially impossible for small species such as guppies while heavy tropical rainfalls cause considerable downstream migration, effectively flushing the majority of guppies into downstream areas. This results in upstream populations that are smaller and typically less genetically diverse than their downstream counterparts (Barson *et al*., 2009).

Despite downstream gene flow, the physical barriers formed by waterfalls generally result in upstream and downstream guppy subpopulations that are markedly divergent in terms of phenotype, behaviour, and life history strategy. Much of this divergence is apparently driven by differing levels of predation above and below the falls. Downstream, near the mouth of the river, guppies are exposed to several large piscivorous fish species such as the pike cichlid, *Crenicichla alta*; the blue acara, *Aequidens pulcher*; and the two-spot sardine, *Astyanax bimaculatus* (Magurran, 2005). These large predatory fishes are excluded from the upstream regions of the river due to their inability to migrate over stream barriers, leaving upstream guppy populations exposed to much lower levels of predation (Reznick, 1996). Often the only fish that preys on guppies in upstream areas is the killifish *Rivulus hartii*, which is capable of moving upstream over small waterfalls and possibly over land during periods of heavy rain (Haskins *et al*., 1961). Unlike larger predatory fishes which will

preferentially prey on adult guppies, the smaller size of *R.hartii* makes it capable only of eating smaller juvenile guppies (Rodd & Reznick, 1997).

Guppies living in high predation areas have typically developed many traits that either facilitate predator avoidance or are the result of the selection pressure caused by predation. High predation males are usually less colourful than their low predation counterparts, and therefore are better camouflaged against the background of stream bed gravel. Males at lower predation sites, free from the dangers posed by large predators, display brighter colours and more conspicuous patterns in response to sexual selection, as females preferentially mate with the most colourful males (Endler, 1980). Under the threat of high predation guppy populations tend to be made up of individuals that mature faster, reproduce more frequently, and have a higher number of smaller offspring compared to populations at low predation sites (Reznick & Endler, 1982). Schooling behaviour is also more common under high predation regimes, probably because it allows for increased vigilance and coordinated escape from predators (Magurran & Seghers, 1990). Furthermore, high predation sites generally contain many small, immature guppies, whereas lower predation sites have an increased proportion of large, mature individuals (Rodd & Reznick, 1997).

Almost all the research on guppies has been undertaken in the steep mountains of the Northern Range, which form the northern portion of Trinidad. Within this region rivers are roughly divided into three aquatic areas – the Caroni, the Oropouche, and the north shore. The Caroni drainage flows towards the

west coast of the island and empties into the Gulf of Paria, which separates Trinidad from the Venezuelan mainland. The Oropouche drainage empties into the Atlantic Ocean on the east coast of the island. Across the north shore there are a number of separate rivers that are unconnected but all flow north into the Caribbean Sea.

1.3 Overview of Thesis

This thesis examines population structure in guppy populations on Trinidad, on both a broad and a fine scale, using molecular genetic techniques. Patterns of population structure will be used to clarify the possible colonization history of the island and identify areas where migration within or between rivers is occurring. Samples included in this study were collected from many sites across the island, including a number of locations on the north shore as well as several rivers within each of the Caroni and Oropouche drainages. This extensive coverage of the island includes some areas that have been outside the scope of previous genetic studies and as a result these data should allow for conclusions about colonization and migration that have not been possible in earlier work.

Chapter 2 consists of a broad geographic survey of guppy populations from 24 rivers and lakes across Trinidad as well as a single river on the nearby island of Tobago. Both nuclear and mitochondrial DNA (mtDNA) are used in this survey, and results suggest that generally populations within each of the two major drainages are more closely related to each other than they are to populations in the other drainage. However, there are exceptions to this pattern, most likely caused by the artificial introduction of guppies from the Caroni into the

Oropouche. With these exceptions taken into account, it is apparent that the Caroni and Oropouche drainages contain populations that are highly divergent from each other, possibly due to the colonization history of Trinidad. Guppy populations in rivers along the north shore of the island seem to have a complex relationship with populations elsewhere on the island, and in some cases their connection to populations in the Caroni and Oropouche is not clear. Nonetheless, a general pattern emerges in which populations on the western side of the north coast are genetically similar to populations in the Caroni watershed, while those on the eastern side are predominantly related to the populations of the Oropouche but show evidence of genetic admixture from the Caroni in several locations.

Chapter 3 is focused on a more detailed investigation of guppy populations within two neighbouring rivers on the north shore of the island, the Marianne and the Paria. While most rivers were sampled only at two sites – one high predation and one low, the Marianne and Paria Rivers were sampled extensively throughout their length to investigate fine-scale patterns of dispersal and population structure. Results suggest that gene flow within rivers occurs mostly in the downstream direction and is somewhat impeded by the presence of waterfalls. There is also evidence of both recent and long-term migration between these two rivers at two separate locations.

Chapter 2: Island-wide Population Structure in the Guppy

2.1 Introduction

2.1.1 Previous Studies

Past studies have frequently demonstrated that Trinidadian guppies from different populations often differ dramatically in terms of behaviour such as schooling and aggression (*e.g*. Magurran & Seghers, 1990), life history traits such as size and fecundity (*e.g*. Reznick & Endler, 1982), and morphological factors such as patterns of colouration (*e.g*. Endler, 1980). Often the most marked differences have been observed when comparing populations subject to high levels of predation to those that coexist with few predators. Experiments designed to investigate population structure using neutral genetic markers have similarly found a great deal of variation among populations. High genetic divergence has often been noted between high and low predation populations in the same river, but perhaps the most notable divide is typically seen when comparing populations between the major watersheds.

The first studies of neutral genetic variation in naturally occurring guppy populations on Trinidad were carried out in the late 1980s and early 1990s using information from allozymes and mtDNA. Carvalho *et al*. (1991) collected allozyme data on fish from multiple sites within each of five rivers – two in the Caroni watershed, one in the Oropouche, and two on the north shore. Their results indicated detectable divergence between sites within each river, which was more pronounced when those sites were separated by an obvious physical barrier such as a waterfall. High genetic divergence was observed between

Caroni, Oropouche, and north watersheds, with an average of 66% of the total genetic diversity attributed to differences between drainages. In addition, the authors observed that populations in the north coast rivers were much more like Caroni populations than like Oropouche. Shaw *et al.* (1991) also used allozymes to study guppies collected in six rivers throughout the Caroni and Oropouche and concluded that populations in rivers within the same drainage were more alike than they were to populations taken from the opposite drainage. Fajen and Breden (1992) sequenced the mtDNA control region in fish from six Trinidadian rivers and similarly saw a clear division between watersheds. Despite this division they confirmed that north shore river populations were much more closely related to those in the Caroni than to those in the Oropouche. All three of these studies share the common conclusion of considerable genetic differentiation between guppies in the Oropouche drainage, which drains into the Atlantic Ocean on the east side of the island, and those in the Caroni drainage, which drains into the Gulf of Paria on the west side of the island.

Past Trinidadian guppy research has also included several artificial introduction experiments in which fish were moved to a new site either within the same river or in a different river. On several occasions high predation guppies were introduced to low predation locales in order to assess behavioural and genetic changes over time, and in one experiment a pregnant female was transplanted into an uncolonized pond to determine whether a new population could be founded by a single individual (Carvalho *et al*., 1996). One of these experiments in particular resulted in a notable exception to the general pattern of

differentiation between Caroni and Oropouche guppies. In 1957, C.P. Haskins moved 200 Guanapo guppies into the Turure River, thereby introducing Caroni genotypes into the Oropouche drainage. The aftereffects of this introduction were still evident many years later, as one allozyme study found that Turure guppies were genetically more similar to Caroni watershed guppies than to Oropouche (Shaw *et al*., 1991), an exception to the normally deep genetic divide between the two drainages.

The natural differentiation between the two drainages may be a reflection of the history of the island – Trinidad and Tobago were both once part of the land mass of South America, only being separated from the mainland by rising ocean levels at the end of the last ice age (Magurran, 2005). Until that time the rivers of Trinidad were likely an extension of the large Orinoco delta that now terminates on the east coast of Venezuela (see Figure 2.1), and the Caroni watershed area probably formed a small part of the northern Orinoco delta (Carvalho *et al.*, 1991). As sea levels rose a large, deep bay developed in what is now the Gulf of Paria, leaving only a small land bridge connecting the island to the mainland. The newly isolated Caroni drainage rivers on Trinidad flowed into this bay, as did northern parts of the Orinoco delta such as the Rio Manamo. At the same time, southern branches of the Orinoco flowed around what is now the south and east of Trinidad, passing by the mouth of the Oropouche watershed (Magurran, 2005). The northern and southern branches of the Orinoco River may have carried two separate *P. reticulata* populations to Trinidad, resulting in the genetically distinct populations that inhabit the modern Caroni and Oropouche watersheds. The

Figure 2.1: Map of a portion of the north-east coast of South America, showing the location of the islands of Trinidad and Tobago and the Orinoco River delta.

same ‘two-arc’ colonization model has also been proposed to explain the distribution pattern of reptile species on Trinidad (Boos, 1984).

Colonization of the Caroni by guppies from the northern branch of the Orinoco may be ongoing – even with the island completely isolated from the continental land mass, individual guppies may occasionally be capable of surviving the 20-30km journey from the Orinoco delta across the Gulf of Paria to the rivers of Trinidad, particularly if they are flushed out of the Orinoco during the rainy season when salinity in the ocean is lowered (Carvalho *et al*., 1991). Although guppies have not been observed crossing the gulf, evidence suggests that individuals from other freshwater fish species have survived this journey. Several specimens of freshwater fish common in South America but not normally found on Trinidad have been caught in rivers on the southwest peninsula of the island, implying that they migrated across the gulf from the Orinoco River or other nearby rivers on the mainland (Alkins & DeSouza, 1984).

The two-arc hypothesis of colonization, as well as the genetic relationship between mainland and Caroni guppies, gained support from Alexander *et al*. (2006), who used both nuclear and mitochondrial DNA sequences to investigate the connection between guppy populations on Trinidad and those found in the rivers of Venezuela, Guyana, and Suriname. The results of this study showed that guppies from rivers in the Caroni drainage were closely related to those sampled in Venezuela, whereas guppies from the Oropouche drainage formed their own genetic cluster not strongly associated with any population found on the mainland but instead loosely linked to more southerly mainland populations from

Guyana and Suriname. This again implies that two separate colonization events were responsible for founding populations in the two watersheds, resulting in a deep genetic divide between populations in the Caroni and those in the Oropouche.

The extent of this division has prompted some to propose that this represents a speciation event, and therefore the guppies found in rivers of the Oropouche drainage, such as the Quare, are actually a different species than the *Poecilia reticulata* found on the rest of the island (Schories *et al*., 2009). Based on sequence divergence in the mitochondrial control region and cytochrome b gene, the authors maintain that this species diverged from *P. reticulata* and a third guppy species, *Poecilia wingei* (Endler's guppy), between 0.4 and 5 million years ago. They have dubbed the newly identified species *Poecilia obscura* and claim it can be distinguished from other guppy species both by genetic differences and through minor morphological differences in the gonopodium and caudal peduncle.

Several recent studies suggest that while the two-arc hypothesis may explain much of the genetic divergence on the island, a more complex history probably underlies the colonization of the northern rivers. The rivers that flow north into the Caribbean Sea differ from others on Trinidad in that they contain a distinctive suite of predators; one typically made up of diadromous fishes common to the Antilles, such as mullets and gobies, rather than the South American species found elsewhere (Reznick, 1996). This difference in predation regime coupled with the inaccessible location of the north coast rivers may

present an obstacle to colonization, as evidenced by several rivers along the shore that are entirely without guppies (Magurran, 2005).

Single nucleotide polymorphism (SNP) and microsatellite data have shown that guppy populations found in at least some of the rivers on the north coast appear to form a genetic cluster that is distinct from populations in either the Caroni or the Oropouche (Willing *et al*., 2010; Suk & Neff, 2009). Despite the formation of a separate northern cluster, SNP based research still showed admixture between the guppies in the north shore Yarra River and those in the rivers of the Caroni drainage, so some amount of ongoing migration may still link these areas (Willing *et al*., 2010). Despite the expected divide between watersheds, evidence of movement between the Caroni and Oropouche was also found in some locations. Although some crossover between the two drainages is likely a result of earlier introduction experiments, some gene flow may be a result of migration from one river to another as a result of flooding, undocumented anthropogenic activity, or relocation of individual fish by avian predators (Suk & Neff, 2009).

2.1.2 This Study

This study aims to further investigate the relationships between *P. reticulata* populations in the Caroni and Oropouche watersheds, as well as those found in rivers across the north coast of Trinidad. Therefore it includes collections from several new sites that have not been included in other population structure studies, as well as samples from rivers such as the Yarra, Marianne, and Paria, which have frequently been sampled in the past. New samples are

largely from rivers on the north shore of the island: both those to the west of the Yarra River and those to the east of the Madamas River (see Figure 2.3 for locations). Also included are populations from Pitch Lake and the Stollmeyer River, a natural asphalt lake and a small river both located on the southwestern peninsula of the island (see Figure 2.2). The data used in this study were taken from 10 microsatellite loci and a DNA sequence from the mitochondrial control region.

Previous work on the population structure of Trinidadian guppies has generally been either focused exclusively on small areas of the island, or has entailed broad-scale comparison of guppies from South America as well as Trinidad. Most previous studies have also made use of a lower number of microsatellites or other, somewhat less variable markers, and few have involved more than one type of genetic marker. The large amount of data generated in the course of this study, owing to both the large number of samples and the use of both nuclear and mitochondrial markers, will provide detailed information about patterns of genetic differentiation both within and among rivers and drainages throughout Trinidad.

This information will allow for testing of the hypothesis that guppies colonized the island of Trinidad in a two-arc pattern, with populations in the Caroni and Oropouche watersheds being established by two separate groups of migrants. If this hypothesis is sound, high levels of genetic differentiation should be found between these two watersheds and populations within each watershed should be more closely related to others in the same watershed than to any

population in the opposite watershed. Furthermore, if the rivers of the north coast were colonized by the same wave of migrants that founded populations in the Caroni, as suggested by Carvalho *et al*. (1991) and Fajen and Breden (1992), then samples collected in the north will be genetically similar to those collected in Caroni drainage rivers. However, rivers on the eastern side of the north shore have not been sampled previously and so it is unclear where the two arcs of colonization meet and whether some north shore river populations may be more closely related to Oropouche populations than to Caroni.

An extension of the two-arc hypothesis is the concept that guppies found in the Oropouche drainage are sufficiently divergent from common Trinidadian guppies to be designated a distinct species, *P. obscura* (Schories *et al*., 2009). If this designation is valid, a clear pattern of divergence should be apparent in the results, indicating that all populations can be unambiguously defined as either *P. reticulata* or *P. obscura*. In this case, both nuclear and mitochondrial DNA data should separate the populations of the Oropouche from those throughout the rest of the island. Strong evidence of a new species could have far-reaching effects, as past genetic studies have typically proceeded under the assumption that guppies collected at various locations on the island are all members of a single common species.

2.2 Materials and Methods

2.2.1 Sample Collection

Guppies were collected from 22 rivers and one lake across the islands of Trinidad and Tobago (listed in Table 2.1). The majority of the included locations

are found in the rivers of the mountainous Northern Range of Trinidad, an area long associated with *P. reticulata* research. Within many rivers, multiple sites – often above and below waterfalls or other barriers – were included in an effort to accurately determine the effects of differing predation levels on population structure and to identify possible gene flow both within and between drainages. At some sites guppies were collected two or three times, over a period of up to eight years, in order to establish whether populations typically remain stable over time or if they undergo frequent demographic changes. In a few cases specific sites were selected for temporal replication because of suspected shifts in demography as a result of documented artificial introductions and other anthropogenic factors. Including multiple sites in each river and all temporal replicates, a total of 72 discrete populations were sampled. Of these, 69 populations were genotyped for microsatellite data.

The mtDNA control region was sequenced in 161 individuals taken from 22 of the sampled rivers. In addition, control region sequences from a further 57 individuals were downloaded from Genbank (accession numbers listed in Appendix 1). These sequences formed a part of the dataset analyzed in Alexander *et al*. (2006), and were taken from fish caught by Felix Breden and John Taylor (Simon Fraser University) between 1988 and 1997. A small number of these sequences were originally analyzed by Taylor & Breden (2000), but most remained unpublished prior to 2006. In this study the Oropouche and Rio Grande Rivers are represented exclusively by sequences taken from Genbank, while the Arima, Aripo, Guanapo, Madamas, Marianne, Paria, Quare, and Yarra

Rivers comprise samples collected for the current study as well as sequences acquired from Genbank. All other rivers with mtDNA data include only samples collected for this work. Including both sequenced and downloaded data, mtDNA information was obtained for a total of 24 rivers.

The average sample size for microsatellites was 40 individuals (range, 15-75). Average sample size for mtDNA was nine individuals (range, 3-16). All samples dated 2002 were collected by Andrew Hendry (McGill University), samples from the Diego Martin River and Tobago were collected by Jim Gilliam (North Carolina State University), those from Pitch Lake, San Souci River, and Shark River were collected by Andrew Furness (University of California, Riverside), and those from the Stollmeyer River were provided by Gregor Rolshausen (McGill University). All other samples were collected under the supervision of Andrew Hendry (McGill University) and Paul Bentzen (Dalhousie University), with the assistance of numerous students, including the author. Detailed information about all samples, including site location, predation level, year of collection, and number of individuals genotyped for microsatellites and mtDNA, is listed in Table 2.1. Site names used in the Marianne and Paria Rivers in this table and throughout the thesis differ from those used in previous studies, and a chart showing alternate names is provided in Appendix 2. Maps illustrating the location of all listed sites are shown in Figures 2.2, 2.3, and 3.1.

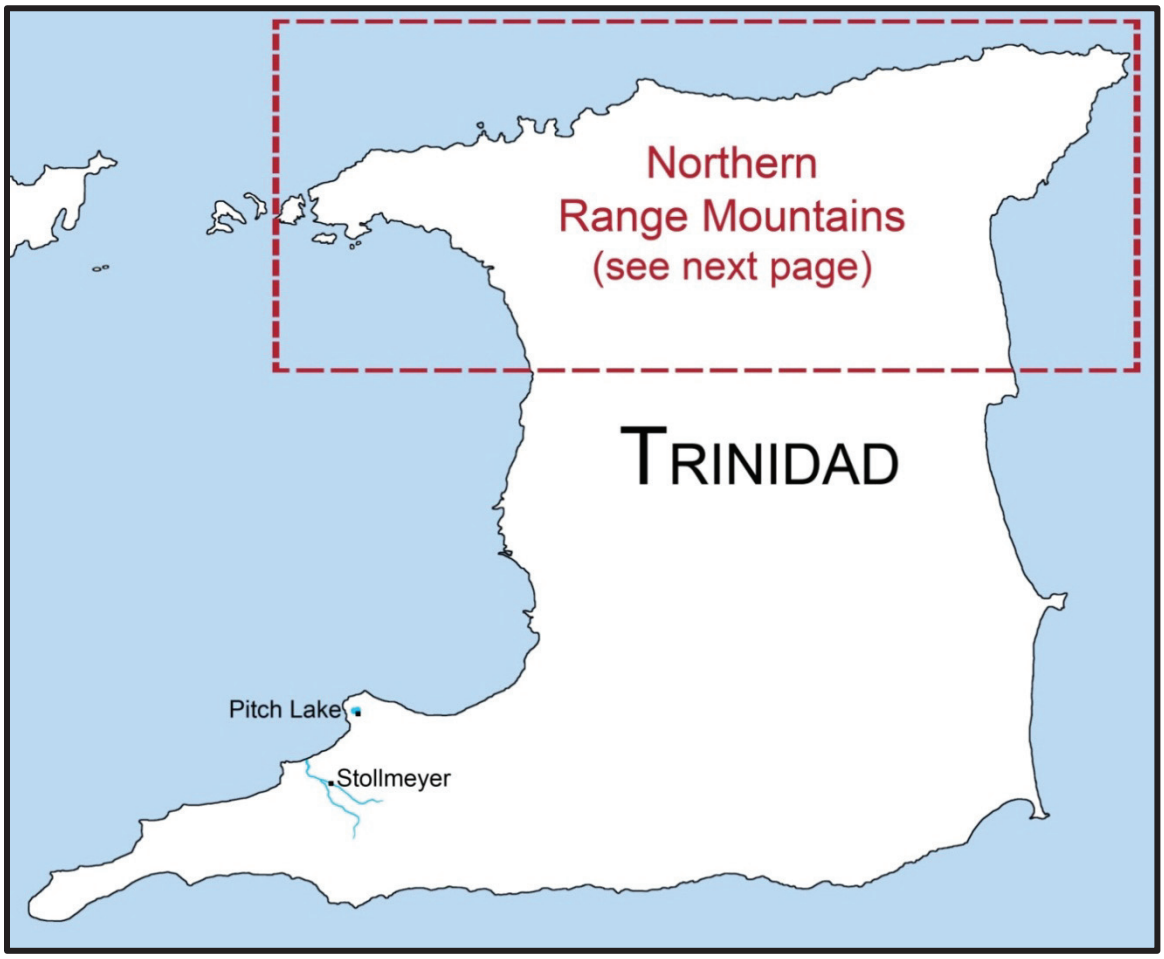


Figure 2.2: The island of Trinidad, with Pitch Lake and Stollmeyer River sites labeled. See Figure 2.3, next page, for a detailed map of sites within the Northern Range Mountains.

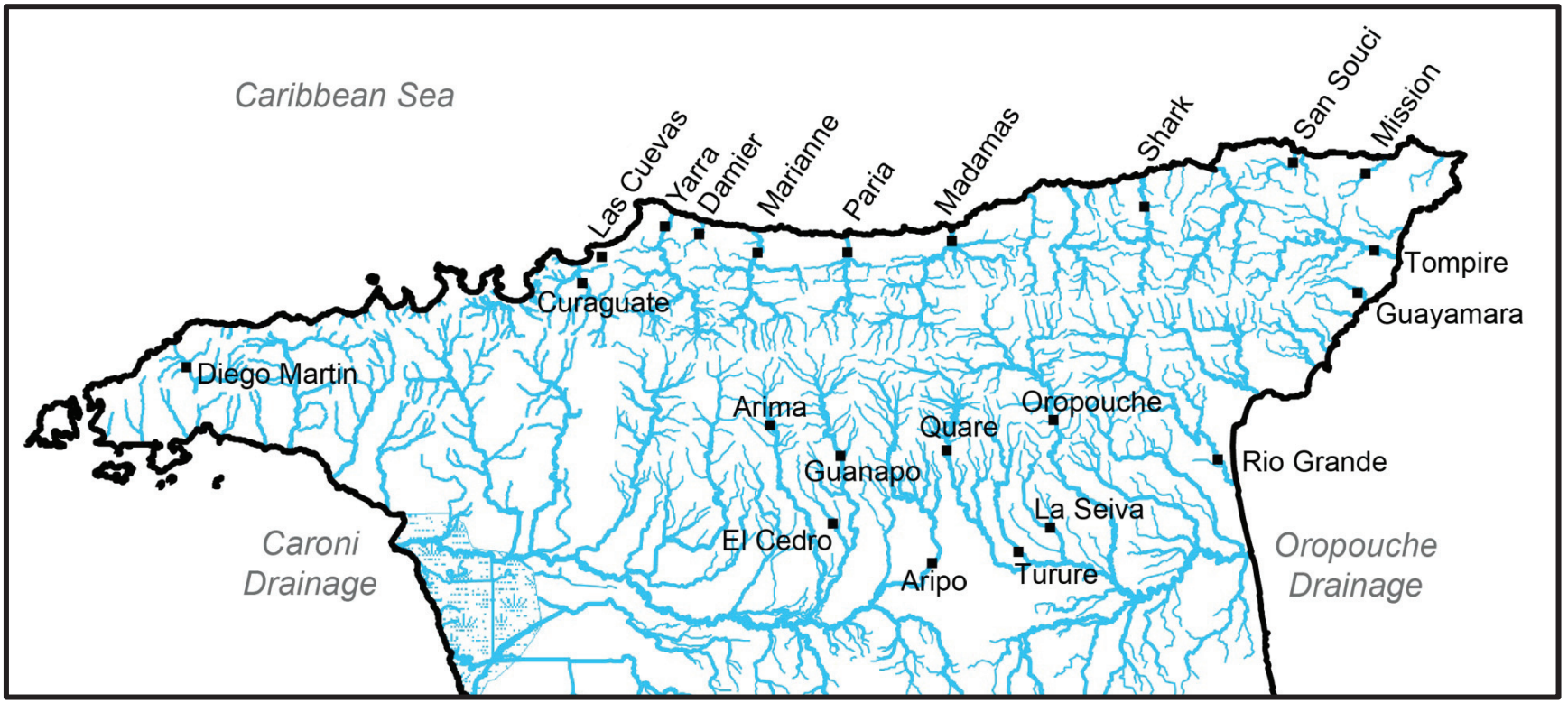


Figure 2.3: Detail map of northern Trinidad with 22 sampled rivers labeled. In many cases individual rivers were sampled at multiple locations in order to assess gene flow and compare differing predation levels.

Table 2.1: List of 74 populations sampled, grouped by drainage or area of the island and listed from west to east. Also shown are predation level, collection site within river, and year of collection (where known). Columns on the right indicate the number of individuals genotyped at microsatellite markers and sequenced for mtDNA. Some mtDNA sequences were taken from individuals collected in years earlier than those listed.

	Location	Site Information	Year	Microsatellites	mtDNA
South Island	**Stollmeyer River**	~	2010	~	7
	Pitch Lake	~	2010	33	7
Caroni Drainage	**Arima River**	Low Predation	2010	45	13
		High Predation	2010	40	
	El Cedro River	Low Predation	2010	45	8
		High Predation	2010	40	
	Guanapo River	Low Predation	2010	45	12
		High Predation	2010	40	
	Aripo River	Low Predation	2010	45	11
		High Predation	2006	44	
		"	2010	40	
North Shore	**Diego Martin River**	~	2009	25	8
	Las Cuevas Bay	~	2010	30	8
	Curaguate River	~	2010	26	~
	Yarra River	Low Predation	2006	44	15
		"	2009	67	
		"	2010	51	
		High Predation	2006	31	
		"	2009	15	
		"	2010	40	
		Upper Yarra	2008	75	
	Damier River	Low Predation	2005	63	~
		"	2009	59	
		"	2010	47	
		High Predation	2005	21	
		"	2009	67	
		"	2010	39	
	Marianne River	Site M-A	2002	40	12
		"	2008	50	
		Site M-B	2006	39	
		Site M-C	2002	40	
		Site M-D	2002	40	
		"	2008	40	
		"	2010	40	

North Shore	**Marianne River** *(continued)*	Site M-E	2002	40	
		"	2010	40	
		Site M-F	2002	40	
		"	2010	38	
	Petite Marianne River *(Marianne Tributary)*	Site PM-A	2006	40	~
		Site PM-B	2002	40	
		"	2010	40	
		Site PM-C	2002	39	
	Paria River	Site P-A	2002	39	12
		Site P-B	2002	40	
		Site P-C	2002	39	
		Site P-D	2004	45	
		Site P-E	2004	40	
		Site P-F	2004	40	
		Site P-G	2002	40	
		"	2010	40	
		Site P-H	2002	40	
	Jordan River *(Paria Tributary)*	Site J-A	2002	38	3
		Site J-B	2002	40	
		Site J-C	2008	47	
	Madamas River	Site 1	2002	33	12
		Site 2	2002	26	
	Shark River	~	2010	35	8
	San Souci River	~	2010	35	8
	Mission River	~	2010	35	8
Oropouche Drainage	**Quare River**	Low Predation	2010	24	16
		High Predation	2006	60	
		"	2008	48	
		"	2010	37	
		Site 3	2008	53	
		Site 4	2008	42	
	Turure River	Low Predation	2010	45	7
		High Predation	2006	44	
		"	2010	40	
	La Seiva River	~	2011	27	9
	Oropouche River	~	?	~	12
East Coast	**Rio Grande River**	~	?	~	3
	Guayamara Bay	~	2010	~	6
	Tompire River	~	2010	29	8
Tobago		~	2009	~	5
	Total			**2824**	**218**

2.2.2 Laboratory Protocol

DNA from samples collected in 2002 was extracted by Erika Crispo using a Qiagen phenol-chloroform extraction protocol. DNA was extracted from fin clips or scales taken from all other specimens by the author. Before extraction,tissue samples were placed in 225μl of digestion buffer (100mM NaCl, 50mM TrisHCl pH8,10mM EDTA, 0.5% SDS) with 2μl proteinase K and incubated overnight at 55°C and 200 rpm in an orbital shaker. Total genomic DNA was isolated by means of a modified glassmilk extraction protocol (as described in Elphinstone *et al*., 2003), adapted for use in a 96 well format on a Perkin Elmer MultiPROBE II liquid handler. Extracted DNA was then stored at -20°C in preparation for further analysis.

A total of 2,824 specimens were genotyped at 10 polymorphic microsatellite loci using primers developed for *P. reticulata* and described in Paterson *et al*. (2005), Watanabe *et al.* (2003), and Shen *et al.* (2007). Primer sequences, sources, and optimal annealing temperatures are described in Appendix 3. DNA was amplified via polymerase chain reaction (PCR) in 5μl volumes comprised of 10-50ng DNA, 0.5μl 10x ThermoPol PCR buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1 % Triton X-100), 200μM dNTP (equal parts dATP, dCTP, dGTP, and dTTP), 200μM fluorescently labelled forward primer, 200μM reverse primer, and 0.5U *Taq* DNA polymerase (New England BioLabs). Fluorescent labelling of the forward primer, in either 700nm or 800nm wavelengths, was carried out by the manufacturer, MWG Biotech AG. Amplification for all loci consisted of an initial denaturing step lasting 4 min at

95°C, followed by 30 cycles of denature for 30s at 95°C, annealing for 30s at a temperature specific to each locus, and extension for 30s at 72°C, followed by a final incubation for 3 min at 72°C. PCRs were carried out in Eppendorf Mastercycler ep thermal cyclers.

After amplification, microsatellite PCR products were visualized by electrophoresis on 8% denaturing polyacrylamide gels run on a LI-COR IR^2 DNA analyzer at a temperature of 50°C. Alleles were scored by hand in reference to a molecular weight size standard ladder included on each gel. All gels also incorporated positive control samples and redundant samples in order to allow comparison of new samples to those with known allele sizes and to help ensure consistent scoring across a large number of populations and loci.

In addition to microsatellite genotyping, 161 samples were selected for sequencing of the mtDNA control region. DNA was amplified in a 10µl PCR containing 10-50ng DNA , 25µl of 10x ThermoPol PCR buffer, 350µM dNTPs, 3 mM $MgCl_2$, 1.25U *Taq* DNA polymerase, and 175µM primer. Primers used were L15926 (Kocher *et al*., 1989) for amplification of the light strand and H16498 (Meyer *et al*., 1990) for amplification of the heavy strand, resulting in a fragment of approximately 490bp. The PCR temperature profile consisted of initial denaturing for 3 minutes at 94°C, 35 cycles of denature at 95°C for 20s, annealing at 52°C for 60s, and extension at 72 for 60s, and a final 3 min incubation at 72°C. To ensure accurate sequencing, excess primers and dNTPs were removed from the PCR products by adding 2U *Exo*I, 1.5U Antarctic

phosphatase, and 100 μM zinc acetate. Purified PCR products were sent to Macrogen USA for sequencing.

2.2.3 Microsatellite Data Analysis

Microsatellite scores were checked for common genotyping errors, such as inaccuracies caused by stutter, large allele drop-out, and null alleles, using Micro-Checker v.2.2.3 (van Oosterhout *et al*., 2004). While scoring errors identified by Micro-Checker were corrected wherever possible, identification of possible null alleles persisted in many populations. For each locus at each site the size range of all alleles, frequency of the most common allele, and estimated frequency of the null allele were found using GENEPOP v.4.0.10 (Raymond & Rousset, 1995; Rousset, 2008). Total number of alleles and allelic richness were calculated using FSTAT v.2.9.3.2 (Goudet, 2001); expected and observed heterozygosities were determined using GENETIX v.4.05 (Belkhir *et al*., 1996-2004). These values are reported in Appendix 4, with more detailed information on allele frequencies in Appendices 4 and 5. Testing for the probability of Hardy-Weinberg equilibrium (HWE) at each locus and at each site was performed in Arlequin v.3.5.1.3 (Excoffier & Lischer, 2010) and the results assessed using sequential Bonferroni correction (Rice, 1989). Only one population, collected in the upper Yarra River, departed significantly from HWE, likely because this sample was made up of individuals collected at several points on a long and sparsely populated stretch of the river.

All 10 loci were checked for signs of selection pressure using LOSITAN (Beaumont & Nichols, 1996; Antao *et al*., 2008), which examines the relationship

between F_{ST} and heterozygosity at each locus and detects loci with unexpectedly high or low F_{ST} values, as these may be under positive or balancing selection. However, it is important to note that testing for F_{ST} outliers can result in overestimation of the number of loci under positive selection if there is complex hierarchical structure between populations (Excoffier et al., 2009; Narum & Hess, 2011). LOSITAN did not find any evidence of balancing selection, but identified four loci that may be undergoing positive selection. Early analysis indicated that while data from three of the identified loci was consistent with the overall data patterns, the locus Pret-46 often provided contradictory information. Inclusion of Pret-46 in analyses frequently resulted in patterns of population structure that were both implausible and inconsistent with the patterns commonly reported in the literature. Additionally, analysis of molecular variance (AMOVA) showed that genetic divergence between populations and regions was often much different at this locus when compared to the other nine loci. Removal of the locus from the microsatellite data typically produced analytical results that were substantially more logical when compared to previous research and to the known geographic and anthropogenic history of Trinidad. Because of this, all data from the locus Pret-46 was excluded from analysis in the remainder of this chapter.

Using data from the remaining nine loci, population structure was estimated from the microsatellite data using several methods. Model-based Bayesian clustering analysis was conducted in STRUCTURE v.2.3.3 (Pritchard *et al*., 2000; Falush *et al*., 2003) in order to identify possible clusters of individuals and to estimate probable admixture within each individual. Due to the

practical limitations of visually presenting the resulting genetic clusters in a very large dataset, a subset of all sampled individuals were chosen for inclusion in this analysis. Where data was available from multiple temporal replicates and multiple sites within a single river, populations were selected to provide maximum coverage of different areas of the river, such that a total of 1,280 individuals from 33 populations were used to represent the entire dataset. Wherever possible, populations sampled in the most recent year of collection (2010) were used. Selecting only these populations allowed for clearer and more concise results without omitting the different predation levels and geographic areas in each river. In STRUCTURE, a burn-in of 50,000 replicates was followed by 50,000 Markov-chain Monte Carlo (MCMC) replicates and three iterations of this parameter set were performed for each number of clusters (K-value) from 20 to 30. The most likely K-value was then found based on the rate of change of the log probability of data (ΔK), as described in Evanno *et al*. (2005), and implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2011).

Two different measures of genetic variation were calculated in order to perform the remainder of the analyses. First, pairwise F_{ST} values were found using Microsatellite Analyser (Dieringer & Schlötterer. 2003). Although F_{ST} has been widely used since the 1940s as a measure of genetic distance in population structure studies, it has several recognized drawbacks. In particular, F_{ST} values tend to be artificially low when heterozygosity levels are high, which can result in low F_{ST} values between what are actually highly divergent populations (Meirmans & Hedrick, 2011). Because of the limitation of F_{ST} a second measure

of genetic distance, $\delta\mu^2$, was also calculated using POPTREE2 (Takezaki *et al*., 2010). This measure may lead to more accurate analyses as it takes into account differing allele sizes due to the stepwise mutation process common to the evolution of microsatellites, and is independent of population size (Goldstein *et al*., 1995).

A dendrogram was created in POPTREE2 (Takezaki *et al*., 2010), using the unweighted pair group method with arithmetic mean (UPGMA) and based on $\delta\mu^2$ genetic distance. In order to make the resulting tree as clear as possible all samples collected within each river were pooled together, regardless of predation level or collection year. The only exceptions to this were Marianne and Paria rivers – where samples were pooled into four groups within the Marianne and its tributary the Petite Marianne, and two groups within the Paria and its tributary the Jordan – due to the large number of individuals taken from those two rivers. With the data pooled a total of 24 distinct populations were used to create the tree, which was viewed in FIGTREE v.1.3.1 (Rambout, 2010). Principal coordinate analysis (PCoA) was carried out using all 69 populations, including multiple sites per river and temporal replicates. PCoAs were based on pairwise genetic distance matrices for both F_{ST} and $\delta\mu^2$ and created in GENALEX v.6.41 (Peakall & Smouse, 2006).

Results of several of the analyses revealed that some populations were consistently clustered into larger groups, possibly revealing a shared colonization history. To determine the relative likelihood of various possible groupings, several analyses of molecular variance (AMOVAs) were performed. Populations

were grouped based on the geographic arrangement of rivers on the island, on proposed genetic relationships taken from the literature, and on the population clusters seen in the phylogenetic tree. This analysis was performed using microsatellite data from all 69 populations. AMOVAs were conducted in Arlequin v.3.5.1.3 (Excoffier & Lischer, 2010).

2.2.4 Mitochondrial DNA Data Analysis

Mitochondrial control region DNA sequences were edited and aligned with CodonCode Aligner v.3.7.1 (http://www.codoncode.com/aligner) using the ClustalW algorithm (Thompson *et al*., 1994). In addition to the 161 samples sequenced for this study, final alignment included 57 previously sequenced control region samples downloaded from GenBank (from Alexander *et al*., 2006, accession numbers listed in Appendix 1). Although the sequences generated during this study were about 490bp in length and sequences acquired from GenBank were over 800bp, only the segment common to all samples was used for analysis, resulting in a fragment of 392bp. After alignment, common haplotypes were identified and a haplotype network created with the software Network (Bandelt *et al*., 1999), which uses a median-joining algorithm to identify and present the most parsimonious haplotype network possible based on the data provided.

2.3 Results

2.3.1 Results of Microsatellite Analysis

Pairwise estimates of genetic differentiation, in the form of F_{ST}, were calculated from nine microsatellite loci and varied from a minimum of zero to a

maximum of 0.65 (Appendix 7). High F_{ST} values were frequently found between populations in different drainages, but were also observed within drainages and even within a single river. Seven pairwise F_{ST} values did not differ significantly from zero (based on $p>0.05$), indicating little or no genetic differentiation between the two populations in those cases (these values are underlined in the F_{ST} table). Non-significant values were generally found either between adjacent populations in the same river or between two samples collected at the same site in different years. The only exception to this was the low predation site of the Damier River, where two out of three temporal replicates were not significantly differentiated from the high predation population of the nearby Yarra River.

The results of Bayesian clustering analysis of 33 populations from across Trinidad were assessed by plotting the rate of change of the log probability of data (ΔK) to determine the most likely number of genetic clusters. This process identified 28 genetic clusters in broad-scale preliminary analysis (K-values from 2 to 30), and 25 clusters in a more focused analysis carried out with an increased number of burn-in and MCMC replicates (K-values from 20 to 30). Plots produced from both analyses are shown in Figure 2.4 and genetic clusters shown in Figure 2.5. Within each population most individuals were wholly or predominately assigned to the same cluster, although there were exceptions to this pattern, such as a several guppies collected in the Madamas River that appear more similar to Marianne and Quare fish than to others from the Madamas. In general, populations under high predation were more genetically diverse than those under low predation. Most samples from the Guanapo River

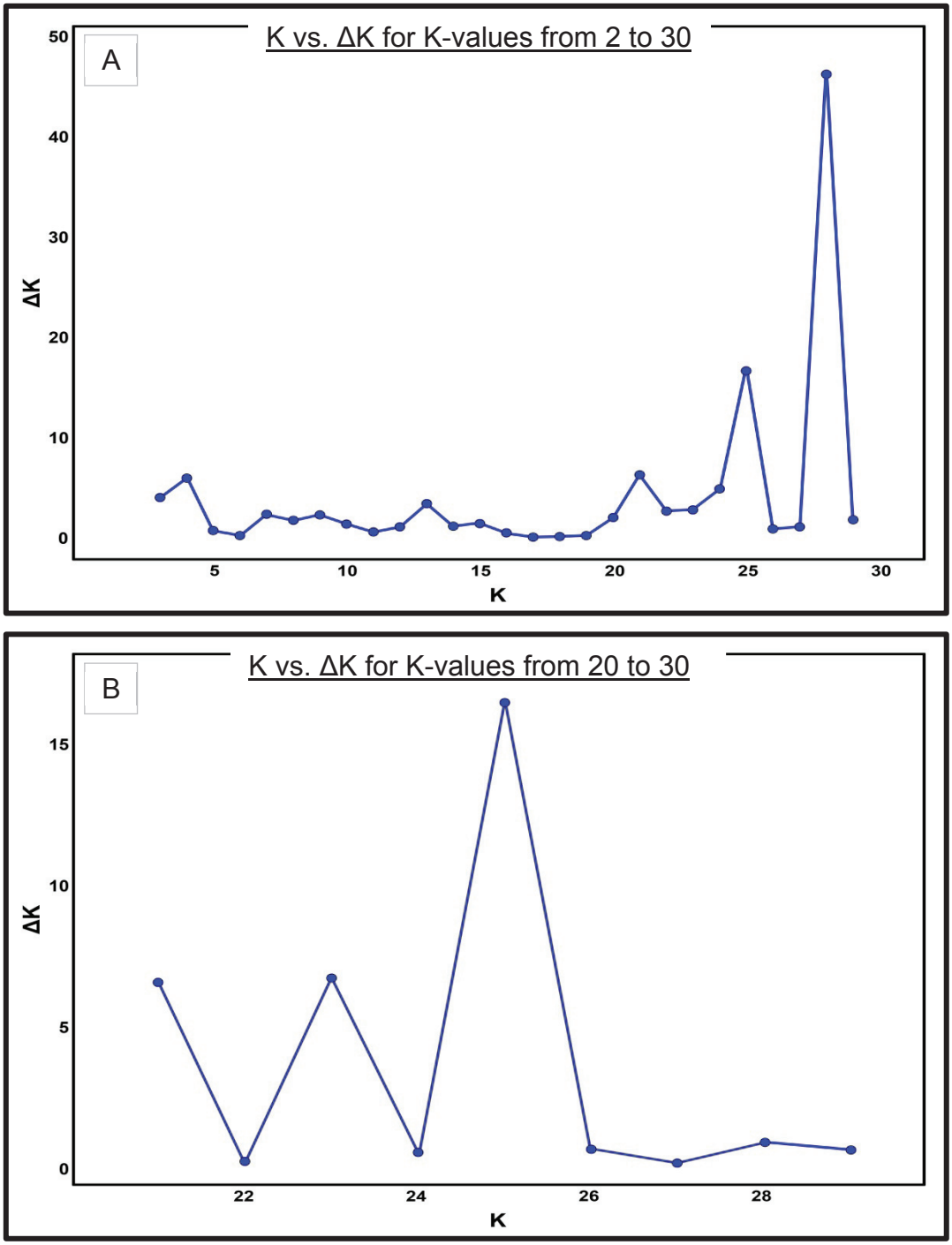


Figure 2.4: ΔK plots for Bayesian clustering analysis of 33 populations from across Trinidad. Plot A shows results of preliminary analysis using K-values from K=2 to K=30, with a peak at K=28. Plot B shows results of analysis with increased MCMC replicates using K-values from K=20 to K=30, with a peak at K=25. Genetic clusters from the run identified in plot B are shown in Figure 2.5.

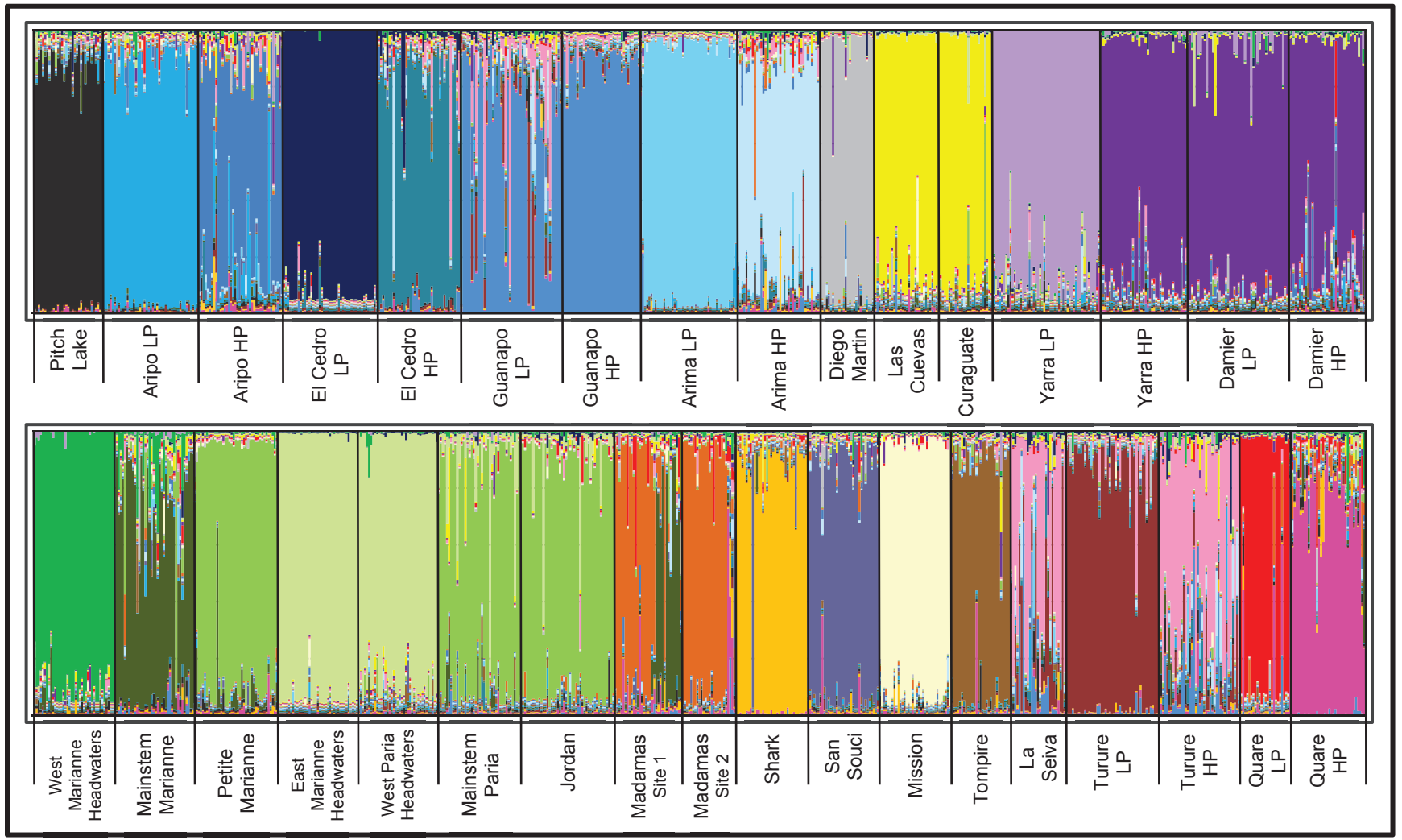


Figure 2.5: Results of Bayesian analysis show population structure in 33 selected populations. Each vertical line represents one individual and is partitioned into coloured blocks that indicate the estimated membership of that individual into each of 25 identified clusters. The designations LP and HP refer to low and high predation sites, respectively.

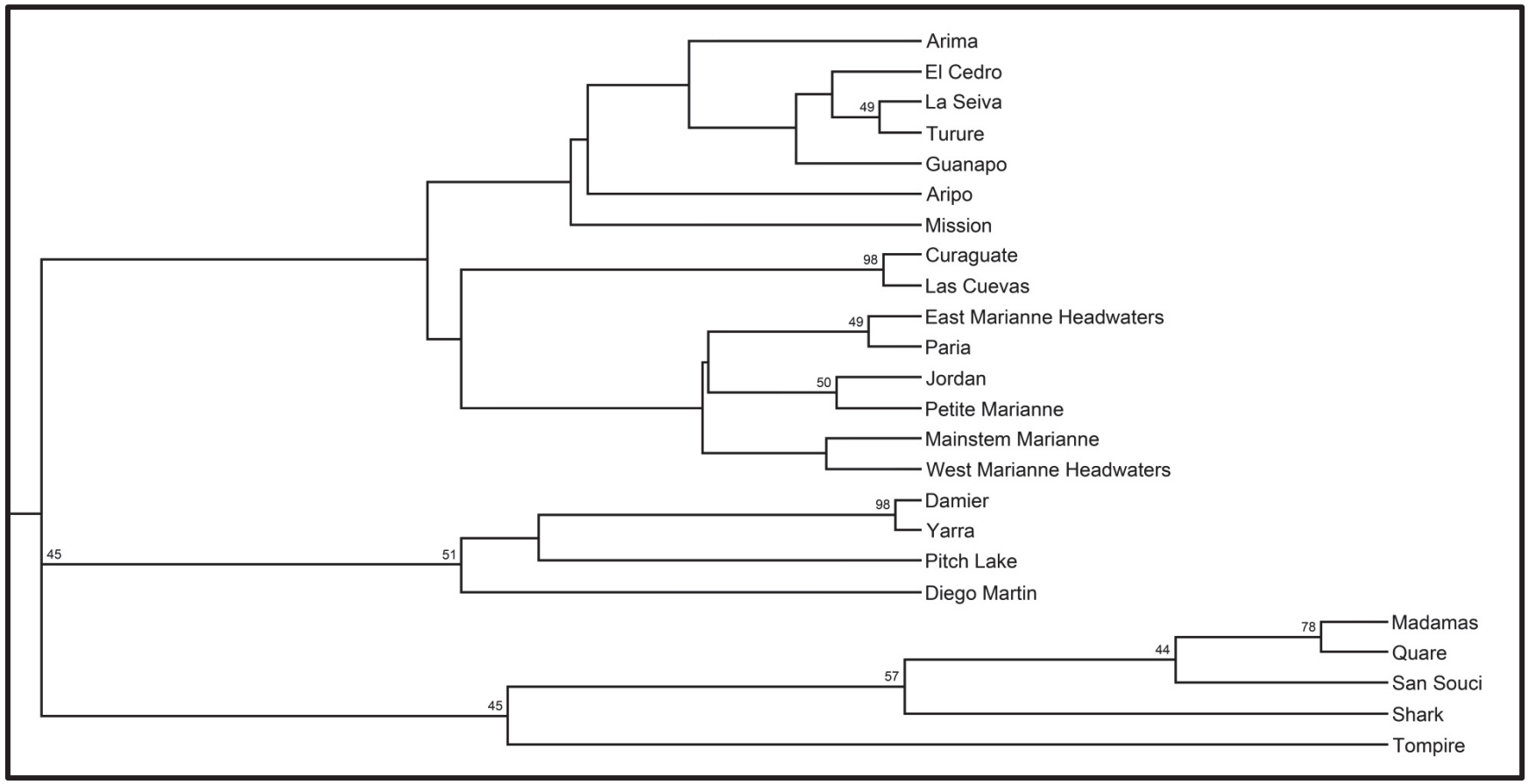


Figure 2.6: Dendrogram of 24 populations based on $\delta\mu^2$ genetic distance and built using the UPGMA tree-building method. Most populations consist of data pooled from all sites and temporal replicates within each river, with the exception of populations from the Marianne and Paria Rivers, where data were pooled into several groups representing different areas of the river. Bootstrap values over 40% (based on 1000 replications) are reported.

clustered together; samples from Las Cuevas Bay and the nearby Curaguate River clustered together; and samples from the Yarra River high predation site and all of the Damier River clustered together. Populations from the Petite Marianne River (a tributary of the Marianne), the Paria River, and the Jordan River (a tributary of the Paria) were all dominated by a single large genetic cluster. Samples taken from the east headwaters of the Marianne and the west headwaters of the Paria also clustered together. There was evidence of admixture from both high and low predation sites of the Turure River in the La Seiva River. The Turure and La Seiva Rivers also showed evidence of admixture from the Guanapo.

The dendrogram, based on $\delta\mu^2$ genetic distance and created with all 2,824 samples grouped into 24 population groups showed some of the same clusters (Figure 2.6). For example, samples from Las Cuevas Bay and the Curaguate River were linked together with a bootstrap value of 98%, as were samples from the Yarra and Damier Rivers. The Turure and La Seiva Rivers, though geographically located near the Quare River within the Oropouche watershed, appear closely related to the rivers of the Caroni watershed, particularly the El Cedro and Guanapo. Within the Marianne and Paria Rivers, genetic similarities were seen between the Petite Marianne (a tributary of the Marianne) and the Jordan (a tributary of the Paria) and also between the east headwaters of the Marianne and the main branch of the Paria River. This tree also revealed a deep divide between two large groups – sites in the Tompire, Shark, San Souci, Quare, and Madamas Rivers, which are all located in the

north-east part of the island, were clearly separated from the remainder of the sampled sites. A subgroup within the larger group was made up of samples from Pitch Lake and the Damier, Yarra, and Diego Martin Rivers, all of which lie along the west coast or the western portion of the north coast of Trinidad. The Mission River was the only exception to this overall pattern, as it was grouped with the rivers of the west-flowing Caroni drainage, despite its position in the northeastern part of the island. While the bootstrap value associated with the connection between the Mission and the rivers of the Caroni was low, the relationship was later corroborated by principal components analysis and analysis of molecular variance.

PCoAs were conducted with all samples separated into 69 populations rather than the 24 used in the phylogenetic tree, adding more detailed information about the relationships between guppies sampled at different sites within each river (Figures 2.7 and 2.8). In some cases all populations within a single river appeared to be genetically similar, while in other cases there was considerable genetic diversity. In both the $\delta\mu^2$ and F_{ST} based PCoAs multiple sites within each of the Quare, Turure, and Damier Rivers were relatively close together, whereas sites within the Yarra, Marianne, and Paria Rivers were more spread out. The upper and lower reaches of the Paria formed distinct, well separated clusters, and sites in the east headwaters of the Marianne were clearly separated from the rest of the river. In the F_{ST} based PCoA the remainder of the Marianne was further split into three widely separated groups representing the west headwaters, the mainstem, and the Petite Marianne tributary.

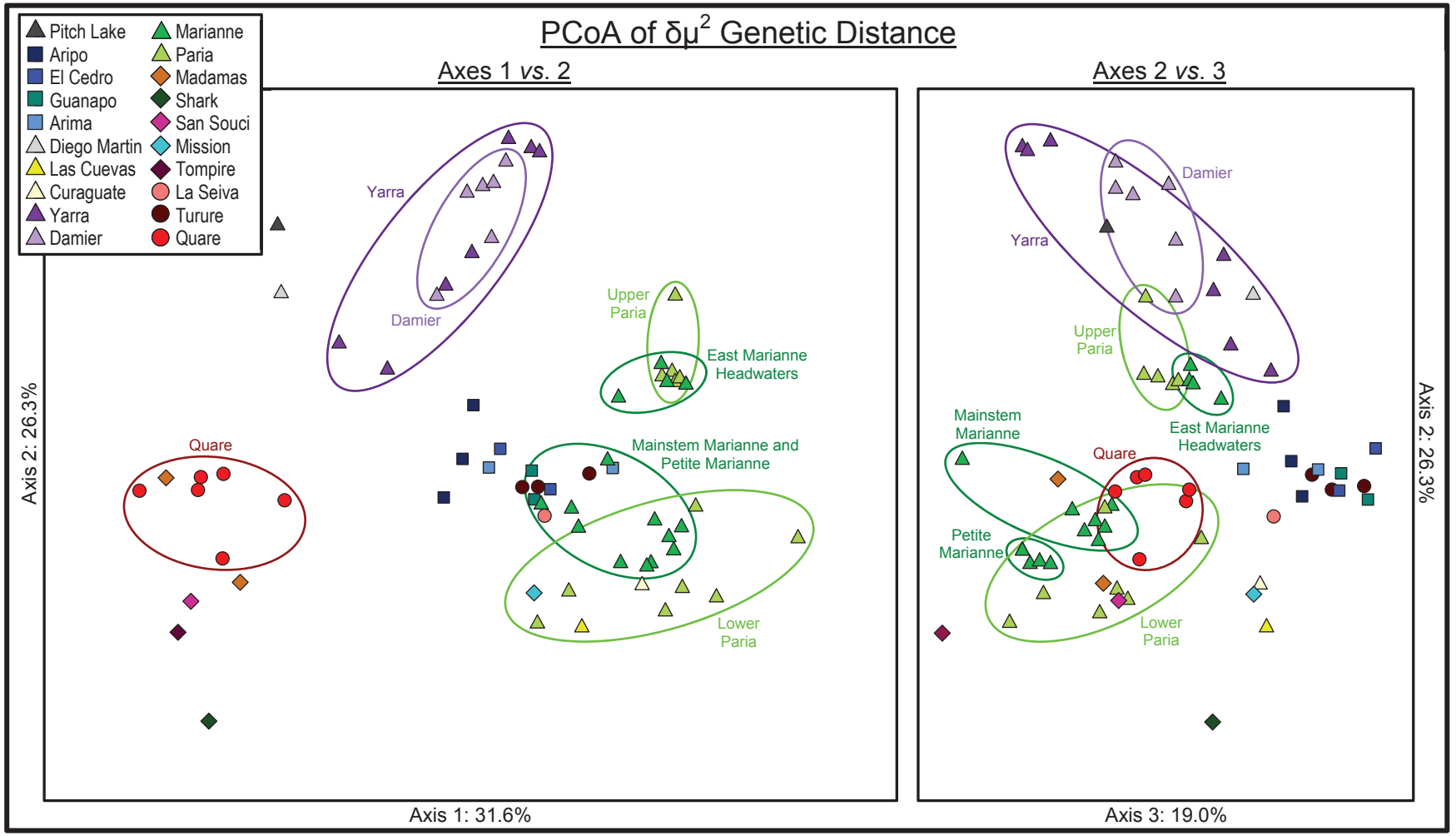


Figure 2.7: PCoA of 69 guppy populations, based on the $\delta\mu^2$ measure of genetic distance. This plot shows three dimensions of genetic variation, describing 76.9% of the total variation present in the dataset.

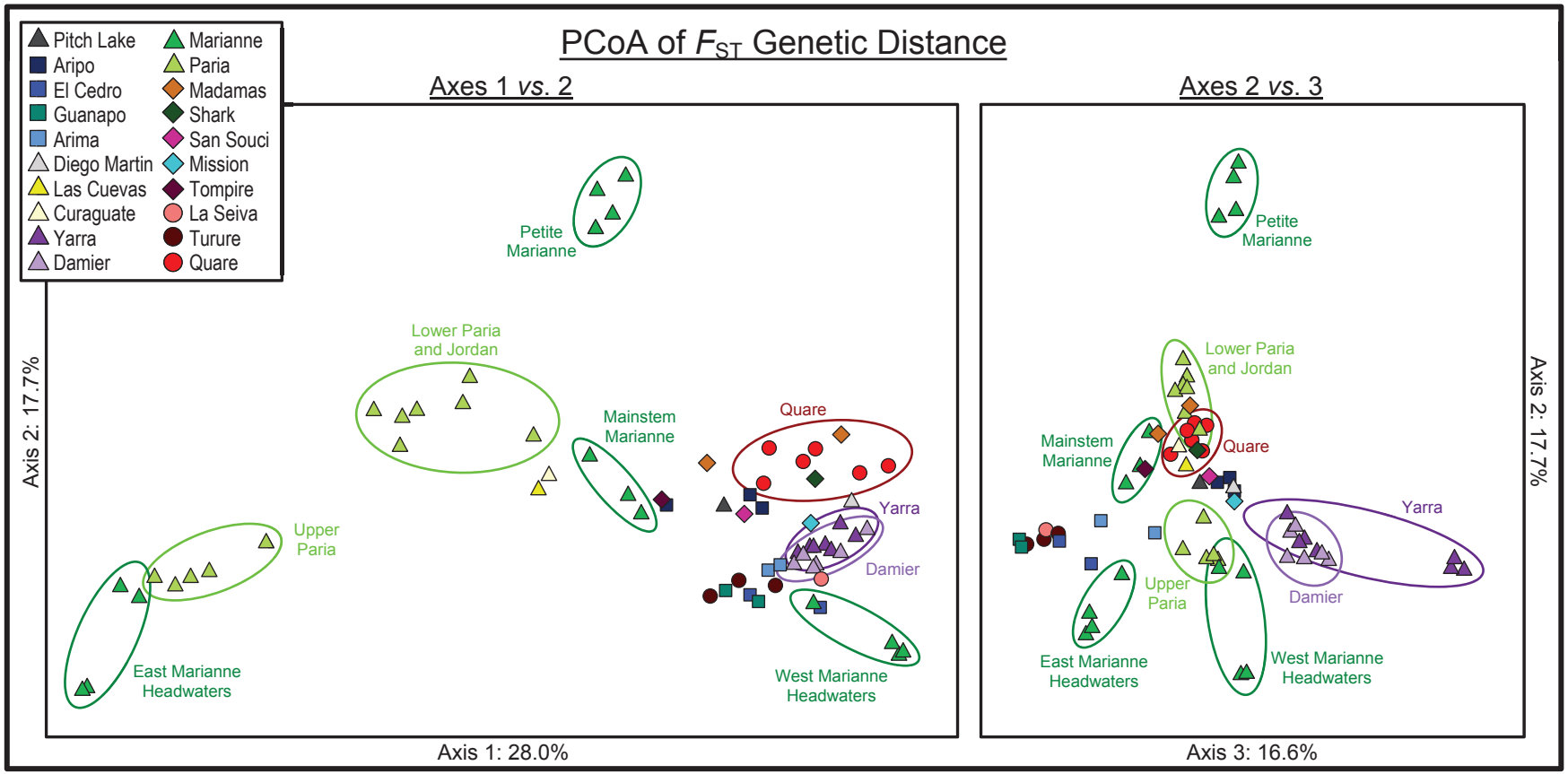


Figure 2.8: PCoA of 69 guppy populations, based on the F_{ST} measure of genetic distance. This plot shows three dimensions of genetic variation, describing 62.2% of the total variation present in the dataset.

Table 2.2: Results of six AMOVAs, showing the percentage of total genetic variation found within individual populations, among all populations, and among groups. Five different groupings were tested, based on proposed patterns of colonization on the island and on the results of other analyses. Groups, as well as the areas they represent, are listed in the table below.

AMOVAs		Sum of Squares	Variance Components	Percentage of Total Variation
#1	No Secondary Structure			
	Within Populations	15127.6	3.14	75.6%
	Among Populations	5093.0	1.01	24.4%
#2	East: Quare, Tompire, La Seiva, Turure West/North: All other rivers			
	Within Populations	15127.6	3.14	74.3%
	Among Populations	4886.7	0.98	23.3%
	Among Groups	206.3	0.10	2.4%
#3	East: Quare, Tompire West/North: All other rivers			
	Within Populations	15127.6	3.14	73.6%
	Among Populations	4898.2	0.99	23.2%
	Among Groups	194.8	0.14	3.2%
#4	East: Quare, Tompire West: Pitch Lake, Aripo, El Cedro, Guanapo, Arima, Diego Martin, La Seiva, Turure North: Las Cuevas, Curaguate, Yarra, Damier, Marianne, Paria, Madamas, Shark, San Souci, Mission			
	Within Populations	15127.6	3.14	74.0%
	Among Populations	4544.1	0.93	21.9%
	Among Groups	548.9	0.17	4.1%
#5	East: Quare, Tompire, San Souci, Shark, Madamas West/North: Aripo, El Cedro, Guanapo, Arima, Las Cuevas, Curaguate, Marianne, Paria, Mission, La Seiva, Turure West Coast: Pitch Lake, Diego Martin, Yarra, Damier			
	Within Populations	15127.6	3.14	73.7%
	Among Populations	4332.4	0.88	20.8%
	Among Groups	760.5	0.23	5.5%
#6	East: Quare, Tompire, San Souci, Shark, Madamas West/North: Aripo, El Cedro, Guanapo, Arima, Mission, La Seiva, Turure Central North: Las Cuevas, Curaguate, Marianne, Paria West Coast: Pitch Lake, Diego Martin, Yarra, Damier			
	Within Populations	15127.6	3.14	74.2%
	Among Populations	3896.5	0.80	19.0%
	Among Groups	1196.4	0.29	6.8%

Both PCoAs suggested a very close genetic relationship between the populations of the Yarra and Damier Rivers. Populations from the upper reaches of the Paria River and the east headwaters of the Marianne River also appeared genetically similar. Populations from the Turure and La Seiva Rivers were quite dissimilar to populations in the nearby Quare and instead were more comparable to those found in the Arima, Aripo, El Cedro, and Guanapo Rivers – all of which are part of the Caroni watershed. The $\delta\mu^2$ PCoA indicated that guppies from Pitch Lake and the Diego Martin River shared some genetic similarity with those from the Damier and Yarra Rivers but were distinct from those found in the Caroni drainage. This PCoA also showed that guppies collected in the Tompire, Shark, San Souci, and Madamas River appeared to be genetically similar to those from the various sites in the Quare.

The first of several AMOVAs was conducted without any grouping of populations to determine how genetic variability was partitioned in the sampled guppy populations. This test showed that the majority of the variation existed within populations, with only 24.4% of the total variation occurring among different populations. Following this, five more AMOVAs were performed using different methods of grouping the populations (results are listed in Table 2.2). The first two of these (AMOVAs #2 and #3) represent the likely results of the 'two-arc' colonization model – under this hypothesis guppies found on the east coast of the island and in the Oropouche watershed would be expected to form a single cohesive group, distinct from guppies both on the western side of the island and across the north. Therefore populations from the Quare, Tompire, La

Seiva, and Turure Rivers were compared to those from the rest of the island, with results showing that 2.4% of total variation was between these two groups. Since all other analyses indicated that populations from the Turure and La Seiva Rivers seemed to be closely related to Caroni drainage guppies despite their physical location in the Oropouche watershed, these two rivers were removed from the Oropouche group and analysis was repeated with only the Quare and Tompire Rivers being compared to all other populations, resulting in 3.2% of total variation between groups.

To test the hypothesis that guppy populations in rivers across the north coast form a distinct cluster separate from population groups on either the east or west coasts, an AMOVA was conducted with eastern, western, and northern rivers treated as three groups (AMOVA #4). Due to the results of the previous test, the Turure and La Seiva Rivers were included within the western river group. This arrangement produced 4.1% of variation among these groups. Finally, two groupings based on the results of the $\delta\mu^2$ dendrogram seen in Figure 2.6 were tested (AMOVAs #5 and #6). Comparing the three major groups seen in that figure resulted in 5.5% of total variation lying among groups. When the largest group was split into two subgroups with the Marianne, Paria, and Curaguate Rivers and Las Cuevas Bay forming their own group, the variation among groups rose to 6.8%, the highest value calculated for any of the AMOVAs tested.

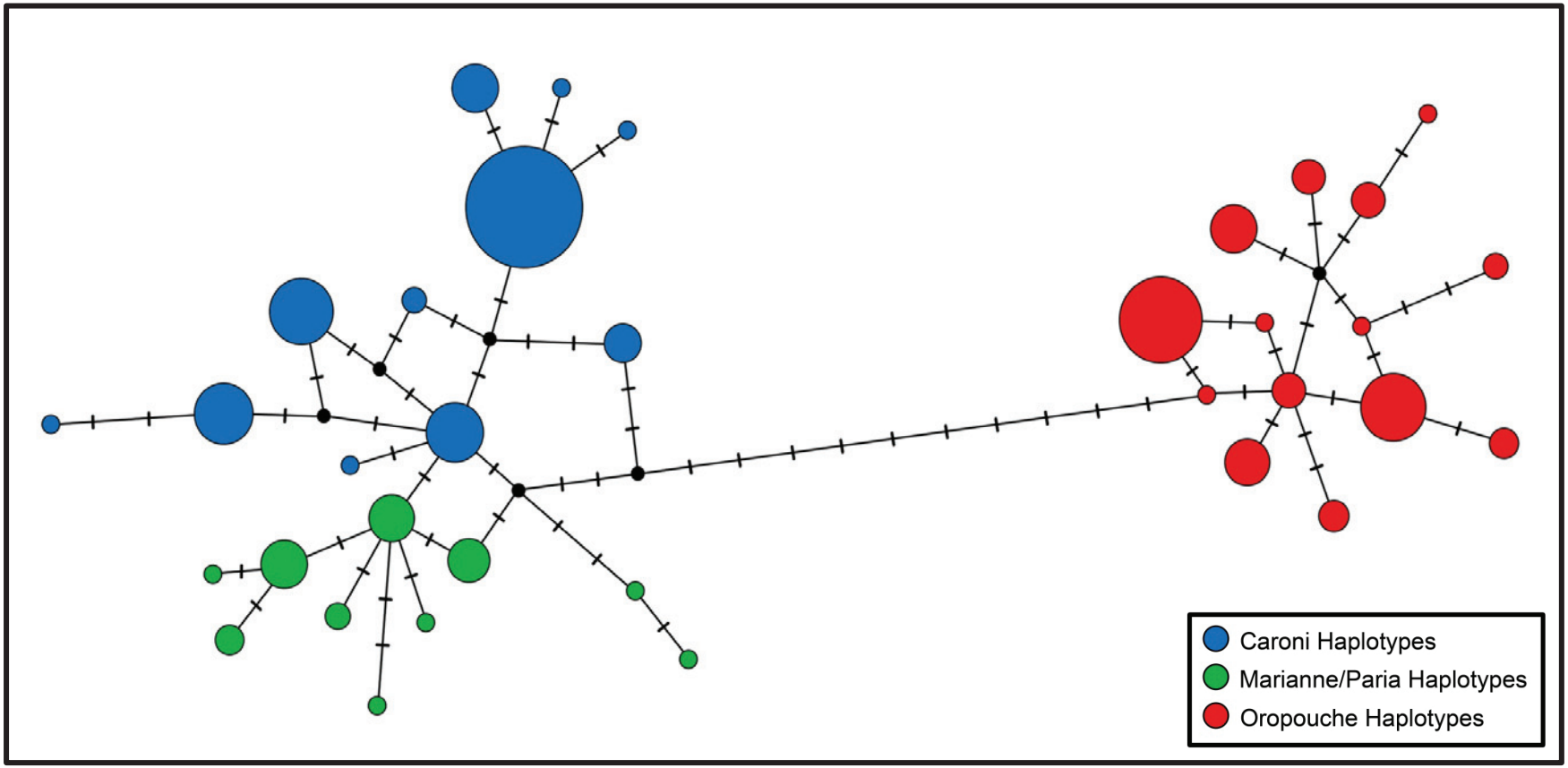


Figure 2.9: Median-joining haplotype network based on a 392bp sequence taken from the mitochondrial control region in 218 fish from 24 sites in Trinidad and Tobago. Each circle represents one of 35 distinct haplotypes, with circle size proportional to the frequency of each. Mutations are represented by a dash on the branch; black dots indicate unobserved intermediate haplotypes.

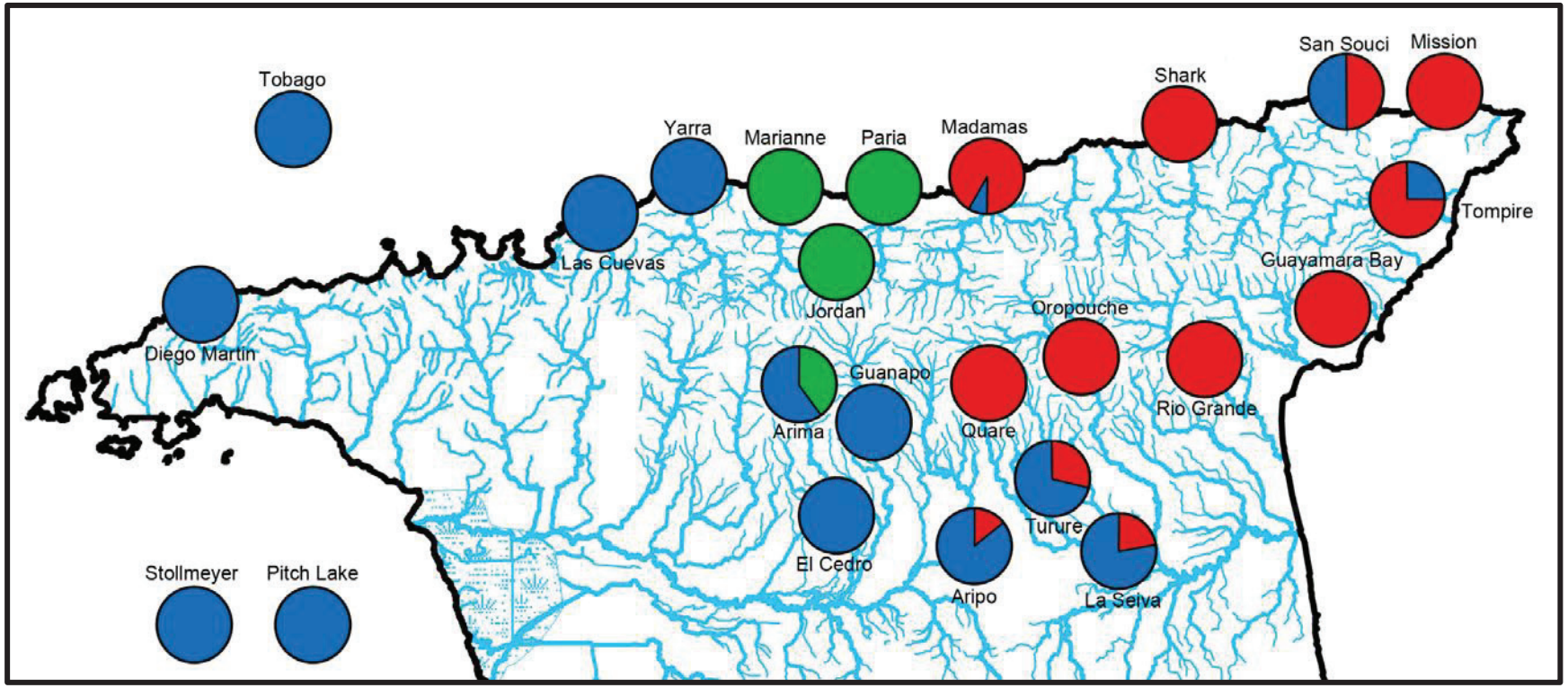


Figure 2.10: Distribution of mtDNA haplotypes across northern Trinidad. Pie charts show the frequency of west/Caroni (*blue*), east/Oropouche (*red*), and Marianne/Paria (*green*) haplotype groups.

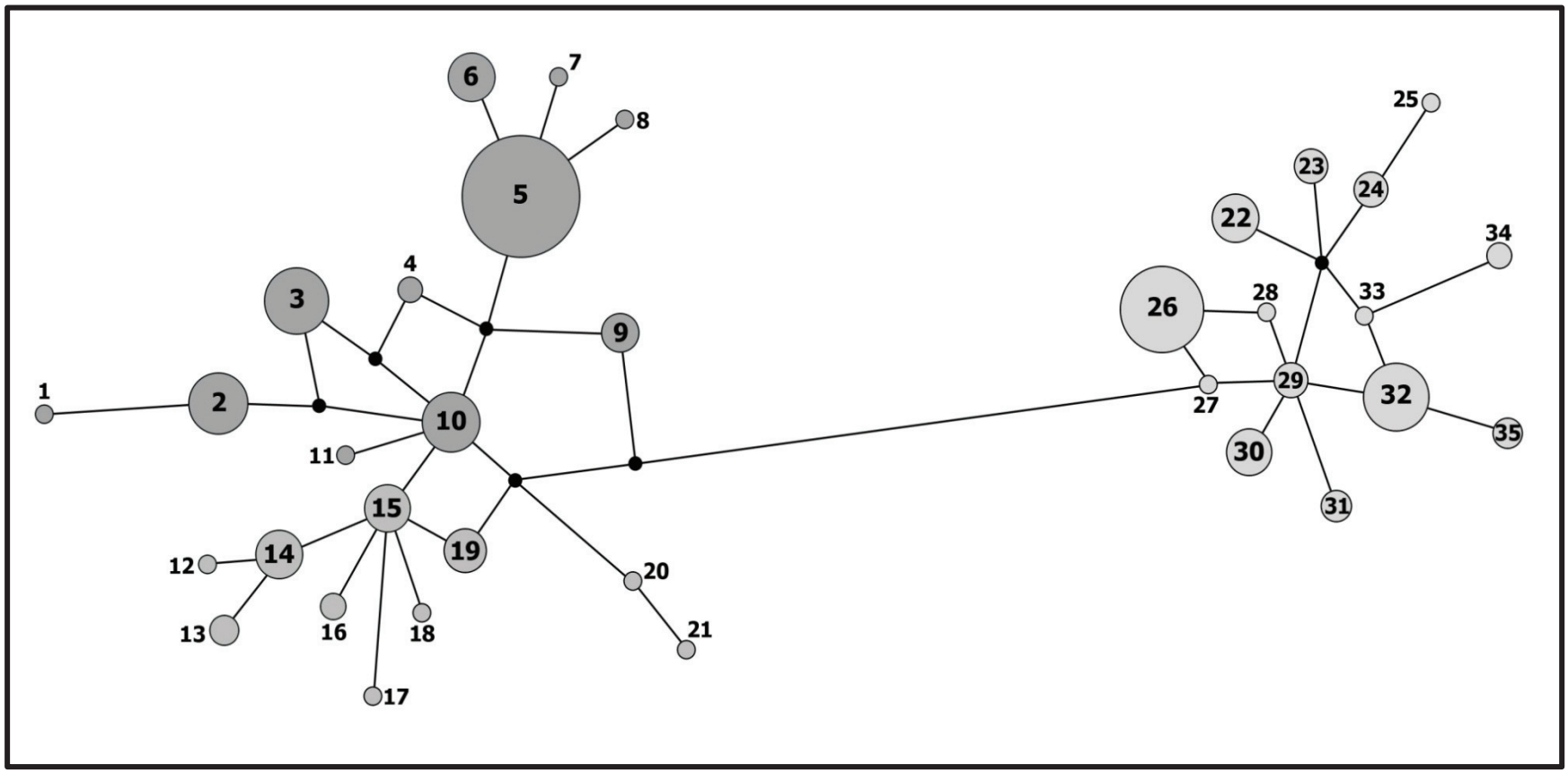


Figure 2.11: Median joining network with each individual haplotype numbered. Details of haplotype distribution, including the total number of individuals carrying each haplotype and the total sample size at each site, can be found in Table 2.3.

Table 2.3: Number of individuals with each of the 35 identified haplotypes found in each of 24 sampling locations. The total number of individuals carrying each haplotype is shown in the right hand column; total sample sizes for each river are shown at the bottom of the table.

Haplotype \ Site	Arima	Aripo	Diego Martin	El Cedro	Guanapo	Guayamara	Jordan	La Seiva	Las Cuevas	Madamas	Marianne	Mission	Oropouche	Paria	Pitch Lake	Quare	Rio Grande	San Souci	Shark	Stollmeyer	Tobago	Tompire	Turure	Yarra	Total
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	**1**
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	**13**
3	2	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	**14**
4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**2**
5	3	7	7	8	12	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	1	**48**
6	2	1	1	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	1	1	-	**8**
7	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
8	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	**5**
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	7	-	-	-	-	**13**
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
12	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
13	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	**3**
14	3	-	-	-	-	-	-	-	-	-	2	-	-	3	-	-	-	-	-	-	-	-	-	-	**8**
15	-	-	-	-	-	-	2	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	**7**
16	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	**2**
17	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**

Haplotype	Arima	Aripo	Diego Martin	El Cedro	Guanapo	Guayamara	Jordan	La Seiva	Las Cuevas	Madamas	Marianne	Mission	Oropouche	Paria	Pitch Lake	Quare	Rio Grande	San Souci	Shark	Stollmeyer	Tobago	Tompire	Turure	Yarra	Total
18	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
19	-	-	-	-	-	-	-	-	-	-	4	-	-	2	-	-	-	-	-	-	-	-	-	-	**6**
20	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
21	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
22	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	**8**
23	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	**4**
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	**4**
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	**1**
26	-	2	-	-	-	-	-	-	-	11	-	-	3	-	-	9	-	-	-	-	-	-	-	-	**25**
27	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**1**
28	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**1**
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	**4**
30	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	**7**
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	**3**
32	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	2	7	-	-	1	-	-	**16**
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	**1**
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	**2**
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	**3**
N	**13**	**11**	**8**	**8**	**12**	**6**	**3**	**9**	**8**	**12**	**12**	**8**	**12**	**12**	**7**	**16**	**3**	**8**	**8**	**7**	**5**	**8**	**7**	**15**	**218**

2.3.2 Results of Mitochondrial DNA Analysis

Alignment and analysis of the 392bp mtDNA sequence revealed 35 haplotypes. Many haplotypes were observed only in a single individual, but several haplotypes were very common and were found in fish from multiple locations. A haplotype network built from these data showed two large and evolutionarily divergent groups, with 21 haplotypes in one group and 14 in the other (Figure 2.9). These groups corresponded roughly to the two sides of the island, with sequences from fish collected on the western half of the north shore and in the west-flowing Caroni drainage generally falling into the first group (shown in blue and green in Figure 2.9), and sequences from fish caught on the eastern half and in the east-flowing Oropouche drainage falling into the second (shown in red). Samples from Pitch Lake, the Stollmeyer River, and Tobago carried haplotypes that fell within the larger west/Caroni group. The five guppies collected on Tobago all carried one haplotype that was unique to that island and differed from the most similar haplotype by several base pair substitutions. Also in the west/Caroni group was a subgroup of haplotypes found almost exclusively in fish collected within the Marianne and Paria Rivers located on the central north shore (shown in green).

At several locations on the north and east coast of Trinidad and in the Oropouche drainage eastern and western haplotypes co-occurred. These locations were the Madamas, San Souci, and Tompire Rivers, which all contained some west/Caroni haplotypes, and the Turure and La Seiva Rivers, where the predominant haplotypes were from the west/Caroni group. Within the

Caroni watershed a small number of east/Oropouche haplotypes were seen in individuals caught in the Aripo River. Also in the Caroni, two haplotypes typically found only in the Marianne/Paria region were observed in fish from the Arima River. The frequencies of west/Caroni, east/Oropouche, and Marianne/Paria haplotypes at various locations across Trinidad are shown in Figure 2.10, numbered haplotypes in Figure 2.11, and exact number of individuals from each site carrying each haplotype is shown in Table 2.3.

2.4 Discussion

2.4.1 Gene Flow between Watersheds

Analyses of both microsatellites and mtDNA consistently revealed evidence of genetic relationships between certain populations in the Oropouche and Caroni watersheds. This is particularly apparent in samples taken from the La Seiva and Turure Rivers, both branches of the Oropouche drainage that flows into the Atlantic Ocean on the east coast of Trinidad. Many individuals collected in these rivers show admixture from western Trinidad's Caroni drainage rivers in the Bayesian clustering analysis, and both rivers are nested deep within the Caroni watershed cluster in the $\delta\mu^2$ tree. In both PCoAs, populations from these two rivers are clustered with those from the Caroni and are distant from populations in another Oropouche river, the Quare. The majority of mtDNA haplotypes found in fish from the La Seiva and Turure are of the west/Caroni type. Furthermore, AMOVAs show slightly increased variation among groups when these rivers are grouped with rivers of the west and north rather than being

included in the eastern group, suggesting a deeper genetic divide between groups when arranged this way.

The presence of Caroni-like genotypes in guppies caught in the Turure River is likely the consequence of an artificial introduction experiment conducted in 1957. At that time C.P. Haskins moved 200 fish from the Caroni drainage into the upper reaches of the Turure, which was formerly devoid of guppies. While Haskins himself stated that these guppies were taken from the lower Arima River, this was based on much later recollection and subsequent research suggests that the source location was actually another nearby Caroni river, the Guanapo (Magurran, 2005). Haskins had been performing other guppy experiments in Trinidad around this time, including the introduction of morphologically distinctive laboratory-bred strains into the headwaters of the Arima and Paria Rivers, followed by several years of observation at downstream locations to determine the extent of their movement and long-term survival (Haskins *et al*., 1961). Unlike those experiments, he never published his findings after moving guppies from the Caroni to Oropouche, and the results of this experiment were not investigated for many years.

Haskins' 1957 experiment exposed the transplanted guppies to both a change in location and a change in predation level, as they were caught in a part of the Caroni rich with large predators and moved to an area containing only the smaller predator *R. hartii* (Carvalho *et al*., 1996). More than 30 years later, an allozyme based study was conducted to determine the long-term genetic effects of this experiment. Results indicated that the introduced guppies had flourished

in their new habitat and over time the newly formed population had also spread throughout the river, mixing with the native population downstream (Shaw *et al*., 1992). More recent studies have confirmed that, while populations from the Oropouche watershed are usually highly differentiated from those in the Caroni, Turure River guppies are an exception and are generally found to be genetically similar to fish collected in the Caroni (Willing *et al*., 2010). Evidence of gene flow from the Caroni has been found even in downstream areas of the Turure far removed from the original introduction site, implying that the transplanted Caroni watershed guppies may be displacing the indigenous population of the river (Suk & Neff, 2009).

Unlike the Turure, previous studies have not included samples from the La Seiva River, but the guppies caught there in 2011 appear to be extremely similar to those from the Turure and dissimilar to all populations taken from the Quare. As the La Seiva and Turure Rivers run adjacent to each other with their headwaters only a short distance apart, it seems reasonable to assume that the reason for this similarity is the immigration of introduced fish or their descendants from the Turure to the La Seiva. This assumption is supported especially in the clustering analysis, where most La Seiva guppies and high predation Turure guppies form a single cluster, and both rivers show evidence of gene flow from the Guanapo.

The close relationship between Caroni and Turure guppies is almost certainly due to a documented anthropogenic introduction, but that is not the only case of gene flow between these two drainages. An east/Oropouche mtDNA

haplotype was found in 2 of the 11 individuals collected in the Aripo River, indicating migration in the opposite direction, from Oropouche to Caroni watersheds. Although this connection is not apparent in the microsatellite data presented here, previous microsatellite and SNP research has also revealed the signature of Oropouche guppies in fish collected in the Aripo (Suk & Neff, 2009; Willing *et al*., 2010). This may be due to the close proximity of the Quare and Aripo Rivers, which lie only 70 meters apart at their closest point (Magurran, 2005). Occasionally a few individuals may be relocated from one river to the other during various human activities or seasonal floods caused by heavy rainfall (Suk & Neff, 2009).

Gene flow from one watershed to another can also be seen on a smaller scale between the Yarra and Damier Rivers on the north shore of the island. Guppies from these neighbouring rivers appear closely related in all analyses, and some F_{ST} values between the two rivers are not significantly different from zero, signifying essentially no genetic divergence. The Yarra River high predation site and all of the Damier River form a single cluster in the Bayesian clustering analysis and the two rivers are connected with a very high bootstrap value in the dendrogram. As with the similarity between the Guanapo and Turure Rivers, this relationship is due to a documented artificial introduction experiment. Guppies were absent from the Damier River until 1996, when D.N. Reznick introduced 200 individuals from a high predation site in the Yarra River into a low predation area of the upper Damier River (Karim *et al*., 2007). Samples from these two rivers are extremely similar, much more so than samples from the

Guanapo and Turure Rivers, likely due to the shorter period of time since this introduction.

2.4.2 Genetic Structure in Guppy Populations across Trinidad

A deep genetic divide can be seen between Oropouche and Caroni guppies in all the analyses performed during this study, provided that samples from the Turure and La Seiva Rivers are treated as part of the larger Caroni drainage population in accordance with their background. While the Bayesian clustering analysis shows admixture from the opposite watershed in some individuals from both drainages, some of this admixture is probably not indicative of ongoing migration between watersheds. In particular, both high and low predation sites of the Guanapo River show signs of admixture from the Turure and La Seiva Rivers. This is probably the result of genetic similarities between native Guanapo fish and the descendants of the Haskins introduction fish, and is unlikely to indicate actual gene flow from the Oropouche to the Caroni. The $\delta\mu^2$ tree and both PCoAs show the Caroni and Oropouche population groups as highly divergent, although this divide is less clear in the F_{ST} based PCoA than in other analyses – possibly because only 62.2% of the total variation in F_{ST} values is described by the three axes of this plot, omitting any divergence between drainages that lies in additional unseen dimensions. Mitochondrial data reinforce the idea of two very separate groups of populations, with most fish taken from the Caroni carrying similar haplotypes that are distinct from the haplotypes common to Oropouche guppies.

This divide is consistent with the 'two-arc' model often proposed for the colonization history of guppies on the island of Trinidad. Under this hypothesis, populations found in rivers on the west coast of the island are assumed to have originated from the northern part of the Orinoco River delta, while populations on the east coast were established by fish from the southern part of the Orinoco (Magurran, 2005). This hypothesis has been applied to explain the results of many genetic studies, as a clear distinction between Caroni and Oropouche guppies has been observed consistently throughout the literature (e.g. Carvalho *et al*., 1991; Shaw *et al*., 1991; Fajen & Breden, 1992; Alexander *et al*., 2006; Willing *et al*., 2010).

The two-arc model may account for population structure patterns in these two major watersheds, but leaves questions about the colonization of rivers along the north coast of Trinidad and the relationship between populations there and those in other rivers on the island. Some studies have concluded that populations in the north are closely related to Caroni watershed populations, essentially forming a single large group highly divergent from populations in the Oropouche (Carvalho *et al*., 1991; Fajen & Breden, 1992). But the results of other studies have shown that north coast river populations may not be as closely related to Caroni populations as originally thought (Suk & Neff, 2009), or that they may actually form a distinct group of their own, completely separate from both Caroni and Oropouche groups (Willing *et al*., 2010).

My findings suggest a complex relationship between populations in the north, and those in the Caroni and Oropouche. Bayesian clustering shows very

little admixture from either drainage in individuals from the north, probably indicating that there is not a great deal of ongoing gene flow into the north shore rivers. The $\delta\mu^2$ dendrogram indicates that even though northern river populations generally do form their own distinct groups, those groups are much more closely related to the Caroni drainage than they are to the Oropouche. The similarity between the Caroni drainage and some northern rivers may be due to a shared history of colonization by the same source population from the Orinoco delta, or it could be a sign of later migration between rivers on Trinidad.

Although many of the north coast river samples exhibit this similarity to Caroni samples, populations from some northern rivers are instead closely linked to those from the Quare River (within the Oropouche watershed), and are highly dissimilar to any found in the Caroni. These populations – taken from the Madamas, San Souci, and Shark Rivers – also lie close to those from the Quare in both PCoAs. Guppies from the Tompire River, which is located on the east coast of the island between the mouth of the Oropouche watershed and the north shore, are also somewhat similar to Quare samples. However, not all the populations in this region are closely related to Quare guppies – the Mission River lies between the San Souci and Tompire, yet microsatellite analysis suggests that the population there is more similar to Caroni drainage guppies than to Oropouche. But in general, guppies from the west side of the north shore appear to be more related to Caroni guppies, while those on the east side are more akin to Oropouche samples. Analysis of mtDNA supports this pattern, with west/Caroni haplotypes found in most fish from the western half of the north

shore and east/Oropouche haplotypes in most fish from the eastern half, including the Mission. It seems that if two-arc colonization did occur, the northern rivers were not entirely colonized by the same wave of immigrants that established populations in the Caroni, although some of the more westerly rivers may have been.

Instead, rivers in the eastern part of the north shore of the island were likely colonized from the Oropouche watershed at some point after that watershed was colonized by immigrants from South America. Despite flowing in opposite directions, several northern rivers and Oropouche drainage rivers have headwaters that lie in close proximity to each other, and migration between rivers might be possible in those areas. Guppies may be able to swim through channels of water formed on the forest floor during periods of heavy flooding, or individual fish may be accidentally relocated to another river because of anthropogenic interference or the actions of predatory birds or bats (Carvalho *et al*., 1991). Artificial introduction has demonstrated that just one pregnant female is capable of establishing a viable new population, perhaps due to the ability of female guppies to store sperm from several males (Carvalho *et al*., 1996). This type of migration seems particularly plausible between the Madamas and Quare Rivers, as populations there appear to be particularly closely related and only a short distance separates the upper reaches of these two rivers. A previous study also found that samples from these two rivers were genetically similar and highly differentiated from guppies in both the Caroni drainage and in neighbouring northern rivers (Alexander *et al*., 2006).

After Oropouche drainage guppies colonized the Madamas they may have then migrated into other rivers along the northeast coast. As with migration from the Quare to the Madamas, guppies could travel over land due to flooding, human activity, or predation. Alternatively, they may have moved from the mouth of one river to the next during the wet season, when heavy rainfall reduces the salinity of the Caribbean Sea (Barson *et al*., 2009). Migration by sea may explain both the colonization of rivers east of the Madamas and the gene flow occurring between other northern rivers, such as the admixture from the mainstem of the Marianne River seen in the Madamas in Bayesian clustering analysis and the presence of west/Caroni mtDNA haplotypes in the San Souci. Guppies in the Mission River, the easternmost north shore river included in this study, are a notable exception to the overall pattern of differentiation on the north coast. Unlike nearby rivers like the San Souci and Tompire, Mission River guppies are similar to Caroni guppies in microsatellite analysis but carry only east/Oropouche mtDNA haplotypes. This suggests that migration from both the Oropouche drainage and the Caroni drainage (or a Caroni-like northern river) has occurred at different times in the past.

The majority of the sites included in this study are located in the Northern Range, either along the north coast or in the Caroni and Oropouche watersheds that drain the southern slopes of these mountains. However, samples were also collected in the Stollmeyer River, in Pitch Lake, and on the island of Tobago. As in a prior study (Fajen & Breden, 1992), only west/Caroni mtDNA haplotypes are evident in Tobago guppies, but it is unclear whether this is because of the natural

colonization history of that island or due to a more recent undocumented introduction. A single haplotype is common to most individuals from the Stollmeyer and Pitch Lake, and given their proximity it seems likely that populations there are closely related, but the Stollmeyer sample was not large enough to allow microsatellite analysis. The dendrogram, PCoAs, and AMOVA show that the population from Pitch Lake is somewhat similar to samples found in the Diego Martin, Yarra, and Damier Rivers. These form a group of coastal rivers somewhat divergent from the more inland Caroni drainage rivers. It is possible that this group represents a separate wave of colonization from mainland South America, or that it is the result of ongoing migration across the Gulf of Paria.

2.4.3 Has Speciation Occurred in the Oropouche Drainage?

The depth of the genetic differentiation between Caroni and Oropouche populations could indicate that these are two separate guppy species. Schories *et al*. (2009) suggest that the guppies found in the Quare and possibly in other east coast rivers belong to the species *P. obscura*, while those in the Madamas and rivers to the west are members of *P. reticulata*. If this is the case, populations in the Quare and Madamas Rivers should be easily distinguished by both microsatellite and mtDNA data. However, analysis of microsatellite data consistently indicates low levels of divergence between these two rivers. They are linked in the $\delta\mu^2$ dendrogram with a bootstrap value of 78%, and lie close together in both in PCoAs. AMOVAs show increased variation among groups when the Madamas is included along with the Quare in the group of eastern

rivers rather than being grouped with Caroni or north coast rivers. This suggests a close genetic relationship and a history of migration between the Quare and Madamas Rivers, which runs counter to the hypothesis that these two rivers contain two different species of guppy. Analysis of mtDNA also failed to find differentiation between the rivers – 11 of 12 Madamas guppies and 9 of 16 Quare guppies all carried the same mtDNA haplotype. Moreover, the same haplotype was found in two fish from the Aripo River, but microsatellite analysis did not find evidence of Oropouche-type guppies in the Aripo.

If speciation has occurred, there should be consistent alignment of fish in natural populations as either *P. reticulata* or *P. obscura*, but guppies found in rivers across the north shore are not always clearly defined as one lineage or the other. For instance, analysis of mtDNA sequences would classify samples collected in the Mission River as *P. obscura* due to their similarity to Quare River guppies, but microsatellite data would characterize the same guppies as *P. reticulata* because of their connection to Caroni drainage populations. Guppies in the Madamas River could not be unambiguously described as either *P. obscura* or *P. reticulata*, since most analyses link Madamas populations to those in the Quare, but Bayesian clustering analysis suggests that a substantial minority of individuals are instead similar to fish from the Marianne River. In addition, one Madamas fish carries a west/Caroni mtDNA haplotype instead of the east/Oropouche haplotype that dominates the Quare. Populations in the San Souci and Tompire Rivers also appear genetically similar to Quare samples in

microsatellite analysis, but as in the Madamas, some individuals in each of these rivers carry west/Caroni mtDNA haplotypes.

Overall, the data collected in this study do not support the designation of a new guppy species in the Oropouche drainage. Although there is ample evidence of high levels of genetic differentiation between Caroni and Oropouche populations, there are also signs of gene flow and introgression between the two lineages, pointing to an absence of natural geographic or biological barriers to interbreeding. In particular, populations in some rivers on the north coast of the island harbour a combination of Caroni-like and Oropouche-like genotypes and could not be readily characterized as either *'obscura*' or *'reticulata'*, hence it is more reasonable to regard them all as *P.reticulata*.

Chapter 3: Structure in the Marianne and Paria Rivers

3.1 Introduction

3.1.1 Elements of Population Structure: Dispersal and Adaptation

Evidence suggests that the genetic relationships among guppy populations from across Trinidad are a reflection of the routes taken by guppies as they colonized the island (Magurran, 2005). Yet colonization history cannot explain the many cases in which there is apparent genetic differentiation among populations found within a single river. Instead, population divergence within rivers is likely a result of constrained gene flow among populations in different areas of the river (Crispo *et al*., 2006). Gene flow may be limited because migrants from guppy populations in upstream regions lack the adaptations necessary to thrive and reproduce in downstream areas, where guppies typically coexist with large piscivorous fishes and have therefore evolved various morphological, behavioural, and life history traits that aid in predator avoidance (Endler, 1980; Magurran & Seghers, 1990; Reznick, 1996). Experiments suggest that guppies adapted to low predation environments experience very high mortality when relocated to downstream sites containing large predators, which may significantly reduce the genetic impact of migrants on high predation populations (Weese *et al*., 2011). Immigrants may also be maladapted for certain ecological conditions, such as stream size, canopy cover, and availability of food, which vary between downstream and upstream sites (Reznick *et al*., 2001). Another possible factor affecting gene flow is the presence of stream barriers

such as waterfalls, which impede migration, particularly in the upstream direction (Becher & Magurran, 2000).

In many of the rivers sampled in this study the small number of collection sites as well as, in some cases, the aftereffects of human-mediated introduction make it impossible to determine whether gene flow between populations is restricted primarily by adaptive divergence or is a result of physical barriers to dispersal. However, two rivers on the north shore of the island, the Marianne and Paria, form an ideal location to study factors influencing gene flow and to investigate fine-scale population structure. These two rivers have not been exposed to the effects of human activity to the same extent as some rivers on the south slopes of the Northern Range (Millar *et al*., 2006), and sites sampled within the rivers vary widely in terms of predation level, ecology, and geography.

3.1.2 Previous Studies

The Marianne and Paria Rivers run adjacent to each other on the north shore of Trinidad, originating high in the Northern Range Mountains and draining into the Caribbean Sea. Certain conditions, such as the density of the rainforest canopy and the width and depth of the water, are similar in both rivers (Hendry *et al*., 2006). The Marianne differs from the Paria though in that it is divided into high and low predation areas by several waterfalls that prevent large predators from entering upstream guppy habitats. As in other north coast rivers, high predation regions in the Marianne are marked by the presence of large piscivorous fish such as gobies, while low predation areas contain only smaller predators such as the killifish *Rivulus hartii* and the freshwater prawn

Macrobrachium crenulatum (Magurran, 2005). In contrast, the Paria is blocked to the upstream movement of diadromous predatory fishes by a single large waterfall near the mouth, resulting in a low predation environment throughout the entire river (waterfalls in both rivers are marked in Figure 3.1).

Guppy populations in the Marianne and Paria have not been as extensively investigated as those in rivers on the south slope of the Northern Range. Nonetheless, recent research has found that within each river populations vary in terms of both morphology and colouration, probably due to differing predation levels and variations in environmental factors such as canopy openness (Hendry *et al*., 2006; Millar *et al*., 2006). A microsatellite-based study of genetic structure in guppy populations within the Marianne River also showed evidence of isolation by distance between populations – the genetic similarity of populations decreased as distance increased (Crispo *et al*., 2006). The same study found that gene flow among populations was considerably impeded by physical barriers and ran predominantly in the downstream direction, presumably because guppies can move downstream over waterfalls but are much less likely to migrate upstream past such large obstacles. Increased genetic diversity was observed in downstream guppy populations when compared to those upstream, likely a result of this tendency towards unidirectional movement (Crispo *et al*., 2006). A later study also noted this pattern of lowered upstream diversity and downstream-biased migration in the Marianne as well as in many of the rivers of the Caroni drainage (Barson *et al*., 2009).

While gene flow may be expected among sites within a single river, some studies conducted in this area have also found evidence of gene flow from one river to another. Willing *et al*. (2010) found that guppies from the Petite Marianne River, a tributary of the Marianne, did not cluster with mainstem Marianne guppies in a population genetic analysis of single nucleotide polymorphisms (SNPs), but instead appeared similar to Paria River guppies. The Petite Marianne has its headwaters very near the Jordan River, a tributary of the Paria (see Figure 3.1 for location), suggesting that the guppies that colonized the Petite Marianne may have originated in the Paria. Migration between watersheds might have been caused by flooding, human interference, or avian predation and has been proposed to explain the movement of guppies between seemingly unconnected rivers in other parts of Trinidad (Suk & Neff, 2009). This type of migration may have also occurred elsewhere in the Marianne drainage, as microsatellite data have indicated a low level of ongoing migration between downstream areas of the Marianne and Yarra Rivers (Barson *et al*., 2009). However, gene flow between those two rivers may be a result of migration via the Caribbean Sea during periods of low salinity rather than a consequence of movement directly from one river to another.

3.1.3 This Study

The objective of this portion of the present study is to investigate the factors that influence patterns of guppy population structure on a fine scale. In Chapter 2, structure was estimated for 25 sites encompassing a broad geographic area, whereas this chapter examines two rivers, the Marianne and

Paria, in greater detail. In order to establish the genetic connections between populations in these two rivers, samples were collected at multiple sites within each river, and temporal replicates were made at some locations. Sites were selected with the intention of encompassing as many areas and major tributaries of both rivers as possible – including the Petite Marianne, mainstem, and east and west headwaters in the Marianne River , and the headwaters, mainstem, Jordan, and an area referred to here as Carl's Tributary in the Paria River. Samples from a total of 20 sites within these two rivers were genotyped at 10 microsatellite loci.

The current study re-examines the conclusions of Crispo *et al*. (2006), making use of a somewhat different and expanded set of microsatellite markers. The results of that study suggest that the genetic structure of guppy populations in the Marianne River is influenced largely by the distance between sites and the presence or absence of stream barriers, rather than by the effects of divergent natural selection caused by differing levels of predation. Similar results are expected in this study, confirming that genetic divergence is low between neighbouring sites when compared to geographically distant sites, that populations in the same area of the river are more alike than those separated by waterfalls, and that selection pressure is not a major reason for reduced gene flow between populations.

Genetic population structure within the Paria River has not been previously studied, but inclusion of the Paria in this study offers an interesting comparison to the Marianne, as the Paria lacks the barrier waterfalls (with the

exception of one waterfall isolating site P-E, Figure 3.1) and variations in predation level seen in the Marianne, but is in other respects very similar. If gene flow is limited predominantly by waterfalls and differing predation levels, then the Paria should show greater gene flow in both the upstream and downstream direction when compared to the Marianne. The same pattern of low genetic divergence between neighbouring sites and increased differentiation over distance expected in the Marianne may also be found in the Paria, but it is possible that the absence of barrier waterfalls in the Paria will diminish this pattern.

Finally, this study will allow for an independent test of the suggestion by Willing *et al*. (2010) of gene flow occurring between the Marianne and Paria watersheds. If migration is occurring between these locations, analysis will reveal that populations in the Petite Marianne tributary are more closely related to those in the Paria than they are to populations in the mainstem of the Marianne. To explore the possibility of migration between rivers at both this location and other locations, several sites in close proximity to the opposite watershed were included in each river. The inclusion of a large number of sites will ideally allow for accurate estimates of gene flow both within and between the Marianne and Paria Rivers.

3.2 Materials and Methods

3.2.1 Sample Collection

Guppies were collected between 2002 and 2010 at 20 sites within the Marianne and Paria Rivers, on the north coast of Trinidad (for site locations see

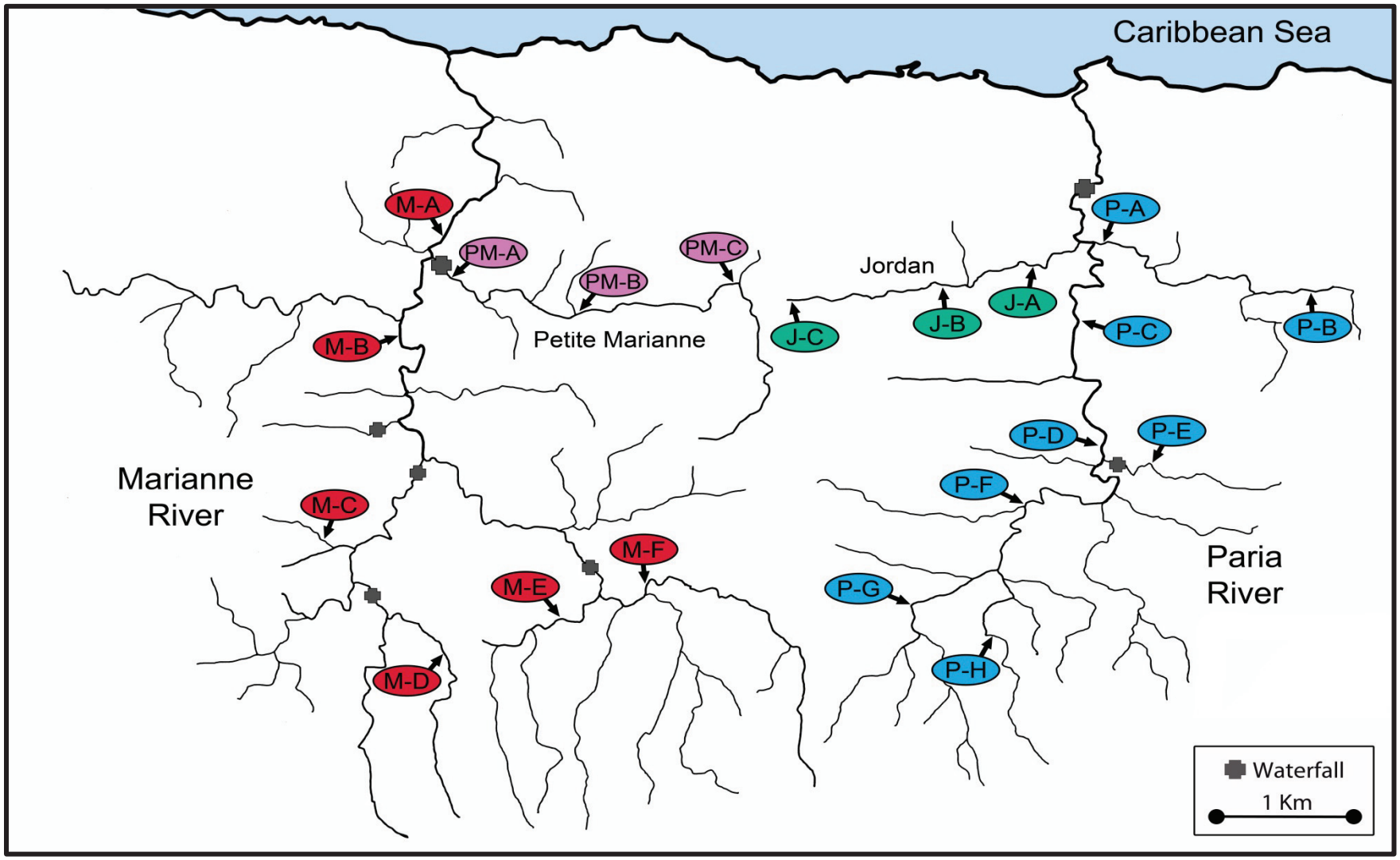


Figure 3.1: Detail map showing sampling sites and waterfalls in the Marianne and Paria Rivers on the north shore of Trinidad. The location of these rivers relative to others on the island can be seen in Chapter 2, Figure 2.3.

Figure 3.1). In order to gauge the stability of populations over time, temporal replicates were included at five sites in the Marianne and one in the Paria. On average, 40 fish were collected at each site and in each temporal replicate (range, 38-50), resulting in a total of 1,094 individuals. Guppies were treated with a lethal overdose of tricaine methanesulfonate (MS-222) and fin clips were stored in 95% ethanol until further analysis. Site locations, year of collection, and number of individuals are listed within the full sample list in Chapter 2, Table 2.1. For clarity, sites in the Petite Marianne, a tributary of the Marianne River, and in the Jordan, a tributary of the Paria River, are listed separately and guppies collected there are referred to throughout as 'Petite Marianne River' and 'Jordan River' samples, respectively.

3.2.2 Laboratory Protocol

Total genomic DNA was extracted from all samples collected in 2002 by Erika Crispo using a Qiagen phenol-chloroform extraction protocol.
DNA from samples collected in later years was extracted by the author using a modified glassmilk protocol (Elphinstone *et al*., 2003) carried out on a Perkin Elmer MultiPROBE II liquid handler. Fin tissue was prepared for extraction by placing it in a mixture of 225μl of digestion buffer (100mM NaCl, 50mM TrisHCl pH8,10mM EDTA, 0.5% SDS) and 2μl proteinase K, and incubating overnight in an orbital shaker held at 55°C and 200 rpm.

A total of 1,094 individuals were genotyped at 10 microsatellite loci. Primers used for genotyping were the same as those used in Chapter 2, and are listed in Appendix 3. DNA was amplified by PCR in 5 μl reactions consisting of

10-50ng DNA, 0.5μl 10x ThermoPol PCR buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1 % Triton X-100), 200μM dNTP, 200μM fluorescently labelled forward primer, 200μM reverse primer, and 0.5U *Taq* DNA polymerase (New England BioLabs). Forward primers were labelled with a fluorescent marker in either 700nm or 800nm wavelength (added by the manufacturer, MWG Biotech AG). PCR protocol for all microsatellite loci consisted of 4 min of denaturation at 95ºC; 30 cycles of denaturation at 95ºC for 30s, annealing at xºC (x being the optimal annealing temperature listed for each primer, given in Appendix 3) for 30s, and extension at 72ºC for 30s; followed by a final incubation at 72ºC for 3 min. All PCRs were conducted in Eppendorf Mastercycler ep thermal cyclers.

After amplification, PCR products were visualized by electrophoresis on 8% denaturing polyacrylamide gels run on a LI-COR IR^2 DNA analyzer at a temperature of 50°C. Gel images generated by this process were scored visually to determine allele sizes in each individual at each microsatellite locus. All images included positive controls, redundant samples, and a molecular weight size standard ladder made up of pUC18 PCR fragments. The inclusion of multiple controls in every image ensured accurate and consistent scoring of all populations across all 10 loci.

3.2.3 Data Analysis

After scoring, all data was assessed with Micro-Checker v.2.2.3 (van Oosterhout *et al*., 2004) to detect potential genotyping errors. Once identified, errors caused by large allele drop-out, null alleles, and inaccurate scoring due to

stutter were rectified wherever possible. The estimated frequency of the null allele, along with allelic size range and frequency of the most common allele, was found with GENEPOP v.4.0.10 (Raymond & Rousset, 1995; Rousset, 2008). Total number of alleles and allelic richness were found with FSTAT v.2.9.3.2 (Goudet, 2001), and both observed and expected heterozygosity rates were calculated for each of the 10 loci in GENETIX v.4.05 (Belkhir *et al*., 1996-2004). Values of all genetic diversity statistics for each locus at each of the 20 Marianne and Paria sites are listed in Appendix 4, and bubble plots of the frequency of each allele at each site can be found in Appendix 6. Because there is low level of genetic differentiation between samples collected in different years at the same site, data from all temporal replicates are pooled in both of these appendices in an effort to concisely present summary statistics and allele frequencies. Observed heterozygosity values were also averaged over all loci for each site and each temporal replicate and these values were plotted onto a map of the Marianne/Paria region in order to determine the effects of site location and presence of waterfalls on population diversity.

All 10 loci were checked for indications of possible selection pressure using LOSITAN (Beaumont & Nichols, 1996; Antao *et al*., 2008). When this analysis was performed using the full dataset in Chapter 2, four loci were identified as potentially under positive selection. As the locus Pret-46 proved problematic when exploring genetic structure over the whole island, it was dropped from further analysis in that chapter. When LOSITAN was run using only the data from the Marianne and Paria Rivers only two loci were recognized

as candidates for positive selection, Pre-38 and G145. While the inclusion of Pret-46 caused seemingly spurious results when used in the analysis of broad scale population structure over the entire island, it showed no such tendency during analysis of only the Marianne and Paria. Instead, the same patterns of population differentiation were seen at all 10 loci (this pattern is visible in the allele frequency plots in Appendix 6). Moreover, exclusion of data collected at the two loci identified by LOSITAN as under possible selection did not appreciably change the overall patterns of population structure, and so data from all 10 loci was used in all analyses in this chapter.

As in Chapter 2, population structure was determined using several different means of measuring genetic distance in order to avoid the pitfalls associated with any given method. Model-based Bayesian clustering analysis was conducted in STRUCTURE v.2.3.3 (Pritchard *et al*., 2000; Falush *et al*., 2003). All 1,094 individuals collected in the Marianne and Paria Rivers were included in this analysis. In STRUCTURE, a burn-in period of 50,000 replicates was followed by 50,000 replicates of the Markov-chain Monte Carlo (MCMC) simulation. Three iterations were performed for each possible number of population clusters (K-value) from 2-27 to accurately determine the most likely number of clusters. Probability of each K-value was found using the Evanno method (Evanno *et al*., 2005) executed in STRUCTURE HARVESTER (Earl & vonHoldt, 2011), which selects the most likely K-value based on the rate of change of the log probability of data (ΔK).

Microsatellite data from the 27 sampled populations was also used to create a neighbour-joining tree based on Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza & Edwards, 1967). This unrooted dendrogram was created in Populations v1.2.32 (Langella, 1999-2010) and viewed in TREEVIEW v1.6.6 (Page, 1996). Principle coordinate analyses (PCoAs) were carried out using both distance between population averages and distance between individuals. First, a PCoA of all populations was created based on F_{ST} values, which were calculated in Microsatellite Analyser (Dieringer & Schlötterer. 2003). A second PCoA was conducted using all 1,094 individuals sampled in the Marianne and Paria Rivers, based on a distance matrix created from the codominant genotypic distance algorithm of GENALEX v.6.41 (Peakall & Smouse, 2006, algorithm described in Smouse & Peakall, 1999). Both PCoAs were generated using GENALEX.

The rate of migration among all sites over the last few generations was calculated using a Bayesian method in BAYESASS v.3.0.1 (Wilson & Rannala, 2003). For the purpose of this analysis data from all temporal replicates was pooled at each site. BAYESASS was run with a burn-in of 1 million MCMC iterations followed by 10 million sample iterations. This process was repeated several times with various starting seed numbers to check for consistent and accurate results, as recommended in the program documentation. The results of all runs were examined to ensure convergence, but only values from the first run were reported. Standard deviations given by the program were used to construct

95% confidence intervals around each estimated rate of migration. Migration rates were considered significant if this interval did not include zero.

In addition to gene flow within each river, movement of individuals from one river to the other was suspected in two locations – between the upper reaches of the Paria and the eastern headwaters of the Marianne and between the Jordan tributary and the Petite Marianne tributary. The connection between rivers almost certainly predates the recent migration rates found in BAYESASS, so historical rates were estimated using the isolation-with-migration coalescent model implemented in IM (Nielsen & Wakeley, 2001; Hey & Nielsen, 2004). As with BAYESASS, several runs were performed in IM using different starting seeds to confirm that results were uniform across runs, but only the values from the longest of the runs were reported. Each run commenced with a burn-in period of 100,000 MCMC iterations and was allowed to proceed until all effective sample sizes were over 50 and trendline plots indicated convergence. This version of IM estimates long-term gene flow between a pair of contemporary populations both descended from a single ancestral population and so can only be used to compare two populations. Therefore, data from several sites were combined to create larger population groups for this analysis. In the upstream area of the two rivers, all individuals collected at sites M-E and M-F in the Marianne River were compared to all those collected at sites P-F, P-G, and P-H in the Paria. Gene flow in the downstream region was assessed by comparing all samples from the Jordan to all samples from the Petite Marianne. Parameters

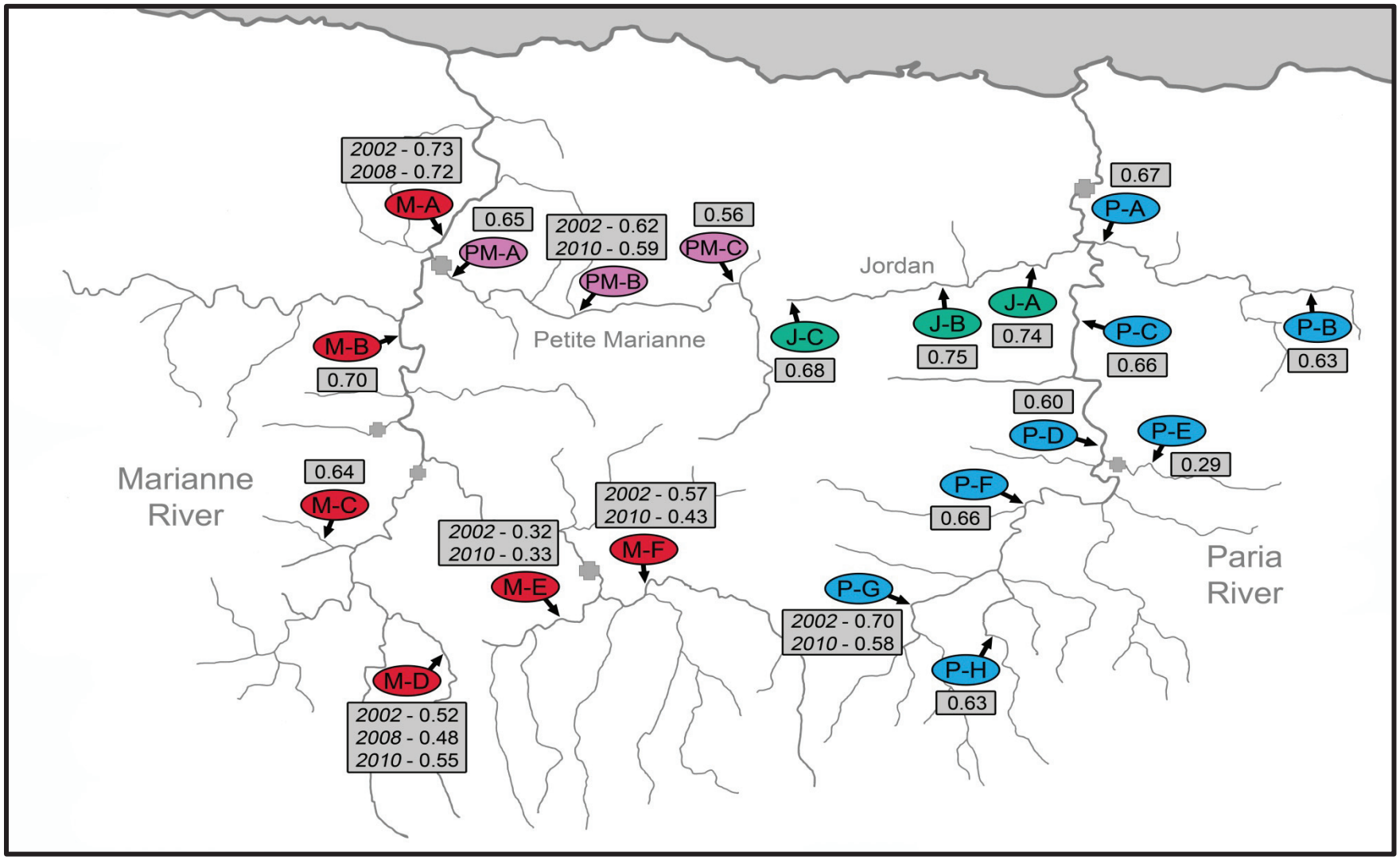


Figure 3.2: Observed heterozygosity levels at each site and each temporal replicate within the Marianne and Paria Rivers.

estimated by IM were converted into number of migrants per generation using the formulas provided in the program manual.

3.3 Results

3.3.1 Genetic Diversity

Observed heterozygosity levels were plotted on a map of the region (Figure 3.2) to allow for easy comparison of heterozygosity and site location. In the Marianne River heterozygosity levels varied from 0.32-0.73, and were generally lower in upstream areas than they were further downstream. Heterozygosity increased from an average of 0.55 in the west headwaters (sites M-C and M-D) and 0.41 in the east headwaters (sites M-E and M-F) to an average of 0.72 in the mainstem (sites M-A and M-B). Genetic diversity also increased along the short length of the Petite Marianne River, rising from 0.56 at PM-C, near the source of the river, to 0.65 at site PM-A, located near the point that the tributary flows into the mainstem at the Petite Marianne Falls. Less variation was seen among sites in the Paria River, where populations had an average heterozygosity of 0.64 in the upper reaches of the river (sites P-F, P-G, and P-H), 0.63 in the mainstem (sites P-C and P-D), and 0.65 in Carl's Tributary (sites P-A and P-B). Levels in the Jordan River were slightly higher and a moderate increase in diversity occurred between the upstream (H_O 0.68) and downstream (H_O 0.74) areas of the tributary. The population at Paria site P-E is separated from other areas of the river by a waterfall and has a very low level of heterozygosity (0.29), which appears to be an anomaly when compared to all other sites throughout the river.

3.3.2 Population Structure

The results of Bayesian clustering analysis in the Marianne and Paria showed evidence of hierarchical population structure within the two rivers. STRUCTURE HARVESTER found the maximum rate of change in the log probability of data (ΔK) at K=4, therefore identifying four probable population clusters. However, ΔK calculated over a large range of K values showed a distinct second peak in the rate of change at K=15, with a value only slightly lower than the maximum (Figure 3.3). Tests of simulated datasets have shown that the Bayesian algorithm underlying STRUCTURE will preferentially identify only the highest level of population clustering in cases where hierarchical structure exists (Evanno *et al*., 2005). Further runs conducted using subsets of the original data confirmed the majority of the clusters seen in the K=15 plot, suggesting that this higher number of population clusters is a reflection of the true population structure of guppies within the rivers. In order to show both broad and fine scale population structure, as well as possible admixture between all populations, both the K=4 and K=15 bar plots were included in Figure 3.4.

The K=4 plot showed a large population cluster that included the west headwaters and mainstem of the Marianne River, another consisting of all locations in the Petite Marianne, one containing individuals from the lower Paria River and the Jordan, and a fourth made up of samples from the upper Paria and the east headwaters of the upper Marianne. Within the lower Paria group many individuals showed evidence of genetic admixture from both the upper Paria and the Petite Marianne groups. Marianne sites M-A and M-B, located in the lower

mainstem of the river, contained a large amount of admixture from the lower Paria and Petite Marianne River groups. All individuals from Paria River site P-E belonged to the lower Paria group, despite the fact that the neighbouring site P-D contained fish predominantly belonging to the upper Paria group.

The K=15 plot revealed more complex relationships among sites, although individuals from the Petite Marianne still formed a single cluster and the downstream region of the Marianne still showed evidence of admixture from other sites. Individuals from Marianne sites M-C and M-D clustered together, as did individuals from Paria sites P-A and P-B and Paria sites P-F and P-G. The upper Paria and upper Marianne no longer formed a single large cluster but admixture from Marianne site M-F was still apparent at several sites upstream in the Paria. In general, guppies throughout the Paria showed more evidence of admixture than those in the Marianne, although all individuals at Paria site P-E formed a distinct cluster with almost no apparent admixture. In both rivers samples collected at a single site over multiple years consistently fell into the same population cluster, usually with little obvious distinction between years. Marianne site M-A was an exception in that the 2008 sample showed considerable admixture from the Petite Marianne and upstream regions of the Marianne mainstem, which was not as evident in the 2002 sample.

The dendrogram, created using Cavalli-Sforza and Edwards chord distance, also showed population stability at sites where data from multiple collection years was available (Figure 3.5). In the Marianne River, all three temporal replicates at site M-D were grouped with bootstrap values of 81%, while

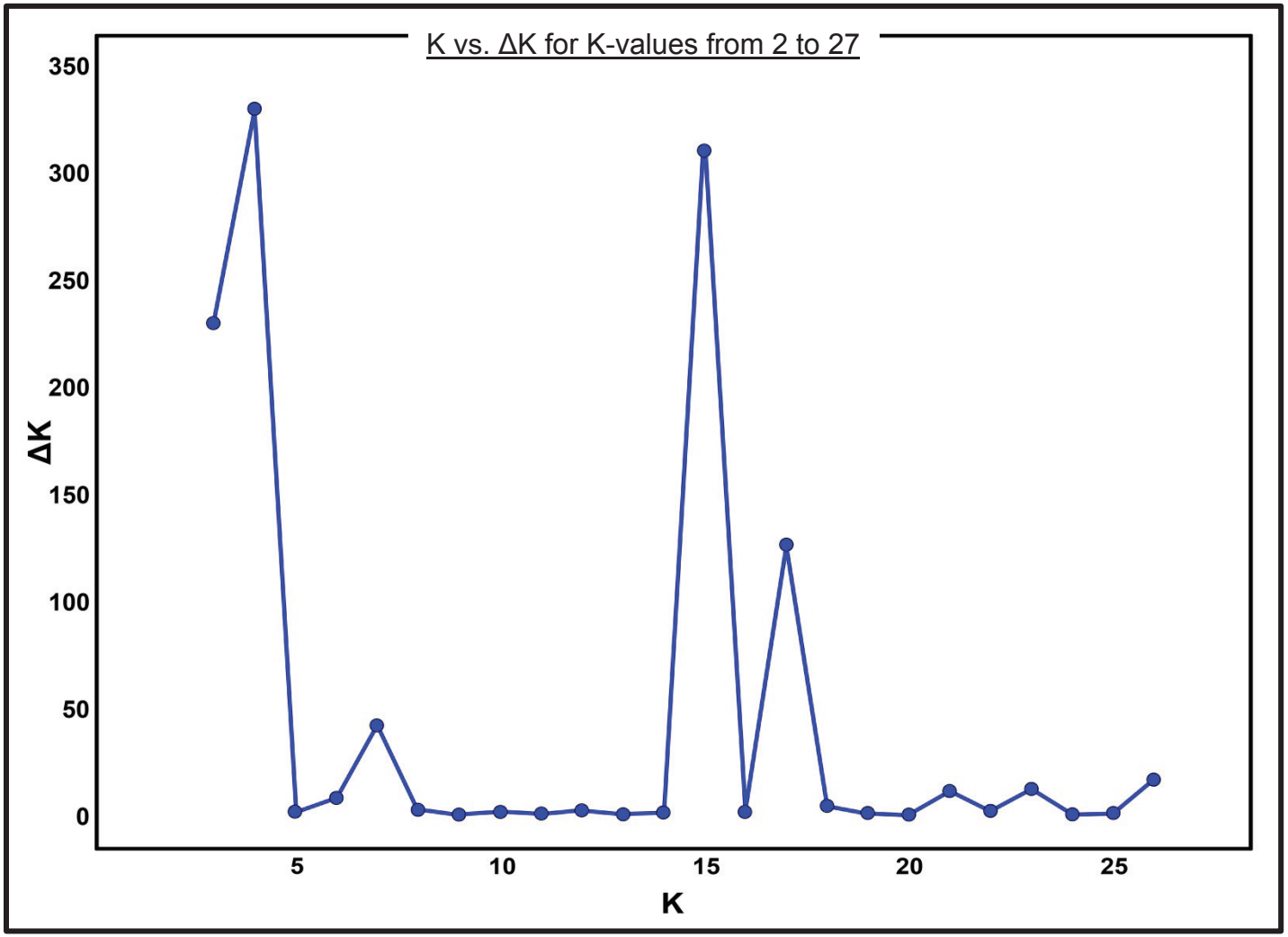


Figure 3.3: ΔK plot for Bayesian clustering analysis of 27 populations from the Marianne and Paria Rivers. K-values from K=2 to K=27 are shown, with a peaks at K=4 and K=15. Genetic clusters from the runs identified in this plot are shown in Figure 3.4.

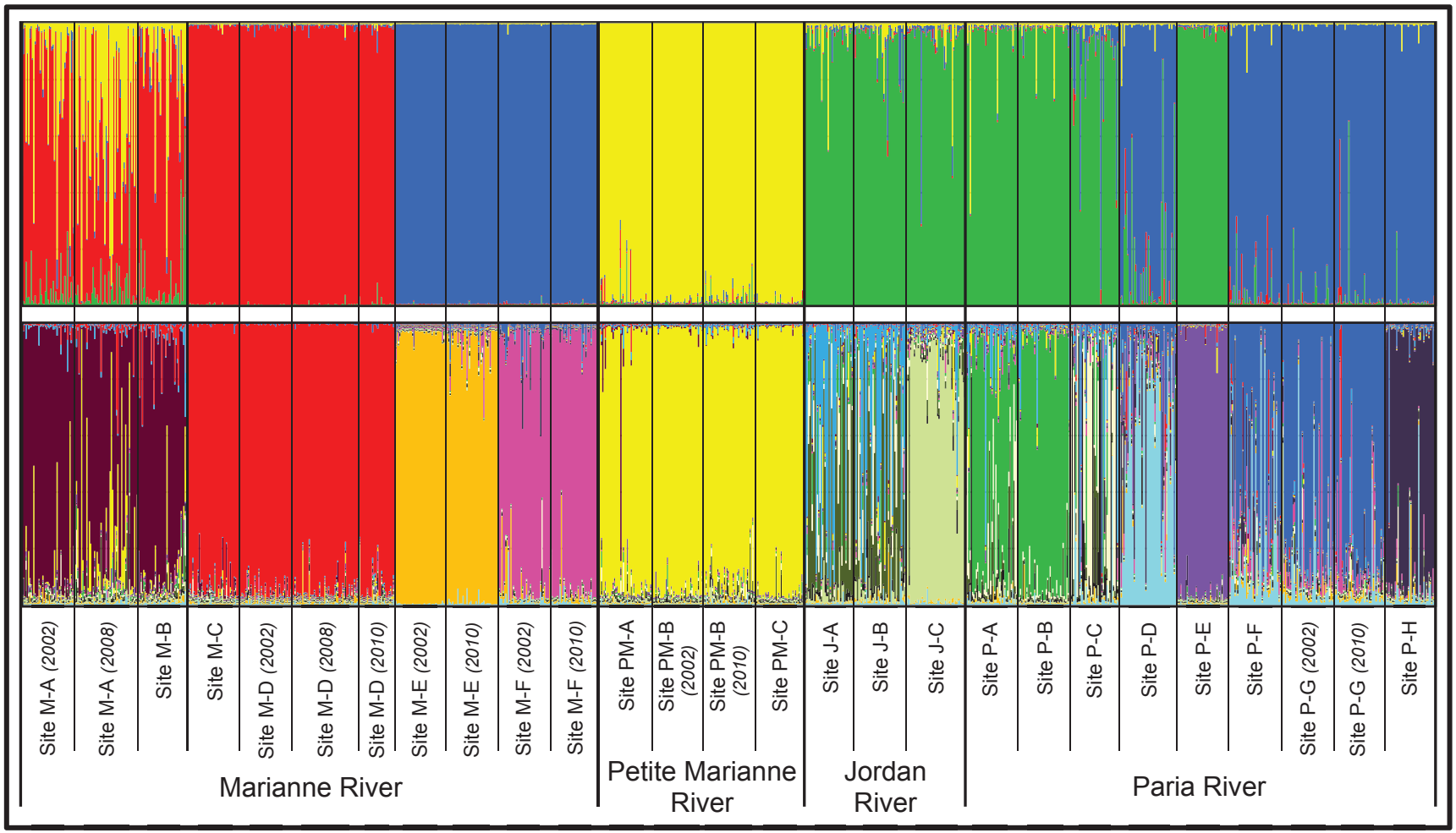


Figure 3.4: Bayesian clustering analysis of 1,094 individuals from 20 locations in the Marianne and Paria Rivers. Each individual is represented by a single vertical line, partitioned into coloured segments to indicate the estimated membership of that individual into each identified cluster. The upper plot shows all individuals grouped into 4 population clusters while the lower shows 15 population clusters.

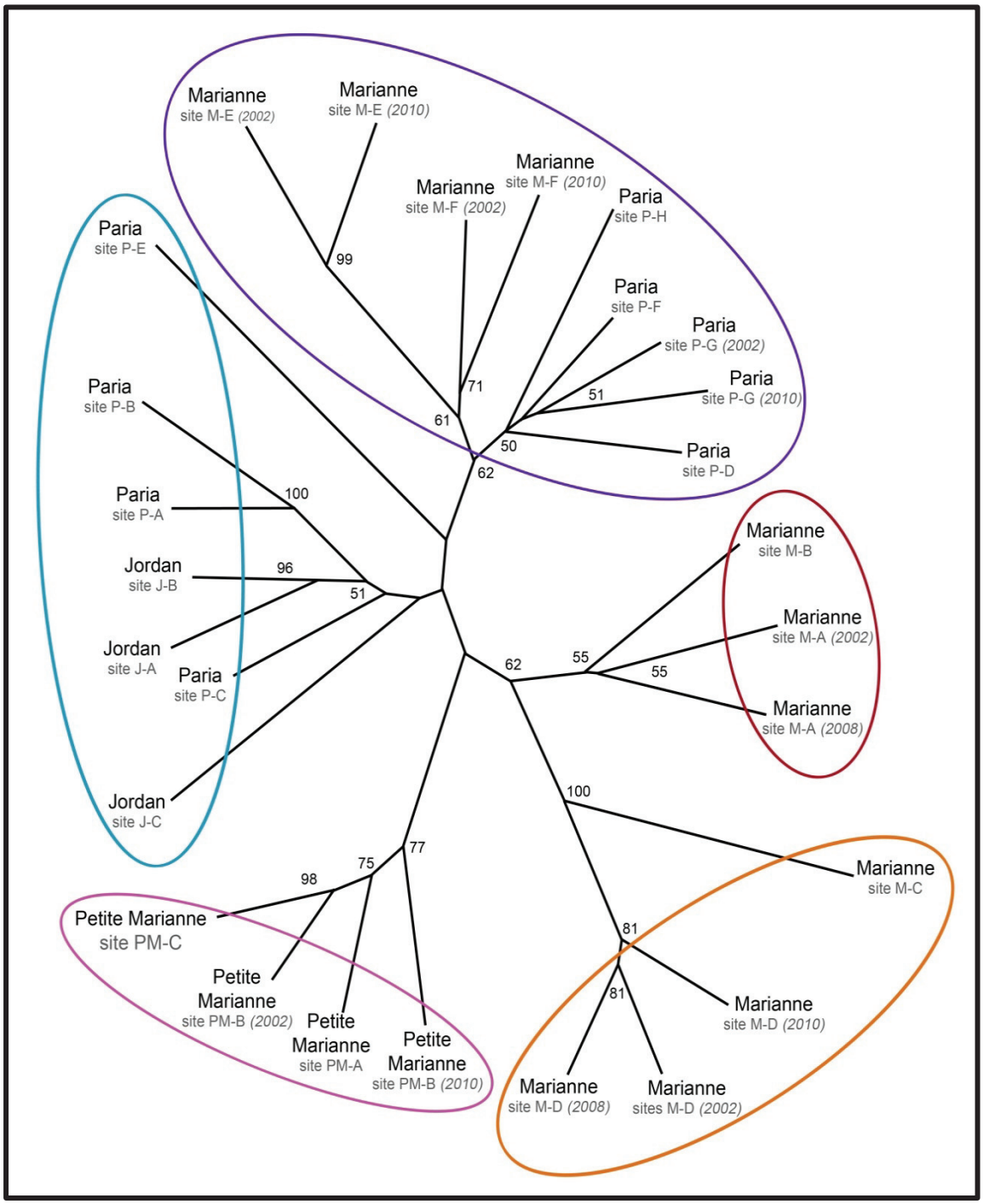


Figure 3.5: Dendrogram of Marianne and Paria populations, based on Cavalli-Sforza and Edwards chord distance. Bootstrap values above 50% (based on 1000 replications) are shown.

replicates at site M-E were linked with a bootstrap of 99%, and those at site P-F with a bootstrap value of 71%. The samples collected at Paria site P-G in 2002 and 2010 and those collected at Marianne site M-B in 2002 and 2008 were also grouped together, but had an associated bootstrap value of only 51% and 55% respectively, and the Paria samples did not appear well differentiated from populations at other sites in the upper Paria River. Replicates at Petite Marianne site PM-B were not as closely related, and the guppies collected in 2002 were not directly linked to those caught in 2010 but were instead more closely connected to samples taken from the adjacent site PM-C.

Five relatively distinct population groups were visible on the dendrogram. The Marianne River was clearly divided into four areas – the Petite Marianne (highlighted in pink in Figure 3.5), the mainstem (in red), the west headwaters (in orange), and an east headwaters group that was closely associated with populations from several sites in the upstream region of the Paria River (in purple). The latter group included populations from the upper Paria sites P-D, P-F, P-G, and P-H, while the remainder of the Paria sites formed a separate group composed of populations from the lower mainstem and the tributaries Jordan and Carl's Tributary (highlighted in blue). Although included in the lower Paria group, the population collected at Paria site P-E was not closely related to any other population and was separated from the rest of the group on its own branch of the tree. The position of this population in the dendrogram is somewhat ambiguous as the bootstrap value associated with this branch was very low, indicating that the topology of the tree in this area was not consistent throughout different

iterations. Therefore, it is possible that the population from site P-E is more closely related to the upstream Paria and Marianne group than it appears in this figure.

The same five groups seen in the dendrogram were also evident in the F_{ST} based PCoA (Figure 3.6). While population clusters were generally well-defined in the PCoA, there was considerable genetic differentiation between populations within some clusters. In the east Marianne headwaters there was some distance between sites M-E and M-F, although collections from different years at each site lay very close together. In the west headwaters, site M-C appeared somewhat divergent from the three replicates at site M-D. Samples taken from the Petite Marianne River formed a group that was separate from both the Marianne and Paria Rivers but appeared slightly more genetically similar to populations in the Paria than to those from the rest of the Marianne. Populations sampled in both the Jordan River and Carl's Tributary were extremely close to the population taken from the mainstem of the Paria River. As in the dendrogram, Paria site P-E was noticeably isolated from both upper and lower Paria population groups (this is particularly evident in the axis 2 *vs*. 3 view), and in this case it was not clear whether this population was a member of either of these two larger groups.

A second PCoA, which incorporated each of the 1,094 sampled individuals as discrete data points, showed some of the same patterns of differentiation as the F_{ST} based PCoA (Figure 3.7). Individuals from the west headwaters and mainstem of the Marianne River formed two large, diffuse

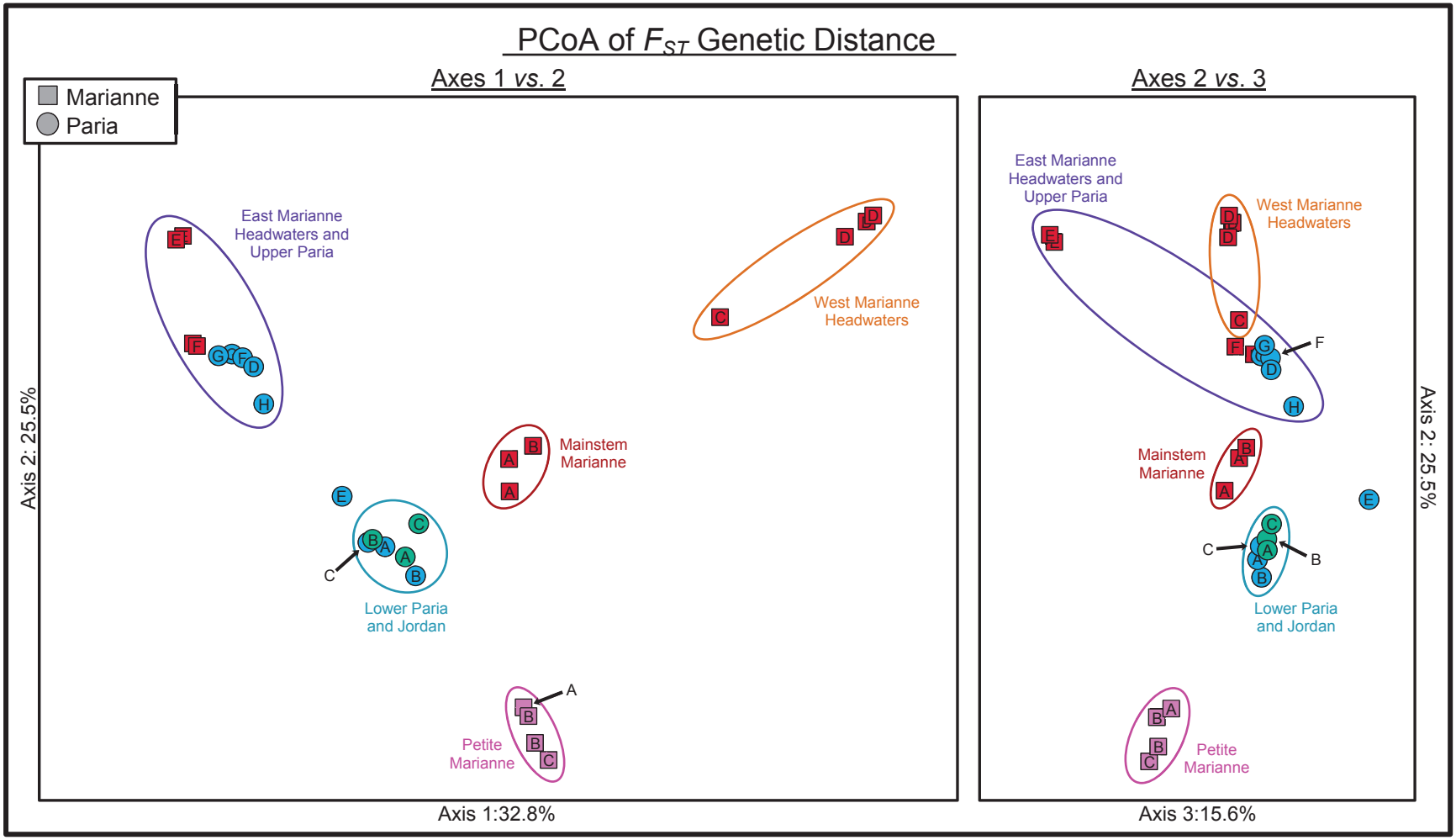


Figure 3.6: PCoA of 27 guppy populations in the Marianne and Paria Rivers, based on the F_{ST} measure of genetic distance. Three dimensions of genetic variation are shown, describing 73.9% of the total variation present in the dataset.

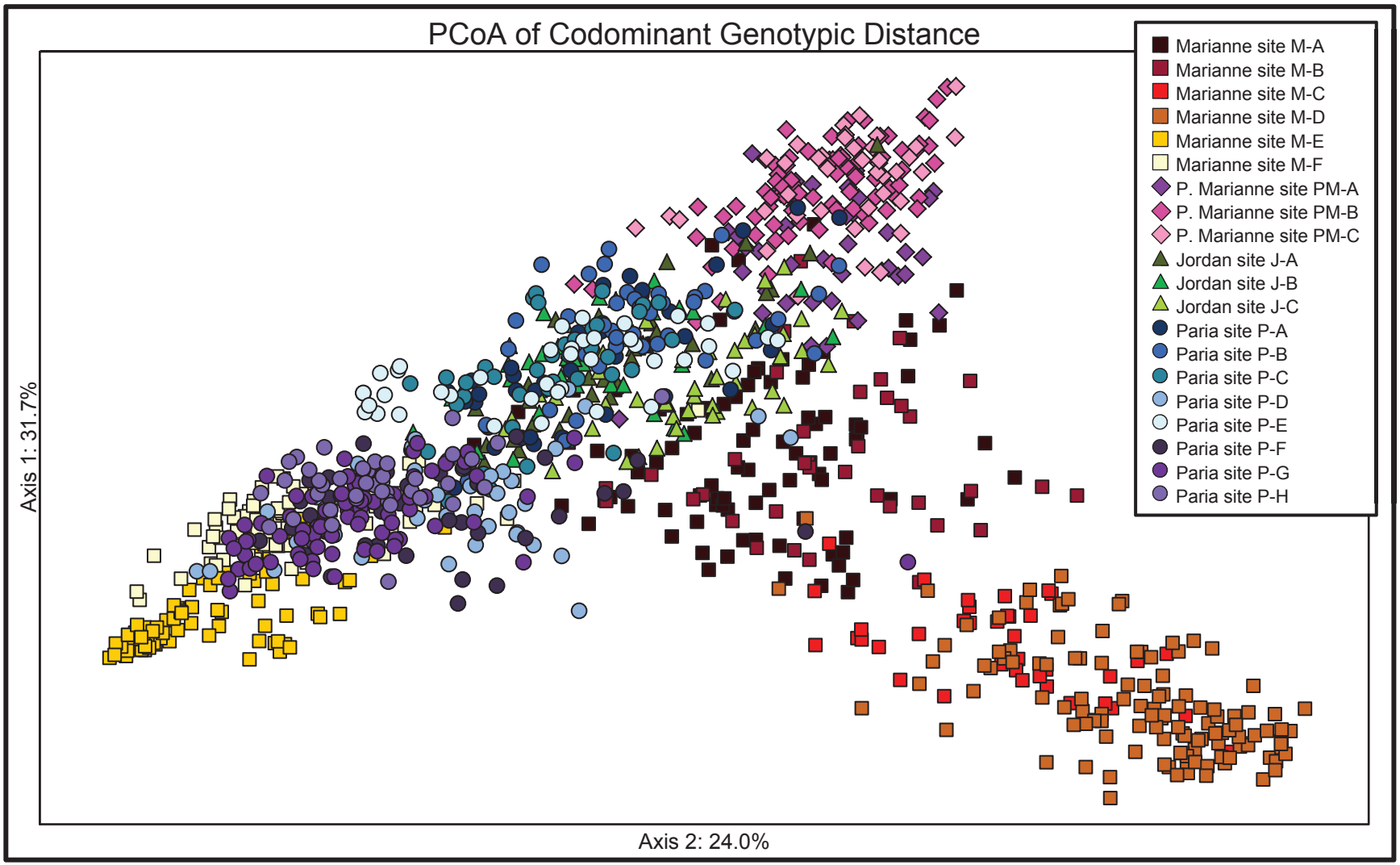


Figure 3.7: PCoA of all 1,094 individuals sampled in the Marianne and Paria Rivers, based on codominant genotypic distance. Two dimensions of genetic variation are shown, describing 55.7% of the total variation.

groups to the right of the figure. Those from the east headwaters were separate from that group and formed a tighter cluster on the left. Fish from the Paria were roughly divided into a downstream group seen in the center of the figure, and an upstream group seen on the left, with some overlap between these groups. Considerable overlap was also seen between the guppies from the upper reaches of the Paria and those collected in the east headwaters of the Marianne. Individuals collected in the Jordan River appeared to be closely related to those in the downstream area of the Paria. Petite Marianne River guppies formed a distinct group near the top of the figure, but some individuals appeared genetically similar to fish from the mainstem of the Marianne while others appeared similar to Jordan and lower Paria guppies.

3.3.3 Migration Within and Between Rivers

Estimated rates of recent migration showed evidence of movement both within and between rivers, although non-immigrants still made up the majority of all populations (Table 3.1). In both rivers movement ran predominantly in the downstream direction, but in some areas considerable migration occurred in the upstream direction. For example, evidence of migration from Marianne site M-A was found at Marianne site M-B, and migrants from Jordan site J-B were seen at Paria site P-C. Within the Petite Marianne River, migration appeared to occur in both upstream and downstream directions, and a small amount of migration from the Petite Marianne to the mainstem of the Marianne was also detected. Significant migration was observed from the east headwaters of the Marianne River to the upper Paria River, suggesting that approximately 10% of the guppies

Table 3.1: Estimated recent migration rates between all sites in the Marianne and Paria Rivers, obtained using BAYESASS. Values that differ significantly from zero, based on 95% confidence intervals, are in bold and underlined. Values along the diagonal represent the proportion of non-immigrants at each site.

Migration from → / *Migration to ↓*	Marianne M-A	Marianne M-B	Marianne M-C	Marianne M-D	Marianne M-E	Marianne M-F	Petite Marianne PM-A	Petite Marianne PM-B	Petite Marianne PM-C	Jordan J-A	Jordan J-B	Jordan J-C	Paria P-A	Paria P-B	Paria P-C	Paria P-D	Paria P-E	Paria P-F	Paria P-G	Paria P-H
Marianne M-A	**0.896**	0.003	0.004	0.003	0.003	0.003	0.003	**0.047**	0.003	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Marianne M-B	**0.221**	**0.675**	0.005	0.006	0.005	0.005	0.006	0.006	0.006	0.006	0.010	0.005	0.006	0.005	0.006	0.006	0.005	0.006	0.005	0.006
Marianne M-C	0.006	0.006	**0.885**	0.015	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.005
Marianne M-D	0.002	0.003	0.003	**0.955**	0.002	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.002	0.002
Marianne M-E	0.003	0.003	0.003	0.003	**0.935**	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.003	0.004	0.003	0.003	0.004	0.004
Marianne M-F	0.004	0.004	0.003	0.003	0.004	**0.933**	0.004	0.003	0.003	0.004	0.004	0.003	0.003	0.004	0.003	0.003	0.003	0.004	0.004	0.003
Petite Marianne PM-A	0.011	0.005	0.005	0.006	0.005	0.005	**0.673**	**0.224**	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.005
Petite Marianne PM-B	0.003	0.003	0.004	0.003	0.003	0.003	0.003	**0.937**	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.003	0.003	0.003
Petite Marianne PM-C	0.005	0.005	0.005	0.005	0.005	0.005	0.005	**0.236**	**0.673**	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Jordan J-A	0.005	0.005	0.005	0.006	0.005	0.006	0.005	0.006	0.005	**0.673**	**0.228**	0.006	0.006	0.007	0.006	0.006	0.005	0.005	0.006	0.005
Jordan J-B	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.006	0.006	**0.886**	0.007	0.006	0.009	0.006	0.006	0.006	0.005	0.006	0.006
Jordan J-C	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.009	0.005	0.005	0.055	**0.850**	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.005
Paria P-A	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.008	0.006	0.005	0.034	0.005	**0.674**	**0.199**	0.005	0.005	0.005	0.005	0.005	0.006
Paria P-B	0.006	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.006	0.006	0.009	0.006	0.006	**0.890**	0.006	0.006	0.006	0.006	0.006	0.006
Paria P-C	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	**0.213**	0.005	0.005	0.012	**0.676**	0.005	0.005	0.005	0.016	0.005
Paria P-D	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	**0.675**	0.005	0.005	**0.236**	0.006
Paria P-E	0.006	0.006	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.006	0.006	**0.894**	0.006	0.006	0.006
Paria P-F	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.005	**0.673**	**0.233**	0.005
Paria P-G	0.003	0.003	0.004	0.007	0.007	**0.098**	0.003	0.003	0.003	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.004	0.003	**0.835**	0.003
Paria P-H	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	**0.216**	**0.689**

collected at Paria site P-G may have had recent immigrant ancestry from the nearby Marianne site M-F.

This point of contact between the east headwaters of the Marianne and the upstream area of the Paria was analyzed further for indications of longer-term migration. Results showed an average of 10 individuals per generation moving from the headwaters of the Marianne to the upper reaches of the Paria (90% highest posterior density range, 4-27), and an average of only one individual per generation migrating in the opposite direction (90% HPD range, 0-3). As evidence of migration between the Marianne and Paria watersheds was found in downstream tributaries in a previous study (Willing *et al*., 2010), long-term migration between the Petite Marianne River and Jordan River was also estimated. Results of this analysis indicated a migration rate of one migrant (90% HPD range, 0-2) from the Petite Marianne to the Jordan, and zero migrants (90% HPD range, 0-1) from the Jordan to the Petite Marianne.

3.4 Discussion

3.4.1 Population Structure within Rivers

Within the Marianne River, the average heterozygosity at guppy locations in the mainstem of the river is 1.5 times higher than it is in upstream areas, and 1.2 times higher than in the Petite Marianne tributary, indicating increased genetic diversity in the lower reaches of the river. This is likely due to the generally unidirectional movement of guppies, resulting in downstream populations that receive a large number of migrants and upstream populations that remain isolated and typically have lower population sizes. Low diversity at

upstream sites may also indicate founder effects resulting from the colonization of these areas by only a few individuals (Crispo *et al*., 2006). Indications of the increased diversity caused by downstream migration can be seen in the Bayesian clustering analysis, which shows many individuals with admixture at the downstream Marianne sites M-A and M-B; and in the individual-based PCoA, where guppies at these sites appear more genetically diverse than those found upstream. In contrast to the Marianne River, populations in the Paria do not seem to be substantially more diverse at downstream sites than they are upstream. Instead, heterozygosity levels are high throughout the river and a large amount of admixture can be seen at most sites in both the upper and lower Paria in the Bayesian clustering analysis. The individual-based PCoA also shows a great deal more overlap between Paria populations than between Marianne populations, suggesting that populations in the Paria are not divided by the high levels of genetic differentiation found in the Marianne. This is probably because the lack of physical barriers in the Paria allows a relatively larger number of migrants to move among sites. Bayesian estimates of recent migration rates confirm movement among sites in several areas of the Paria River, including the Jordan and Carl's Tributary.

Increased heterozygosity in downstream guppy populations has been observed in many previous studies, both within the Marianne River (Crispo *et al*., 2006), and in other rivers across Trinidad. Shaw *et al*. (1991, 1994) analyzed allozyme data and found that, in general, lowland habitats contained more genetically diverse guppy populations than those found in upland areas. Other

microsatellite based studies have also found higher heterozygosity in downstream populations when compared to upstream populations within the Caroni drainage (van Oosterhout *et al*., 2006; Barson *et al*., 2009; Suk & Neff, 2009). In addition, introduction experiments have provided strong evidence that guppies frequently migrate downstream and that this influx can affect the populations found in the lower reaches of the river (Haskins *et al*., 1961; Becher & Magurran, 2000). However, prior research has also shown that the distinction between upstream and downstream sites is less pronounced when no barrier waterfalls are present in the river (Carvalho *et al*., 1991), as in the Paria River. Within the Paria, only one population included in this study is separated from the rest of the river by a waterfall – site P-E. The sample collected at this site differs substantially from other Paria River samples in that it has a very low level of heterozygosity, shows almost no evidence of admixture in the Bayesian clustering analysis, and lies far from any other Paria site in both the dendrogram and F_{ST}-based PCoA. The waterfall appears to present a barrier to migration, causing this population to become isolated and highly divergent.

Despite the increase in genetic diversity along the length of the Marianne River, Bayesian estimation of recent migration rates indicates only a low level of movement from the Petite Marianne to the mainstem of the river and no significant migration from the upper to the lower Marianne. The low estimated downstream migration rates in the Marianne River may be attributed to several causes. First, the samples included in this study were collected over the course of eight years, from 2002 to 2010. Because the Bayesian method implemented

in BAYESASS only estimates migration over the past few generations (Wilson & Rannala, 2003), and wild guppies can have 2-3 generations per year (Magurran, 2005), it may be difficult to obtain accurate estimates of migration between sites if the population samples were not collected within the same time frame. Moreover, while many guppies are presumably swept into the mainstem from upstream sites, they may survive only at low rates because they are not adapted to high levels of predation (Weese *et al*., 2011), which could result in lowered migration levels in all analyses. Alternately, the increased diversity of downstream sites could be influenced by factors other than migration from other parts of the river. Guppies in downstream sites are exposed to a wide array of predators, which may result in behavioural adaptations that reduce sexual selection and therefore increase population diversity (Shaw *et al*., 1991). Thus the higher migration rates and reduced differences in genetic diversity in the Paria River as compared to the Marianne may be influenced by both the consistently low predation level found throughout the river and the ease of movement between sites not separated by physical barriers.

Although estimated migration rates showed little downstream movement in the Marianne, a high level of upstream migration was found within the mainstem of the river, where approximately 22% of individuals at site M-B were identified as migrants from site M-A. This upstream migration suggests that in the absence of stream barriers such as waterfalls, neighbouring sites may exchange migrants both with and against the flow of the river. This tendency is also seen in the Petite Marianne River, where almost 24% of guppies at site PM-C were

immigrants from further downstream in the tributary. An earlier study also found both upstream and downstream migration in areas of the Marianne River not separated by waterfalls, including movement from near the mouth of the river into the area referred to here as site M-B, and migration in both directions in the Petite Marianne (Crispo *et al.*, 2006). In the Paria River upstream migration is also seen – about 22% of individuals at site P-H are identified as migrants from site P-G, and 21% of individuals at site P-C are classified as migrants from Jordan site J-B. However, as guppies in various regions of the Paria are generally continuously distributed instead of forming discrete populations at each site, these migration rates likely represent simply the direction of gene flow between different areas of the river, not movement from one specific site to another.

Within both rivers sites in close geographic proximity appear more similar than sites separated by distance. In Bayesian clustering analysis, guppies in the west headwaters of the Marianne River, at sites M-C and M-D, form a single population cluster. All guppies collected in the Petite Marianne River also form a distinct cluster separate from other areas of the river. In the Paria, the entire river is clearly split into an upstream and a downstream group in the K=4 clustering analysis, and nearby sites continue to cluster together in the K=15 analysis. Both the dendrogram and F_{ST} based PCoA also show a distinct grouping of upstream and downstream populations in the Paria River, and furthermore indicate that upstream sites may be more similar to populations in

the nearby upper Marianne River than they are to more distant populations within the Paria itself.

Comparison of population structure in these two rivers shows evidence of higher levels of gene flow within the Paria than within the Marianne, suggesting that the presence of waterfalls and differing predation levels are major elements in impeding gene flow between populations. Because there tends to be covariance between stream barriers and the factors creating selection pressure, it is difficult to separate the possible influence of maladaptation of immigrants due to divergent natural selection from the effect of stream barriers. However, genetic differences between guppy populations in similar environments indicate that selection does not contribute to population structure to the same degree as geographic features that block dispersal. This is particularly evident in the population at Paria site P-E, which lies in a small tributary very near the mainstem site P-D, but separated from it by a waterfall. As the entire Paria River lacks large fish predators and these sites are in close proximity and thus likely physically alike, divergent natural selection would not be expected to create substantial genetic differentiation between these populations. And yet populations at these two sites appear genetically dissimilar in all analyses, presumably due solely to the low potential for gene flow caused by the presence of a barrier to dispersal. This supports the findings of Crispo *et al*. (2006), who concluded that geography played a larger role than selection in limiting gene flow and forming patterns of population structure in the Marianne.

3.4.2 Demographic Changes over Time

Temporal replicates in both the Marianne and Paria Rivers suggest that populations are relatively stable over time at most sites. This is especially evident in upstream regions of the Marianne – both F_{ST} values and Bayesian clustering analysis show very little difference between the replicates taken at Petite Marianne site M-B (F_{ST}, 0.07), or between those taken at Marianne sites M-D (Average F_{ST}, 0.02). Higher F_{ST} values are found between replicates taken at Marianne sites M-E (F_{ST}, 0.16) and M-F (F_{ST}, 0.15), but Bayesian clustering indicates that these replicates are more similar to each other than they are to populations at any other site. The dendrogram shows that, in the upper Marianne, samples collected at the same site in different years are closely related. Replicates at sites M-D and M-F are connected with high bootstrap values; those collected at site M-E in 2002 and 2010 are linked with a bootstrap of 99% and are markedly separated from any other site in the Marianne. The F_{ST}-based PCoA shows very little divergence between replicates, and like the dendrogram it reveals the genetic distance between the population at Marianne site M-E and those in other parts of the upper Marianne. Multiple collections at Petite Marianne site PM-B are not directly connected in the dendrogram, but instead appear more related to populations in other parts of the tributary. Nonetheless, this may not signify a noteworthy difference between these two replicates, as both the Bayesian clustering and F_{ST}-based PCoA analyses indicate that the populations found at all sites in the Petite Marianne share a high degree of genetically similarity.

Populations found downstream in the Marianne appear to be considerably less stable than those in the upstream area. When 2002 and 2008 samples collected at Marianne site M-A are compared, the latter sample contains more individuals with signs of admixture from upstream population groups, suggesting differing levels of gene flow over time. In the dendrogram these replicates are directly connected, but with a low bootstrap value of only 55%. In the PCoA one of the two replicates (the 2002 sample) lies much closer to the population at site M-B than to the replicate collected in a later year, and estimated migration rates imply that guppies could be moving between sites M-A and M-B regularly. High admixture in the 2008 sample may be a result of the extensive flooding that occurred on the north shore of Trinidad in 2005 and 2006, which likely swept many downstream populations out to sea and temporarily increased migration from upstream to downstream (Weese *et al*., 2011). Heterozygosity levels from multiple years suggest that while this flooding may have raised migration rates, fish are regularly flushed into the mainstem from farther upstream.

As with Marianne site M-A, Bayesian clustering analysis also suggests that the population at Paria site P-G experiences fluctuations in the number of migrants it receives from other sites in different years, although the difference is not as pronounced as in the Marianne. In the dendrogram the two replicates at Paria site P-G are linked, but the bootstrap value is low and the population does not appear substantially dissimilar to populations at other sites in the upstream region of the Paria. The F_{ST} value between replicates is low (0.03), but is comparable to the F_{ST} found between many sites in the upper Paria region. This

is supported by the F_{ST}-based PCoA, which shows that while the replicates are not separated by a great deal of genetic distance, neither sample collected at this site is easily distinguished from other populations in the upper reaches of the river.

3.4.3 Gene Flow between the Marianne and Paria Rivers

While migration seems to occur primarily among sites within the same river drainage, there is also evidence of migration from one river to the other. In particular, gene flow between the east headwaters of the Marianne River and the upper region of the Paria River is apparent in the results of most analyses. Bayesian clustering analysis of populations from across the island shows that the east Marianne and west Paria samples clustered together into a single group (Chapter 2, Figure 2.5). While fine-scale analysis of only the Marianne and Paria Rivers provides the resolution to divide these populations into separate clusters in the K=15 plot, the higher level of hierarchical structure still groups them together. The dendrogram links the upper Marianne and upper Paria with a bootstrap value of 62%, and the F_{ST}-based PCoA also suggest a strong connection between the rivers in this area, with the population at Marianne site M-F appearing very closely related to that at Paria sites P-F and P-G. There is also a great deal of overlap between upstream populations in the Marianne and Paria in the individual-based PCoA, with individuals from Marianne sites M-E and M-F showing more similarity to guppies from populations at Paria sites P-F, P-G, and P-H than to those from other areas of the Marianne. Bayesian estimation of recent migration shows that at Paria site P-G about 1 in 10 guppies are recent

migrants (or their descendants) from the upper Marianne River. Historical migration calculated using the coalescent method also indicates long-term movement of guppies from the Marianne to the Paria in this area, at a rate of approximately 10 individuals per generation.

The east headwaters of the Marianne River and the upstream region of the Paria River are located adjacent to each other, near the village of Brasso Seco. Because the upper reaches of both rivers are so close to an inhabited area, guppies may be accidentally transferred between them, although it seems unlikely that this alone would be sufficient to create the consistently high level of migration found in this analysis. Alternately, guppies may be capable of moving from one river to the other through channels of water formed on the forest floor during seasonal floods. There is evidence that individual fish may have migrated in the same way between river drainages in other parts of the island, such as from the Quare to the Aripo (Suk & Neff, 2009). This type of movement appears to be a regular occurrence in the upper Marianne and Paria area, as migration rates and signs of admixture suggest that guppies from the east headwaters of the Marianne are more likely to migrate to the Paria than to move to other parts of the Marianne itself.

Other researchers have proposed that guppies in north shore rivers may be able to move between rivers via the Caribbean Sea when salinity levels are lowered by heavy rainfall (Barson *et al*., 2009). However, that type of movement would relocate fish from the lower reaches of one river to the lower reaches of another, and both the large waterfall at the mouth of the Paria and a smaller

barrier near the mouth of the Marianne would prevent guppies from moving upstream from there. Thus it is unlikely that migration by sea is responsible for the genetic similarities between upstream regions of the rivers as seen in this study. Based on the genetic similarity of east Marianne headwaters populations and upper Paria populations, it is possible that this area of the Marianne was originally colonized by guppies from the Paria watershed. A large waterfall separates the east headwaters of the Marianne River from the mainstem, probably preventing colonization of the headwaters from the mainstem of the Marianne. Recent migration rates suggest that although originally migrants may have moved from the Paria to the Marianne, gene flow now occurs predominantly from Marianne to Paria.

Gene flow was also detected between the Marianne and Paria Rivers at the tributaries Petite Marianne and Jordan, although the connection there was less pronounced than that upstream. Island-wide Bayesian clustering analysis placed the Petite Marianne sample in the same group as individuals caught in the Jordan and the mainstem of the Paria (Chapter 2, Figure 2.5). The dendrogram of populations from rivers across the island also linked the Jordan and Petite Marianne, with a bootstrap value of 50% (Chapter 2, Figure 2.6). In Bayesian clustering of only Marianne and Paria guppies, several individuals in the Jordan River show evidence of admixture from the Petite Marianne population group, although this may not be the result of ongoing migration but may instead simply be evidence of a genetic relationship between Jordan and Petite Marianne guppies. Both of the Marianne/Paria PCoAs suggest that populations from the

Petite Marianne are somewhat more closely related to populations in the Jordan and lower Paria than they are to guppies collected in the mainstem or upstream regions of the Marianne. While recent migration rates do not show significant movement between the Petite Marianne and Jordan in either direction, estimation of long-term migration does suggest a low level of migration between these tributaries. Unlike the east Marianne headwaters and upper Paria, these two tributaries are located deep in the forest, far from any human settlement. Therefore, while guppies may have been moved between the upper reaches of the Marianne and Paria due to anthropogenic activities, such activities are almost certainly not responsible for transferring guppies between the Petite Marianne and the Jordan.

The relationship between Petite Marianne and Jordan River guppies, as well as the genetic divergence between guppy populations in the Petite Marianne River and the mainstem Marianne, has been observed in previous work. SNP-based research found that guppies taken from the Petite Marianne appeared to be related to populations from the Paria rather than populations from elsewhere in the Marianne (Willing *et al*., 2010). Furthermore, an earlier microsatellite study of Marianne populations showed that guppies collected in the Petite Marianne formed a distinct genetic cluster, with little apparent gene flow between the tributary and other parts of the Marianne watershed (Crispo *et al*., 2006). As in the east headwaters, the Petite Marianne River is separated from the lower Marianne by a large waterfall that most likely renders movement from the mainstem of the river into the tributary impossible for guppies. Instead,

populations in the Petite Marianne may have arisen from guppies that dispersed from the nearby Jordan River. As populations in these two tributaries appear well differentiated in most analyses and there is no evidence of recent migration, this colonization probably predates the genetic connection between the upstream Marianne and Paria.

The current analysis shows signs of admixture from the Petite Marianne in the Jordan and a high level of heterozygosity in the Jordan River, implying that guppies may be capable of migrating from the Marianne watershed to the Paria watershed between these two tributaries. However, the headwaters of the Jordan are at a higher elevation than the headwaters of the Petite Marianne and the two tributaries are separated by a large, steep hill (A. Hendry, personal communication). There is little chance that any guppy could successfully move uphill from the Petite Marianne to the Jordan, and thus evidence of migration in this direction is most likely an artifact indicating only that there are genetic similarities between these rivers arising from the occasional movement of guppies from the Jordan to the Petite Marianne.

Chapter 4: Conclusion

This study was undertaken in an effort to investigate the genetic structure among Trinidadian guppy populations from across Trinidad and Tobago, with particular emphasis on two rivers located on the north shore of Trinidad, the Marianne and Paria. Analysis incorporated guppies taken from a large number of locations, and included multiple sites and temporal replicates within some rivers as well as samples collected in regions of Trinidad that have not previously been considered during genetic surveys of the species. My research shows that the relationships among populations are complex, but clear patterns emerge in many areas, reflecting both contemporary gene flow and historical routes of colonization.

Prior studies conducted in Trinidad have consistently demonstrated a deep genetic divide between guppy populations in the west-flowing Caroni drainage and the east-flowing Oropouche drainage, and have attributed this divide to a two-arc model of colonization of the island (Carvalho *et al*., 1991; Shaw *et al*., 1991; Fajen & Breden, 1992; Alexander *et al*., 2006; Willing *et al*., 2010). However, there has been a degree of uncertainty regarding the relationship between the populations in these two drainages and those in rivers across the north coast of the island – some suggest that northern river guppies are directly related to those in the Caroni, while others have proposed that they form a distinct third cluster separate from either major drainage. As in previous work, my results also exhibit the division between Caroni and Oropouche guppies, with the exception of one river with a known history of artificial

introduction. Additionally, I found that while some rivers along the north shore do contain Caroni-type guppies, this only appears to be true of the rivers on the western side of the north coast. Most guppies in the rivers of the northeastern part of the island are instead related predominantly to populations in the Oropouche watershed. Nonetheless, many individuals in northeastern rivers show signs of genetic admixture from the Caroni. This admixture is especially apparent in guppies collected in the Mission River on the north shore, all of which carry Oropouche-like mtDNA haplotypes and yet have Caroni-like microsatellite genotypes.

Despite the extent of genetic differentiation between guppy populations in the Caroni and Oropouche drainages, evidence does not support the designation of Oropouche guppies as the new species *Poecilia obscura* (proposed by Schories *et al.*, 2009). Both microsatellite and mtDNA data indicate substantial gene flow between the Quare River, located in the Oropouche watershed, and the Madamas River, which is situated on the north shore and is known to contain a population of common Trinidadian guppies (*Poecilia reticulata*). Moreover, several north shore populations consist of individuals with both Caroni-like and Oropouche-like genotypes and would prove difficult to classify as either *P. obscura* or *P. reticulata*.

Detailed study of guppy populations in the Marianne and Paria Rivers revealed evidence of downstream-biased gene flow within rivers, as well as increased divergence and decreased migration between populations separated by waterfalls when compared to those with no physical barriers to migration.

Signs of gene flow from one river to the other were also found, indicating movement of guppies between rivers. While evidence of migration between two tributaries of the lower Marianne and Paria has been reported in other work (Willing *et al*., 2010), my results additionally show a previously unsuspected point of migration between sites in the upper reaches of each river. This is presumably the result of either movement over land or relocation of individual fish by humans, predatory animals, or birds.

The Trinidadian guppy has long been considered a model species in the study of evolution and adaptation. This thesis represents a contribution to the current knowledge of guppy population structure, adding to the understanding of both the natural history of the species and the consequences of deliberate or accidental introduction events. Collection sites include rivers on both the far west and far east sides of Trinidad's north shore, areas where little was previously known about the origin of guppy populations or their association with populations in other regions of the island. Inclusion of these rivers allows an extension of the two-arc hypothesis often used to describe the historic colonization of the island by guppies. My results also show some unexpected connections between rivers, such as gene flow from the Quare to the Madamas River, and migration between the upper reaches of the Marianne and Paria Rivers. In addition to enhancing the knowledge of migration patterns of guppies in Trinidad, the results of this research could be useful in predicting the effects of seasonal flooding and anthropogenic interference on other freshwater fish species and in other areas of the world.

References

Alexander, H.J., Taylor, J.S., Sampson, S.W., Breden, F. 2006. Parallel evolution and vicariance in the guppy (*Poecilia reticulata*) over multiple spatial and temporal scales. *Evolution*. 60(11): 2352-2369.

Alkins, M., De Souza, G. 1984. Two new freshwater fish records for Trinidad and some comments on the zoogeography of the southern peninsula. *Living World.* 1983/1984: 8-12.

Antao, T., Lopes, A., Lopes, R.J., Beja-Pereira, A., Luikart, G. 2008. LOSITAN: a workbench to detect molecular adaptation based on a *F*st-outlier method. *BMC Bioinformatics*. 9: 323.

Bandelt, H-J., Forster, P., Röhl, A. 1999. Median-joining networks for inferring interspecific phylogenies. *Mol. Biol. Evol.* 16(1): 37-48.

Barson, N.J., Cable, J., van Oosterhout, C. 2009. Population genetic analysis of microsatellie variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events, and population bottlenecks. *J. Evol. Biol*. 22(3): 485-497.

Beamont, M.A., Nichols, R.A. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proc. R. Soc. Lond. B. Biol. Sci*. 263: 1619-1626.

Becher, S.A., Magurran, A.E., 2000. Gene flow in Trinidadian guppies. *J. Fish Biol*. 56: 241-249.

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F. 1996-2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France).

Boos, H.E.A. 1984. A consideration of the terrestrial reptile fauna on some offshore islands north west of Trinidad. *Living World*. 1983/1984: 19-26.

Carvalho, G.R., Shaw, P.W., Hauser, L., Seghers, B.H., Magurran, A.E. 1996. Artificial introductions, evolutionary change and population differentiation in Trinidadian guppies (*Poecilia reticulata*: Poeciliidae). *Biol. J. Linn. Soc*. 57: 219-234.

Carvalho, G.R., Shaw, P.W., Magurran, A.E., Seghers, B.H. 1991. Marked genetic divergence revealed among populations of the guppy *Poecilia reticulata* (Poeciliidae), in Trinidad. *Biol. J. Linn. Soc.* 42: 389-405.

Cavalli-Sforza, L.L., Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution*. 3: 550-570.

Crispo, E., Bentzen, P., Reznick, D.N., Kinnison, M.T., Hendry, A.P. 2006. The relative influence of natural selection and geography on gene flow in guppies. *Mol. Ecol*. 15: 49-62.

Dieringer, D., Schlötterer, C. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes*. 3(1): 167-169.

Earl, D., vonHoldt, B.M. 2011. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* DOI: 10.1007/s12686-011-9548-7

Elphinstone M.S., Hinten G.N., Anderson M.J., Nock C.J. 2003. An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. *Mol. Ecol. Notes.* 3: 317-320.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 34(1): 76-91.

Evanno, G., Regnaut, S., Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol*. 14: 2611-2620.

Excoffier, L., Hofer, T., Foll, M. 2009. Detecting loci under selection in a hierarchically structured population. *Heredity*. 103: 285-298.

Excoffier, L., Lischer, H.E.L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*. 10: 564-567.

Fajen, A., Breden, F. 1992. Mitochondrial DNA sequence variation among natural populations of the Trinidadian guppy, *Poecilia reticulata*. *Evolution*. 46(5): 1457-1465.

Falush, D., Stephens, M., Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*. 164: 1567-1587.

Ghosh, S.K., Dash, A.P. 2007. Larvivorous fish against malaria vectors: a new outlook. *Trans. R. Soc. Trop. Med. Hyg*. 101: 1063-1064.

Goldstein, D.B., Linares, A.R., Cavelli-Sforza, L.L., Feldman, M.W. 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. *Proc. Natl. Acad. Sci. U.S.A.* 92: 6723-6727.

Gordon, S.P., Reznick, D.N., Kinnison, M.T., Bryant, M.J., Weese, D.J., Räsänen, K., Millar, N.P., Hendry, A.P. 2009. Adaptive changes in life history and survival following a new guppy introduction. *Am. Nat*. 174(1): 34-45.

Goudet, J. 2001. FSTAT: a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available online from: http://www2.unil.ch/popgen/softwares/fstat.htm.

Haskins, C.P., Haskins, E.F., McLaughlin, J.J.A., Hewitt, R.E. 1961. Polymorphism and population structure in *Lebistes reticulates*, an ecological study. In: *Vertebrate Speciation* (ed. W.F. Blair). University of Texas Press. Austin, Texas.

Hendry, A.P., Kelly, M.L., Kinnison, M.T., Reznick, D.N. 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *J. Evol. Biol*. 19(3): 741-754.

Hey, J., Nielsen, R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*. 167: 747-760.

Karim, N., Gordon, S.P., Schwartz, A.K., Hendry, A.P. 2007. This is not déjá vu all over again: male guppy colour in a new experimental introduction. *J. Evol. Biol*. 20(4): 1339-1350.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6196-6200.

Langella, O. 1999-2010. Populations 1.2.32. Available online from: http://www.bioinformatics.org/groups/?group_id=84.

Magurran, A.E. 2005. *Evolutionary Ecology: The Trinidadian Guppy*. Oxford University Press, Oxford, UK.

Magurran, A.E., Phillip, D.A.T. 2001. Evolutionary implications of large-scale patterns in the ecology of Trinidadian guppies, *Poecilia reticulata*. *Biol. J. Linn. Soc.* 73: 1-9.

Magurran, A.E., Seghers, B.H. 1990. Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour*. 118(3/4): 214-234.

Meirmans, P.G., Hedrick, P.W. 2011. Assessing population structure: F_{ST} and related measures. *Mol. Ecol. Resour.* 11: 5-18.

Meyer, A., Kocher, T.D., Basasibwaki, P., Wilson, A.C. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*. 347: 550-553.

Millar, N.P., Reznick, D.N., Kinnison, M.T., Hendry, A.P. 2006. Disentangling the selective factors that act on male colour in wild guppies. *Oikos*. 113: 1-12.

Narum, S.R., Hess, J.E. 2011. Comparison of F_{ST} outlier tests for SNP loci under selection. *Mol. Ecol. Resour*. 11(Suppl.1): 184-194.

Nielsen, R., Wakeley, J. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*. 158(2): 885-896.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* 12: 357-358.

Paterson, I.G., Crispo, E., Kinnison, M.T., Hendry, A.P., Bentzen, P. 2005. Characterization of tetranucleotide microsatellite markers in guppy *(Poecilia reticulata*). *Mol. Ecol. Notes*. 5: 269-271.

Peakall, R., Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*. 6: 288-295.

Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155: 945-959.

Rambout, A. 2010. FIGTREE v.1.3.1. Institute of Evolutionary Biology, University of Edinburgh.

Raymond M., Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity*. 86: 248-249.

Reznick, D. 1996. Life history evolution in guppies: a model system for the empirical study of adaptation. *Neth. J. Zool.* 46(3-4): 172-190.

Reznick, D., Butler, M.J., Rodd, H. 2001. Life history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am. Nat*. 157(2): 126-140.

Reznick, D., Endler, J.A. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution*. 36(1): 160-177.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution.* 43(1): 223-225.

Rodd, F.H., Reznick, D.N. 1997. Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology*. 78(2): 405-418.

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Mol. Ecol. Resour. 8*: 103-106.

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

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

Shaw, P.W, Carvalho, G.R., Magurran, A.E., Seghers, B.H. 1994. Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. *J. Fish Biol*. 45: 875-888.

Shaw, P.W., Carvalho, G.R., Seghers, B.H., Magurran, A.E. 1992. Genetic consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad. *Proc. R. Soc. Lond. B. Biol. Sci*. 248: 111-116.

Shen, X., Yang, G., Liao, M. 2007. Development of 51 genomic microsatellite DNA markers of guppy (*Poecilia reticulata*) and their application in closely related species. *Mol. Ecol. Notes*. 7: 302-306.

Smouse, P.E., Peakall, R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*. 82: 561-573.

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

Takezaki, N., Nei, M., Tamura, K. 2010. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Mol. Biol. Evol*. 27(4): 747-752.

Taylor, J.S., Breden, F. 2000. Slipped-strand mispairing at non-contiguous repeats in Poecilia reticulata: a model for minisatellite birth. *Genetics*. 155: 1313-1320.

Thompson, J.D., Higgins, D.G., Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22(22): 4673-4680.

van Oosterhout, C., Hutchinson W.F., Wills D.P.M., Shipley P. 2004. Program note: MICRO-CHECKER software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*. 4: 535–538.

van Oosterhout, C., Joyce, D.A., Cummings, S.M., Blais, J., Barson, N.J., Ramnarine, I.W., Mohammed, R.S., Persad, N., Cable, J. 2006. Balancing selection, random genetic drift, and genetic variatrion at the major histocompatibility complex in two wild populations of guppies (*Poecilia reticulata*). *Evolution*. 60(12): 2562-2574.

Watanabe, T., Yoshida, M., Nakajima, M., Taniguchi, N. 2003. Isolation and characterization of 43 microsatellite DNA markers for guppy (*Poecilia reticulata*). *Mol. Ecol. Notes*. 3: 487-490.

Weese, D.J., Schwartz, A.K., Bentzen, P., Hendry, A.P., Kinnison, M.T. 2011. Eco-evolutionary effects on population recovery following catastrophic disturbance. *Evol. Appl*. 4(2): 354-366.

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

Wilson, G.A., Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*. 163: 1177-1191.

Appendix 1: Genbank accession numbers for mitochondrial control region sequences from 57 samples included in mtDNA analysis. All accession numbers were taken from Alexander *et al*., 2006. ***** Indicates a sample originally analyzed in Taylor & Breden, 2000.

Location		Accession Numbers
Arima River	*Lower*	AF170265*, AY135450, AY135452
	Mid	AF170266*, AY135475, AY135477
	Upper	AF228623*, AY135460, AY135476
Aripo River	*Lower*	AY135470
	Upper	AF170268*, DQ102585, DQ102586
Guanapo River		AF170267*, AY135449, AY135472
Madamas River		AF170262*, AF529248, AF529254
Marianne River		AF193901, AY135456, AY135462, AY135463 AY135467
Oropuche River	*Lower*	AF529245, AF529249, AF538279, DQ102558
	Mid	AF170259*, AF529244, AF529247, AF529250, AF529256
	Upper	AF170260*, AF529255, AF529257
Paria River		AF193902, AY135448, AY135453
Jordan River		AF228624*, AY135459, AY135474
Quare River	*Lower*	AF193897, AF529251, AF529253
	Upper	AF529246, AF529252, DQ102584
Rio Grande		AF170258*, AF170269*, AF170270*
Yarra River	*Lower*	AF170264*, AY135461, AY135471
	Upper	AF170263*, AY135455, AY135464

Appendix 2: List of site names used throughout this thesis in the Marianne and Paria Rivers, showing the corresponding names frequently used for these sites in previous studies. Numerical site names were used in Crispo *et al.* (2006), Hendry *et al.* (2006), and Millar *et al.* (2006).

River	Site Name in Current Study	Site Name in Previous Work
Marianne River	M-A	Marianne site 7
	M-B	Marianne site 15
	M-C	Marianne site 1
	M-D	Marianne site 16
	M-E	Marianne site 4
	M-F	Marianne site 3
Petite Marianne River	PM-A	Marianne site 11
	PM-B	Marianne site 10
	PM-C	Marianne site 9
Jordan River	J-A	Paria site 12
	J-B	Paria site 14
	J-C	Paria site 18
Paria River	P-A	Paria site 3
	P-B	Paria site 1
	P-C	Paria site 13
	P-D	Paria site 15
	P-E	Paria site 16
	P-F	Paria site 17
	P-G	Paria site 7
	P-H	Paria site 8

Appendix 3: Microsatellite primers used in this study. Listed are the forward and reverse sequences, repeat motif, optimal PCR annealing temerature, GenBank accession number, and source publication for each of the ten primers used.

Locus	*Primer Sequence (5'-3')* ***F:** forward, **R:** reverse*	*Repeat Sequence*	*Optimal T_A*	*GenBank #*	*Source Publication*
Pre9	**F:** TTGCAAGTCAGTTGATGGTTG **R:** TGCCCTAGGGATGAGAAAAG	$(CAGA)_{13}$	60°C	AY830941	Paterson *et al*. (2005)
Pre13	**F:** ACAGTACTGTCTGTCTGTCT **R:** TGTTTGAGACACTCATGGTGAAG	$(CTGT)_{18}$	65°C	AY830942	Paterson *et al*. (2005)
Pre15	**F:** CTGAGGGACCAGGATGTTAAG **R:** CCATAAACACGCAAACCAAC	$(GATG)_{16}$	65°C	AY830943	Paterson *et al.* (2005)
Pre26	**F:** GCTGACCCCAGAAAAGTGG **R:** TGGGACTTTCATGAGACTTGG	$(GATG)_{19}$	60°C	AY830946	Paterson *et al.* (2005)
Pret-27	**F:** CACACGGGCTCTCATTTTT **R:** CTGTGTTTGTGTTCGGTCGTA	$(GT)_{53}$	60°C	AB100321	Watanabe *et al.* (2003)
Pret-28	**F:** ACATCGGCGTCCTCACCT **R:** GGGGGTTGAAACACATCCA	$(GT)_{32}$	60°C	AB100322	Watanabe *et al.* (2003)
Pret-38	**F:** AGGGAAAAGGAAAGAAAGAA **R:** CGAACAAGCCCAAATCTA	$(GT)_{19}$	50°C	AB100328	Watanabe *et al.* (2003)
Pret-46	**F:** AACCCTAATGACTCCCAACA **R:** CGACCCACCAGTAATCCAA	$(CA)_{27}$	60°C	AB100334	Watanabe *et al.* (2003)
Pret-80	**F:** GGAAGGGAGGGGAGGAT **R:** CACTTCAGCAGGGCAGACTA	$(GT)_{14}(GA)_{11}$	60°C	AB100354	Watanabe *et al.* (2003)
G145	**F:** TCTCCAAACCTCCCCTGTA **R:** GACGAGCCTCTGCTTCTTC	$(GT)_{11}$	60°C	DQ855588	Shen *et al.* (2007)

Appendix 4: Population statistics, including predation level, total number of fish sampled within each river (N), number of alleles (A), allelic richness (A_E), size range of alleles (R), size (S) and frequency (F) of the most common allele, expected (H_E) and observed (H_O) heterozygosity, and estimated frequency of the null allele (Null). At sites where sampling occurred in multiple years, data from all replicates is pooled.

Population		Locus									
		Pre9	**Pre13**	**Pre15**	**Pre26**	**Pret-27**	**Pret-28**	**Pret-38**	**Pret-46**	**Pret-80**	**G145**
Arima River Low Predation N=45	A	13	6	16	16	13	8	7	8	8	6
	A_E	9.6	5.8	12.1	10.7	8.7	6.7	5.0	5.5	6.3	4.8
	R	178-186	90-114	172-292	98-170	180-192	146-178	120-148	144-236	194-220	338-352
	S	182	114	200	114	190	146	148	234,236	200	352
	F	0.79	0.91	0.34	0.53	0.56	0.90	0.67	0.47	0.73	0.52
	H_E	0.35	0.18	0.81	0.64	0.60	0.19	0.51	0.56	0.43	0.55
	H_O	0.36	0.13	0.88	0.71	0.57	0.18	0.62	0.57	0.42	0.51
	PHWE	0.562	0.007	0.569	0.154	0.911	0.323	0.041	0.300	0.543	0.708
	Null	0.000	0.000	0.000	0.003	0.009	0.000	0.000	0.027	0.000	0.012
Arima River High Predation N=40	A	3	5	10	6	5	5	4	3	5	3
	A_E	2.5	2.9	7.7	4.9	4.0	2.9	3.7	2.9	3.7	2.9
	R	158-234	98-194	164-288	106-222	172-232	148-176	132-168	218-244	200-222	338-348
	S	178	110	164	114	192	148	148	234	214	346
	F	0.33	0.43	0.30	0.18	0.29	0.37	0.61	0.70	0.42	0.36
	H_E	0.83	0.73	0.88	0.89	0.83	0.77	0.57	0.47	0.75	0.72
	H_O	0.74	0.13	0.74	0.91	0.77	0.78	0.43	0.39	0.62	0.62
	PHWE	0.285	0.000	0.203	0.782	0.243	0.724	0.001	0.153	0.133	0.151
	Null	0.039	0.348	0.086	0.000	0.034	0.000	0.075	0.079	0.069	0.040

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Aripo River Low Predation N=45	A	11	12	14	12	8	9	14	6	7	4
	A_E	6.3	7.8	8.7	8.3	5.1	6.3	8.0	3.2	5.2	2.6
	R	162-226	90-138	196-284	106-218	180-204	150-182	128-182	156-240	200-214	338-350
	S	186	110	212	190	180	176	174	234	212	348
	F	0.55	0.36	0.41	0.38	0.74	0.57	0.51	0.88	0.64	0.91
	H_E	0.65	0.79	0.78	0.79	0.44	0.64	0.70	0.23	0.55	0.17
	H_O	0.70	0.69	0.82	0.91	0.40	0.75	0.71	0.20	0.51	0.13
	PHWE	0.175	0.012	0.724	0.952	0.290	0.954	0.864	0.497	0.370	0.083
	Null	0.000	0.037	0.000	0.000	0.049	0.000	0.000	0.040	0.026	0.065
Aripo River High Predation N=84	A	18	20	28	20	21	24	24	11	14	9
	A_E	9.5	11.2	15.1	11.1	12.5	11.5	11.8	6.6	7.9	5.2
	R	158-242	86-206	184-308	114-234	148-206	140-216	120-184	166-240	194-220	334-354
	S	186	106	200,204	194	180	156	142	234	204,212	348
	F	0.26	0.22	0.10	0.29	0.14	0.23	0.22	0.55	0.20	0.66
	H_E	0.86	0.90	0.95	0.87	0.92	0.88	0.90	0.66	0.85	0.54
	H_O	0.76	0.71	0.95	0.84	0.84	0.75	0.76	0.63	0.77	0.45
	PHWE	0.302	0.001	0.062	0.377	0.000	0.000	0.000	0.317	0.013	0.007
	Null	0.062	0.091	0.000	0.023	0.050	0.061	0.072	0.044	0.050	0.046
Curaguate River N=26	A	14	9	16	9	10	6	7	12	7	3
	A_E	11.5	8.3	13.9	8.2	8.7	5.8	5.3	10.2	6.1	2.3
	R	154-254	94-126	164-312	142-182	200-220	156-172	120-180	188-220	186-216	336-244
	S	190	98	276	154	212	158	122	204	212	342
	F	0.21	0.33	0.16	0.25	0.33	0.42	0.65	0.19	0.30	0.94
	H_E	0.89	0.80	0.91	0.84	0.83	0.66	0.54	0.88	0.78	0.11
	H_O	0.95	0.80	1.00	0.44	0.75	0.54	0.54	0.78	0.68	0.11
	PHWE	0.967	0.690	0.855	0.000	0.384	0.096	0.509	0.674	0.238	1.000
	Null	0.000	0.000	0.000	0.215	0.067	0.039	0.000	0.065	0.053	0.000

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Damier River Low Predation N=169	A	11	20	32	20	19	25	12	26	11	9
	A_E	8.5	10.2	13.3	9.9	10.6	14.2	7.7	13.2	6.2	5.8
	R	162-214	94-206	128-344	106-230	176-220	164-238	122-178	140-230	194-216	332-356
	S	174	122	192	118	186	210	138	156	204	244
	F	0.18	0.25	0.24	0.39	0.21	0.08	0.31	0.16	0.27	0.33
	H_E	0.87	0.88	0.91	0.81	0.89	0.94	0.81	0.93	0.79	0.78
	H_O	0.83	0.69	0.81	0.81	0.84	0.95	0.75	0.71	0.76	0.77
	PHWE	0.353	0.000	0.018	0.001	0.807	0.033	0.004	0.000	0.706	0.151
	Null	0.023	0.099	0.050	0.000	0.028	0.000	0.038	0.107	0.010	0.023
Damier River High Predation N=127	A	13	25	35	17	17	23	11	22	8	11
	A_E	8.8	10.7	12.7	9.8	9.9	13.0	6.7	10.4	5.8	5.3
	R	162-222	102-230	128-340	106-222	176-218	170-214	122-178	142-236	196-216	332-356
	S	198	122	192	118	214	210	138	192	204	344
	F	0.17	0.29	0.22	0.38	0.24	0.12	0.29	0.21	0.41	0.28
	H_E	0.87	0.87	0.91	0.82	0.87	0.93	0.80	0.88	0.74	0.75
	H_O	0.87	0.68	0.87	0.72	0.83	0.88	0.75	0.76	0.71	0.77
	PHWE	0.617	0.000	0.025	0.865	0.361	0.349	0.019	0.000	0.053	0.001
	Null	0.006	0.109	0.022	0.040	0.018	0.033	0.046	0.055	0.013	0.010
Diego Martin River N=25	A	5	10	6	8	7	11	3	6	5	6
	A_E	4.2	8.7	4.9	6.8	5.7	7.5	3.0	5.9	4.2	4.9
	R	190-238	94-186	184-256	170-214	162-218	150-222	122-150	168-218	188-212	336-350
	S	190,214	114	184	170	162	182	122	170	198	338
	F	0.42	0.25	0.54	0.34	0.56	0.44	0.64	0.34	0.48	0.52
	H_E	0.64	0.85	0.64	0.80	0.63	0.73	0.53	0.79	0.62	0.64
	H_O	0.56	0.63	0.56	0.80	0.56	0.75	0.44	0.86	0.52	0.67
	PHWE	0.372	0.007	0.335	0.400	0.226	0.962	0.367	0.969	0.028	0.182
	Null	0.013	0.130	0.024	0.000	0.018	0.000	0.067	0.000	0.068	0.021

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
El Cedro River Low Predation N=45	A	4	3	4	5	2	2	3	2	2	3
	A_E	2.4	2.3	3.3	3.5	2.0	2.0	2.3	1.8	2.0	2.9
	R	186-214	102-130	248-260	106-202	176-190	166-172	140-150	234-238	214-220	340-344
	S	214	110	252	106	176	172	146	238	214	344
	F	0.93	0.88	0.67	0.63	0.78	0.87	0.52	0.94	0.78	0.62
	H_E	0.14	0.22	0.48	0.54	0.34	0.23	0.51	0.10	0.35	0.51
	H_O	0.14	0.20	0.47	0.51	0.34	0.27	0.58	0.11	0.31	0.49
	PHWE	1.000	0.472	0.068	0.069	1.000	1.000	0.451	1.000	0.663	0.254
	Null	0.000	0.030	0.040	0.052	0.001	0.000	0.000	0.000	0.032	0.010
El Cedro River High Predation N=40	A	10	6	12	7	10	10	8	3	6	5
	A_E	7.7	4.9	9.1	4.6	6.7	6.5	5.9	3.0	4.5	4.9
	R	158-230	86-150	216-300	106-202	176-206	150-194	140-180	234-238	206-220	338-346
	S	158	110	252	106	176	174	146	234	220	342
	F	0.35	0.53	0.30	0.71	0.53	0.58	0.36	0.54	0.45	0.39
	H_E	0.76	0.64	0.83	0.47	0.68	0.63	0.75	0.55	0.61	0.75
	H_O	0.80	0.65	0.77	0.41	0.59	0.59	0.70	0.50	0.52	0.62
	PHWE	0.613	0.022	0.402	0.292	0.373	0.416	0.061	0.070	0.748	0.001
	Null	0.000	0.021	0.000	0.045	0.051	0.000	0.000	0.091	0.024	0.096
Guanapo River Low Predation N=45	A	12	9	17	14	13	15	8	2	7	4
	A_E	7.4	6.3	11.9	8.9	8.6	9.7	4.4	2.0	4.4	3.7
	R	158-230	86-122	220-296	102-214	158-200	142-184	120-162	234-238	200-224	340-348
	S	158	86	236	182	188	166	148	234	220	342
	F	0.54	0.36	0.19	0.36	0.33	0.28	0.80	0.88	0.44	0.70
	H_E	0.68	0.77	0.91	0.81	0.83	0.86	0.35	0.21	0.62	0.47
	H_O	0.68	0.69	0.97	0.77	0.82	0.80	0.31	0.24	0.51	0.52
	PHWE	0.498	0.053	0.959	0.407	0.110	0.400	0.088	1.000	0.266	0.833
	Null	0.012	0.039	0.000	0.000	0.000	0.000	0.021	0.000	0.066	0.000

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Guanapo River **High Predation** **N=40**	A	8	7	12	5	11	6	1	2	4	3
	A_E	6.2	6.5	8.6	4.5	8.4	4.7	1.0	2.0	2.8	2.9
	R	158-230	86-114	228-280	106-182	176-200	146-184	148	234-242	200-220	340-344
	S	158	110	244	178	190	170	148	234	220	342
	F	0.35	0.27	0.28	0.59	0.24	0.51	1.00	0.74	0.71	0.46
	H_E	0.76	0.82	0.84	0.59	0.85	0.65	0.00	0.38	0.43	0.58
	H_O	0.75	0.76	0.87	0.49	0.71	0.59	0.00	0.29	0.52	0.36
	PHWE	0.805	0.747	0.898	0.042	0.055	0.303	~	0.177	0.678	0.000
	Null	0.000	0.031	0.000	0.062	0.069	0.010	~	0.080	0.000	0.159
La Seiva River **N=27**	A	14	13	17	15	19	14	8	6	8	5
	A_E	10.7	10.0	12.6	9.2	13.2	10.4	5.7	4.5	5.4	5.0
	R	158-238	86-286	172-288	106-222	150-228	136-180	122-148	220-240	194-222	340-348
	S	158	110	244	106	190	166	148	234	214	342
	F	0.33	0.26	0.22	0.56	0.17	0.40	0.57	0.76	0.63	0.33
	H_E	0.85	0.85	0.90	0.67	0.91	0.81	0.62	0.41	0.57	0.78
	H_O	0.81	0.67	0.96	0.70	0.96	0.67	0.44	0.40	0.58	0.65
	PHWE	0.234	0.008	0.494	0.391	0.668	0.032	0.124	0.473	0.132	0.067
	Null	0.000	0.096	0.000	0.000	0.000	0.063	0.099	0.041	0.000	0.081
Las Cuevas Bay **N=30**	A	17	12	16	7	11	15	8	21	6	5
	A_E	12.5	9.9	13.5	6.7	9.1	8.2	6.0	13.5	5.1	4.1
	R	178-258	94-166	248-320	158-182	200-220	152-228	120-204	170-228	188-214	336-344
	S	190	98	296	158	206,212	158	120	206,212	212	342
	F	0.17	0.22	0.14	0.30	0.18	0.40	0.40	0.13	0.52	0.77
	H_E	0.91	0.87	0.92	0.80	0.87	0.77	0.71	0.92	0.65	0.39
	H_O	0.93	0.96	0.94	0.20	0.83	0.69	0.48	0.86	0.66	0.29
	PHWE	0.228	0.489	0.275	0.000	0.236	0.115	0.001	0.673	0.131	0.004
	Null	0.000	0.000	0.000	0.335	0.007	0.024	0.129	0.028	0.007	0.111

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Madamas River site 1 N=33	A	9	21	15	15	20	17	6	3	5	6
	A_E	7.4	14.6	11.2	10.1	13.2	12.6	5.7	2.1	3.3	4.9
	R	166-210	110-262	204-284	122-226	154-226	142-204	148-166	142-154	206-216	336-346
	S	166	178,214	256,260	154	188	152,166	148	142	210	342
	F	0.45	0.11	0.16	0.28	0.19	0.16	0.29	0.94	0.76	0.60
	H_E	0.74	0.93	0.90	0.83	0.90	0.91	0.80	0.11	0.39	0.57
	H_O	0.68	0.93	0.76	0.72	0.83	0.72	0.54	0.04	0.44	0.42
	PHWE	0.156	0.118	0.006	0.000	0.000	0.001	0.000	0.019	0.057	0.004
	Null	0.032	0.016	0.078	0.004	0.037	0.097	0.135	0.119	0.000	0.102
Madamas River site 2 N=26	A	9	12	13	12	10	14	4	5	6	5
	A_E	5.9	10.4	10.9	9.8	8.0	11.5	3.9	3.4	3.9	4.4
	R	158-198	166-246	216-280	106-226	160-204	150-218	158-166	144-212	196-220	336-344
	S	166	222	240	170	188,190	152	164	144	210	338
	F	0.75	0.32	0.16	0.34	0.23	0.32	0.47	0.88	0.70	0.60
	H_E	0.43	0.84	0.90	0.83	0.83	0.86	0.63	0.23	0.46	0.58
	H_O	0.42	0.76	0.91	0.68	0.73	0.79	0.78	0.13	0.44	0.62
	PHWE	0.244	0.172	0.072	0.000	0.080	0.416	0.163	0.013	0.072	0.078
	Null	0.000	0.000	0.004	0.029	0.064	0.043	0.000	0.105	0.000	0.034
Marianne River site M-A N=90	A	13	22	22	13	24	14	9	25	7	6
	A_E	9.6	13.2	11.7	9.0	14.1	9.2	4.9	11.3	5.2	3.4
	R	154-218	102-190	156-284	130-186	178-238	150-182	148-172	148-226	202-216	334-346
	S	186	142	244,252	174	194	162	148	160	210	342
	F	0.19	0.13	0.14	0.24	0.16	0.18	0.39	0.28	0.32	0.75
	H_E	0.89	0.93	0.91	0.86	0.93	0.88	0.70	0.87	0.75	0.41
	H_O	0.84	0.93	0.82	0.42	0.88	0.86	0.67	0.78	0.68	0.40
	PHWE	0.644	0.062	0.026	0.000	0.033	0.644	0.080	0.029	0.525	0.039
	Null	0.019	0.003	0.044	0.241	0.032	0.000	0.000	0.045	0.036	0.038

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Marianne River site M-B N=39	A	13	15	16	12	22	12	6	16	6	3
	A_E	9.6	11.3	11.9	9.4	13.7	9.6	5.0	11.3	5.8	3.0
	R	154-202	102-178	188-304	126-186	188-242	146-182	140-164	150-228	204-220	336-342
	S	186	142	260,268	174	210	166	148	158	204	342
	F	0.17	0.16	0.18	0.29	0.17	0.21	0.56	0.26	0.37	0.74
	H_E	0.88	0.91	0.89	0.85	0.92	0.88	0.62	0.88	0.75	0.42
	H_O	0.95	0.74	0.36	0.65	0.87	0.92	0.61	0.70	0.74	0.43
	PHWE	0.166	0.005	0.000	0.020	0.060	0.052	0.235	0.002	0.309	0.486
	Null	0.000	0.080	0.281	0.122	0.036	0.000	0.028	0.081	0.000	0.000
Marianne River site M-C N=40	A	9	16	11	9	12	9	5	6	3	4
	A_E	5.1	10.8	8.0	6.7	7.4	5.9	3.3	4.3	1.6	3.0
	R	162-206	98-190	192-248	146-198	210-248	132-184	148-160	152-188	204-212	334-342
	S	162	166	228	174	222	184	148	152	204	342
	F	0.46	0.16	0.26	0.51	0.27	0.29	0.60	0.47	0.97	0.49
	H_E	0.64	0.90	0.84	0.69	0.81	0.79	0.52	0.65	0.05	0.55
	H_O	0.69	0.85	0.94	0.70	0.77	0.70	0.44	0.76	0.05	0.53
	PHWE	0.668	0.181	0.397	0.707	0.000	0.142	0.012	0.866	1.000	0.079
	Null	0.000	0.030	0.000	0.010	0.103	0.057	0.035	0.000	0.000	0.051
Marianne River site M-D N=120	A	8	14	7	11	13	14	4	4	5	4
	A_E	6.2	7.9	5.3	7.9	8.3	7.8	2.2	2.2	2.9	2.0
	R	162-206	98-158	212-236	142-182	200-232	132-176	148-156	152-232	190-214	338-344
	S	190	142	216	154	214	150	148	152	204	338
	F	0.21	0.31	0.36	0.21	0.23	0.43	0.92	0.89	0.87	0.93
	H_E	0.82	0.81	0.76	0.86	0.85	0.77	0.14	0.20	0.24	0.13
	H_O	0.77	0.83	0.75	0.85	0.75	0.61	0.12	0.18	0.17	0.13
	PHWE	0.062	0.157	0.691	0.740	0.000	0.000	0.000	0.420	0.000	0.012
	Null	0.028	0.006	0.000	0.016	0.061	0.099	0.040	0.025	0.067	0.000

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Marianne River site M-E N=80	A	3	5	8	4	8	8	3	7	1	4
	A_E	1.7	4.2	3.9	3.1	4.4	5.8	1.3	5.1	1.0	1.8
	R	170-186	98-134	140-304	138-182	176-216	168-182	154-160	172-230	212	340-348
	S	178	102	228	146	216	172	154	226	212	342
	F	0.96	0.53	0.78	0.78	0.56	0.36	0.99	0.45	1.00	0.96
	H_E	0.07	0.64	0.37	0.36	0.59	0.75	0.03	0.69	0.00	0.08
	H_O	0.07	0.66	0.40	0.38	0.55	0.48	0.03	0.64	0.00	0.05
	PHWE	1.000	0.255	0.521	0.951	0.000	0.000	1.000	0.068	~	0.056
	Null	0.000	0.000	0.000	0.000	0.056	0.139	0.000	0.000	~	0.063
Marianne River site M-F N=78	A	6	8	12	6	12	5	4	11	3	1
	A_E	4.3	6.7	7.8	5.1	7.7	4.5	2.6	6.3	1.4	1.0
	R	178-206	94-134	188-304	134-186	174-216	168-176	154-162	210-250	212-230	342
	S	182	106	212	138	212	172	154	218	212	342
	F	0.32	0.36	0.32	0.37	0.37	0.44	0.66	0.53	0.99	1.00
	H_E	0.73	0.80	0.81	0.72	0.80	0.70	0.47	0.68	0.03	0.00
	H_O	0.69	0.72	0.76	0.68	0.73	0.44	0.42	0.59	0.01	0.00
	PHWE	0.435	0.017	0.009	0.187	0.000	0.000	0.000	0.023	0.008	~
	Null	0.020	0.019	0.005	0.027	0.037	0.136	0.075	0.063	0.000	~
Petite Marianne River (Marianne tributary) site PM-A N=39	A	7	16	15	7	9	9	7	11	5	2
	A_E	5.2	11.5	10.7	5.8	7.9	5.4	4.1	8.3	3.5	2.0
	R	174-198	102-182	224-284	146-174	174-238	146-170	138-168	194-216	204-218	336-342
	S	182	142,146	256	174	232	160	164	208	212	336
	F	0.33	0.15	0.16	0.60	0.29	0.61	0.85	0.28	0.58	0.64
	H_E	0.74	0.90	0.89	0.60	0.83	0.58	0.27	0.83	0.53	0.46
	H_O	0.73	0.88	0.91	0.50	0.71	0.54	0.22	0.88	0.58	0.56
	PHWE	0.908	0.137	0.777	0.179	0.044	0.319	0.007	0.164	1.000	0.410
	Null	0.000	0.000	0.005	0.104	0.061	0.015	0.000	0.003	0.000	0.000

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Petite Marianne River (Marianne tributary) site PM-B N=80	A	7	21	18	6	13	11	6	14	8	2
	A_E	5.5	11.0	11.4	4.9	9.3	6.4	3.0	9.3	3.8	2.0
	R	174-198	110-222	224-292	142-174	174-238	148-172	138-168	194-226	198-218	336-342
	S	182	138,150	248	150	232	162	164	210	212,216	336
	F	0.30	0.14	0.15	0.53	0.22	0.37	0.88	0.19	0.46	0.86
	H_E	0.76	0.91	0.91	0.65	0.88	0.75	0.22	0.88	0.58	0.23
	H_O	0.79	0.85	0.92	0.28	0.84	0.58	0.24	0.81	0.52	0.19
	PHWE	0.588	0.092	0.824	0.000	0.402	0.000	1.000	0.000	0.172	0.115
	Null	0.000	0.031	0.000	0.226	0.018	0.078	0.000	0.037	0.017	0.055
Petite Marianne River (Marianne tributary) site PM-C N=40	A	7	17	14	13	10	7	3	12	4	2
	A_E	5.4	11.0	10.9	8.2	8.6	4.8	1.7	8.7	3.1	2.0
	R	170-198	110-234	228-280	142-206	174-242	154-168	164-168	194-224	208-218	336-342
	S	182	150	244	150	232	160	164	208	216	336
	F	0.37	0.17	0.18	0.55	0.26	0.64	0.97	0.23	0.50	0.88
	H_E	0.74	0.90	0.90	0.68	0.85	0.55	0.05	0.85	0.56	0.21
	H_O	0.77	0.74	0.84	0.34	0.76	0.50	0.06	0.88	0.51	0.24
	PHWE	0.919	0.024	0.013	0.000	0.066	0.100	1.000	0.536	0.583	1.000
	Null	0.001	0.068	0.034	0.193	0.058	0.058	0.000	0.011	0.013	0.000
Mission River N=35	A	5	5	6	6	6	3	5	4	7	7
	A_E	3.6	4.1	5.0	4.4	4.3	2.8	3.6	2.9	5.4	5.5
	R	166-230	106-162	232-304	162-214	170-206	148-176	138-172	170-194	206-234	338-358
	S	178	134	244	170	204	148	138	190	230	356
	F	0.76	0.76	0.60	0.52	0.61	0.77	0.83	0.90	0.53	0.63
	H_E	0.40	0.39	0.59	0.64	0.55	0.37	0.30	0.19	0.65	0.57
	H_O	0.41	0.47	0.48	0.77	0.48	0.42	0.27	0.21	0.55	0.40
	PHWE	0.371	1.000	0.219	0.408	0.001	0.458	0.566	1.000	0.082	0.018
	Null	0.000	0.000	0.083	0.000	0.083	0.000	0.046	0.000	0.172	0.118

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Paria River site P-A N=39	A	10	20	15	6	13	13	6	15	5	2
	A_E	7.5	13.4	10.5	5.6	9.9	8.7	4.4	10.8	4.8	1.6
	R	170-210	102-214	228-300	138-158	204-234	150-188	154-166	188-222	198-220	336-342
	S	182	134,194	268	142	220	158	164	200	220	342
	F	0.30	0.11	0.26	0.30	0.28	0.26	0.47	0.28	0.42	0.97
	H_E	0.80	0.93	0.87	0.78	0.86	0.84	0.67	0.87	0.73	0.06
	H_O	0.66	0.94	0.85	0.42	0.78	0.84	0.54	0.83	0.76	0.06
	PHWE	0.086	0.895	0.713	0.000	0.039	0.355	0.012	0.015	0.768	1.000
	Null	0.072	0.000	0.000	0.203	0.053	0.000	0.063	0.008	0.000	0.000
Paria River site P-B N=40	A	7	21	12	6	9	11	6	15	5	2
	A_E	5.2	12.2	9.4	4.2	7.2	7.1	4.2	10.3	4.6	1.4
	R	174-210	98-206	228-292	134-158	206-224	150-188	154-166	190-222	198-220	342-344
	S	186	190	260	154	220	158	164	200	220	342
	F	0.49	0.19	0.18	0.60	0.32	0.41	0.68	0.23	0.42	0.99
	H_E	0.68	0.90	0.88	0.58	0.81	0.75	0.50	0.88	0.72	0.03
	H_O	0.70	0.92	0.87	0.26	0.92	0.68	0.36	0.87	0.72	0.03
	PHWE	0.737	0.915	0.002	0.000	0.273	0.198	0.009	0.331	0.876	1.000
	Null	0.000	0.000	0.011	0.212	0.000	0.068	0.093	0.018	0.003	0.000
Paria River site P-C N=39	A	10	21	20	7	11	12	5	13	6	1
	A_E	7.0	12.4	12.3	6.2	8.0	10.0	3.7	9.7	5.3	1.0
	R	158-206	86-198	188-328	130-154	208-232	150-186	148-164	198-226	198-220	342
	S	182	98,106	216	142	222	174	164	200	216	342
	F	0.40	0.15	0.18	0.30	0.39	0.15	0.67	0.27	0.28	1.00
	H_E	0.77	0.91	0.91	0.81	0.79	0.89	0.49	0.86	0.79	0.00
	H_O	0.74	0.94	0.92	0.18	0.76	0.87	0.46	0.84	0.87	0.00
	PHWE	0.537	0.000	0.501	0.000	0.754	0.224	0.824	0.323	0.219	~
	Null	0.000	0.000	0.002	0.347	0.036	0.010	0.015	0.017	0.000	~

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Paria River site P-D N=45	A	10	13	17	9	12	13	5	13	3	1
	A_E	8.2	9.7	10.7	6.5	8.5	9.2	3.8	8.3	2.8	1.0
	R	170-206	86-206	164-296	130-182	174-220	150-182	138-160	182-248	210-214	342
	S	178	110	220	134	210	174	160	222	212	342
	F	0.41	0.20	0.16	0.39	0.42	0.29	0.45	0.31	0.83	1.00
	H_E	0.79	0.88	0.90	0.77	0.78	0.84	0.66	0.82	0.29	0.00
	H_O	0.69	0.76	0.82	0.68	0.76	0.77	0.57	0.76	0.21	0.00
	PHWE	0.044	0.040	0.181	0.096	0.293	0.034	0.189	0.210	0.059	~
	Null	0.045	0.060	0.038	0.064	0.008	0.060	0.058	0.037	0.094	~
Paria River site P-E N=40	A	1	2	4	3	6	3	4	5	3	3
	A_E	1.0	2.0	3.3	2.8	3.7	1.9	3.0	4.5	2.4	2.5
	R	170	86-102	252-264	138-154	206-220	146-158	146-160	210-222	204-212	336-342
	S	170	86	260	150	220	158	160	218	212	342
	F	1.00	0.76	0.41	0.77	0.64	0.96	0.84	0.62	0.91	0.92
	H_E	0.00	0.36	0.66	0.38	0.51	0.09	0.27	0.56	0.17	0.15
	H_O	0.00	0.48	0.60	0.44	0.41	0.06	0.24	0.52	0.14	0.05
	PHWE	~	0.279	0.385	0.701	0.004	0.046	0.513	0.040	0.033	0.026
	Null	~	0.000	0.032	0.000	0.046	0.000	0.043	0.035	0.078	0.137
Paria River site P-F N=40	A	11	11	17	10	10	12	4	15	6	1
	A_E	7.9	9.4	11.0	7.5	6.7	8.5	3.5	9.9	4.2	1.0
	R	158-210	94-138	176-332	118-182	176-220	148-180	148-160	182-250	204-232	342
	S	178	102	224	134	212	174	160	218	212	342
	F	0.26	0.26	0.20	0.29	0.49	0.39	0.58	0.21	0.79	1.00
	H_E	0.83	0.87	0.89	0.81	0.71	0.80	0.57	0.87	0.37	0.00
	H_O	0.83	0.95	0.83	0.76	0.82	0.72	0.51	0.82	0.36	0.00
	PHWE	0.471	0.500	0.074	0.278	0.724	0.290	0.063	0.641	0.770	~
	Null	0.000	0.000	0.035	0.024	0.000	0.053	0.070	0.034	0.020	~

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Paria River **site P-G** **N=80**	A	11	14	18	9	11	10	5	16	9	3
	A_E	7.1	9.5	9.6	6.6	6.0	6.6	3.1	9.7	4.2	1.8
	R	158-206	90-198	176-316	118-210	176-220	154-184	148-162	152-250	196-216	338-344
	S	178	106	216,220	134	216	176	160	218	212	342
	F	0.35	0.22	0.16	0.32	0.26	0.27	0.53	0.30	0.75	0.96
	H_E	0.78	0.88	0.88	0.79	0.70	0.82	0.54	0.86	0.42	0.08
	H_O	0.81	0.77	0.92	0.74	0.68	0.79	0.50	0.86	0.37	0.04
	PHWE	0.301	0.042	0.636	0.063	0.040	0.004	0.000	0.609	0.022	0.002
	Null	0.000	0.068	0.000	0.039	0.012	0.029	0.047	0.034	0.038	0.094
Paria River **site P-H** **N=40**	A	9	12	9	5	8	8	3	12	4	2
	A_E	7.1	9.0	6.4	5.0	5.4	6.2	2.7	8.0	3.2	1.5
	R	174-206	90-198	176-240	130-146	200-218	166-180	154-162	186-234	204-214	336-342
	S	182	110	176	134	210	174	160	218	212	342
	F	0.26	0.24	0.51	0.34	0.59	0.56	0.69	0.30	0.59	0.97
	H_E	0.82	0.86	0.68	0.77	0.61	0.64	0.45	0.81	0.54	0.05
	H_O	0.90	0.84	0.68	0.73	0.67	0.72	0.46	0.86	0.41	0.05
	PHWE	0.002	0.057	0.743	0.702	0.063	0.574	0.334	0.886	0.089	1.000
	Null	0.000	0.024	0.000	0.024	0.009	0.000	0.000	0.000	0.089	0.000
Jordan River **(Paria tributary)** **site J-A** **N=38**	A	8	21	26	6	9	16	5	18	7	3
	A_E	5.6	13.8	14.7	5.3	7.1	10.0	4.5	12.6	6.2	2.5
	R	174-210	94-202	212-368	134-154	208-236	150-192	154-168	190-226	198-220	336-342
	S	182	94,110,126,134	272	146	222	150	164	206	214	342
	F	0.38	0.10	0.13	0.29	0.39	0.24	0.43	0.20	0.31	0.76
	H_E	0.72	0.93	0.94	0.78	0.77	0.87	0.66	0.91	0.81	0.37
	H_O	0.79	0.97	0.89	0.45	0.86	0.92	0.54	0.91	0.69	0.37
	PHWE	0.948	0.712	0.135	0.000	0.931	0.559	0.179	0.396	0.035	1.000
	Null	0.000	0.000	0.036	0.180	0.000	0.000	0.041	0.000	0.068	0.003

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Jordan River (Paria tributary) site J-B N=40	A	7	24	23	6	11	13	6	17	7	3
	A_E	5.7	13.4	13.6	5.1	7.9	9.6	4.4	12.6	5.9	1.9
	R	174-206	94-206	224-360	134-154	208-232	150-186	148-168	190-228	198-220	336-342
	S	186	110	260	142	222	150	164	200	216	342
	F	0.41	0.16	0.15	0.30	0.40	0.24	0.42	0.17	0.30	0.96
	H_E	0.73	0.91	0.92	0.77	0.77	0.87	0.66	0.92	0.79	0.09
	H_O	0.75	0.92	0.95	0.41	0.85	0.97	0.72	0.97	0.89	0.09
	PHWE	0.672	0.873	0.161	0.000	0.819	0.849	0.866	0.594	0.176	1.000
	Null	0.000	0.000	0.006	0.203	0.000	0.000	0.000	0.000	0.000	0.000
Jordan River (Paria tributary) site J-C N=47	A	6	7	17	5	8	12	3	13	5	6
	A_E	5.2	6.2	10.6	4.3	6.2	8.6	2.7	9.0	4.3	5.6
	R	178-210	94-146	224-360	134-150	208-224	150-176	154-164	190-224	210-220	336-346
	S	178	138	244	142	222	154	160	218	214	342
	F	0.29	0.31	0.27	0.50	0.51	0.24	0.71	0.36	0.47	0.41
	H_E	0.77	0.78	0.86	0.64	0.69	0.85	0.43	0.81	0.65	0.73
	H_O	0.79	0.72	0.86	0.55	0.70	0.85	0.53	0.75	0.59	0.43
	PHWE	0.689	0.723	0.410	0.438	0.191	0.186	0.372	0.391	0.011	0.000
	Null	0.000	0.041	0.000	0.062	0.010	0.000	0.000	0.005	0.085	0.158
Pitch Lake N=33	A	12	20	12	17	17	15	11	15	7	7
	A_E	10.3	13.6	9.1	12.1	11.5	8.8	8.4	13.1	4.8	4.8
	R	174-230	118-274	212-272	134-206	180-238	166-234	118-166	172-248	206-224	338-362
	S	202	166	228	190	192	224	154	172	212	346
	F	0.16	0.17	0.20	0.18	0.18	0.59	0.32	0.20	0.45	0.65
	H_E	0.90	0.92	0.87	0.91	0.90	0.64	0.82	0.90	0.64	0.54
	H_O	0.94	0.63	0.82	0.84	0.85	0.64	0.85	0.87	0.57	0.42
	PHWE	0.651	0.000	0.634	0.709	0.239	0.666	0.829	0.664	0.405	0.003
	Null	0.000	0.157	0.032	0.029	0.036	0.000	0.000	0.029	0.004	0.086

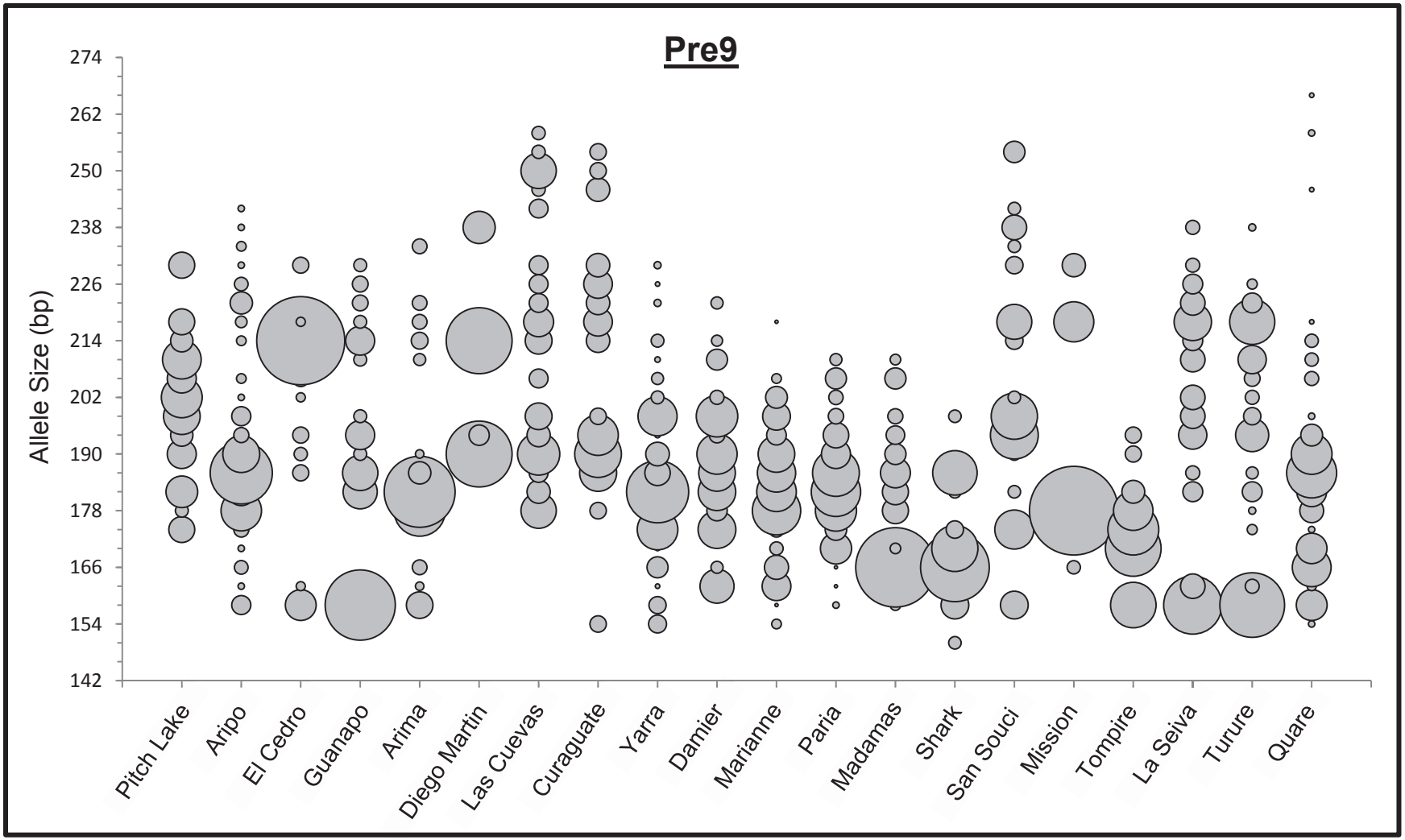
Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Quare River Low Predation N=24	A	6	9	11	10	9	11	8	6	5	5
	A_E	5.5	7.8	9.2	7.7	7.7	9.1	7.0	5.2	4.5	3.9
	R	166-206	86-226	200-284	146-230	170-206	148-216	120-172	144-212	204-220	338-354
	S	186	186	248	166	172	148	158	144	210	346
	F	0.43	0.28	0.26	0.31	0.43	0.26	0.24	0.48	0.42	0.74
	H_E	0.72	0.81	0.84	0.81	0.76	0.85	0.82	0.69	0.68	0.43
	H_O	0.76	0.72	0.95	0.81	0.64	0.89	0.78	0.52	0.68	0.35
	PHWE	0.497	0.012	0.182	0.311	0.028	0.577	0.383	0.135	0.651	0.195
	Null	0.000	0.022	0.000	0.000	0.057	0.000	0.039	0.106	0.003	0.029
Quare River High Predation N=145	A	15	35	29	27	29	40	19	30	15	12
	A_E	8.6	15.6	13.4	12.9	12.0	15.2	11.0	10.5	7.4	7.0
	R	158-218	86-282	180-360	106-234	160-246	136-230	120-184	142-238	180-232	334-362
	S	186	178	240	178	172	152	164	144	210	346
	F	0.23	0.10	0.12	0.13	0.26	0.12	0.18	0.47	0.42	0.47
	H_E	0.86	0.95	0.94	0.93	0.89	0.94	0.90	0.77	0.77	0.73
	H_O	0.83	0.84	0.86	0.90	0.82	0.87	0.79	0.73	0.61	0.50
	PHWE	0.225	0.000	0.001	0.099	0.000	0.001	0.000	0.008	0.000	0.000
	Null	0.017	0.062	0.042	0.024	0.024	0.031	0.067	0.030	0.082	0.159
Quare River site 3 N=53	A	16	21	26	19	20	30	10	19	9	8
	A_E	10.3	14.2	14.0	11.8	10.5	13.0	7.5	8.9	6.3	6.0
	R	154-266	146-286	196-360	138-230	166-228	148-224	120-172	144-232	192-232	338-362
	S	186	186	244	162	172	148	164	144	210	346
	F	0.27	0.12	0.17	0.14	0.30	0.24	0.29	0.56	0.34	0.53
	H_E	0.87	0.94	0.93	0.91	0.86	0.89	0.83	0.67	0.78	0.67
	H_O	0.90	0.95	0.88	0.96	0.86	0.84	0.76	0.60	0.82	0.48
	PHWE	0.596	0.701	0.115	0.563	0.386	0.551	0.710	0.016	0.074	0.000
	Null	0.000	0.000	0.032	0.000	0.000	0.000	0.025	0.028	0.000	0.143

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Quare River site 4 N=42	A	10	18	15	13	14	11	10	10	7	7
	A_E	7.5	13.0	11.1	9.7	6.9	9.0	6.1	5.8	5.4	5.4
	R	158-258	130-294	184-296	162-242	166-222	148-212	120-182	144-224	194-232	338-352
	S	166	182	232	238	172	154	122	144	194	346
	F	0.32	0.17	0.16	0.24	0.59	0.22	0.66	0.74	0.43	0.67
	H_E	0.79	0.92	0.90	0.87	0.61	0.86	0.54	0.45	0.70	0.53
	H_O	0.73	0.85	0.78	0.76	0.51	0.80	0.42	0.42	0.69	0.44
	PHWE	0.033	0.444	0.115	0.000	0.003	0.055	0.000	0.046	0.000	0.211
	Null	0.000	0.035	0.071	0.187	0.050	0.003	0.000	0.039	0.003	0.079
San Sauci River N=35	A	14	4	11	11	13	10	11	13	6	5
	A_E	9.4	4.0	11.0	8.5	8.5	7.6	6.7	10.7	5.7	4.0
	R	158-254	194-214	216-284	118-214	162-224	156-204	120-176	166-234	194-210	334-350
	S	194	210	284	206	186	160	120	166	208	342
	F	0.22	0.54	0.21	0.25	0.29	0.27	0.34	0.25	0.49	0.58
	H_E	0.86	0.60	0.88	0.85	0.82	0.82	0.76	0.88	0.71	0.59
	H_O	0.88	0.17	1.00	0.89	0.77	0.94	0.86	0.92	0.71	0.66
	PHWE	0.865	0.001	0.919	0.687	0.020	0.013	0.882	0.048	0.448	0.619
	Null	0.000	0.271	0.000	0.000	0.029	0.000	0.000	0.020	0.000	0.000
Shark River N=35	A	8	17	16	10	8	5	5	12	12	5
	A_E	5.5	13.2	14.0	7.7	6.3	3.9	3.7	8.8	8.4	3.7
	R	150-198	158-278	208-320	150-202	170-202	144-168	120-176	142-238	178-214	338-354
	S	166	158, 166	252	158	170	148	120	224	196	348
	F	0.46	0.17	0.16	0.44	0.34	0.53	0.80	0.21	0.36	0.68
	H_E	0.71	0.91	0.92	0.75	0.77	0.62	0.34	0.85	0.80	0.49
	H_O	0.68	0.90	1.00	0.71	0.76	0.49	0.27	0.81	0.69	0.42
	PHWE	0.149	0.167	0.465	0.530	0.014	0.051	0.018	0.585	0.001	0.169
	Null	0.000	0.000	0.000	0.044	0.043	0.074	0.053	0.017	0.088	0.017

Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Tompire River N=29	A	7	21	13	12	12	15	3	5	6	4
	A_E	6.0	15.1	10.4	9.6	8.7	11.7	2.6	4.7	4.7	3.7
	R	158-194	98-342	264-340	106-218	162-222	154-246	136-148	168-238	210-228	336-344
	S	170	246	300	106	222	154	140	174	220	342
	F	0.30	0.18	0.23	0.38	0.35	0.39	0.80	0.62	0.44	0.78
	H_E	0.78	0.92	0.88	0.81	0.80	0.81	0.33	0.57	0.69	0.37
	H_O	0.75	0.95	0.86	0.65	0.78	0.78	0.40	0.59	0.64	0.26
	PHWE	0.125	0.059	0.172	0.015	0.737	0.253	1.000	0.140	0.598	0.008
	Null	0.010	0.017	0.000	0.080	0.000	0.046	0.000	0.010	0.018	0.080
Turure River Low Predation N=45	A	5	6	10	9	13	9	6	2	4	3
	A_E	4.4	5.5	7.4	6.1	8.2	6.7	3.9	2.0	3.2	2.9
	R	158-218	90-114	184-280	106-214	166-228	136-174	122-148	234-238	196-214	342-352
	S	158	110	244	106	184	166	148	234	214	342
	F	0.55	0.33	0.22	0.55	0.36	0.40	0.47	0.90	0.74	0.79
	H_E	0.62	0.78	0.84	0.66	0.80	0.76	0.60	0.18	0.42	0.35
	H_O	0.66	0.76	0.82	0.65	0.88	0.67	0.47	0.20	0.43	0.26
	PHWE	0.288	0.284	0.880	0.053	0.503	0.162	0.040	1.000	0.220	0.035
	Null	0.000	0.003	0.022	0.052	0.000	0.071	0.062	0.000	0.031	0.066
Turure River High Predation N=84	A	16	17	27	18	25	23	15	8	12	10
	A_E	9.5	9.1	12.9	9.5	11.9	11.8	5.7	3.8	6.0	6.2
	R	158-238	86-270	168-332	106-218	148-232	136-188	120-164	160-242	194-224	334-352
	S	158	110	260	106	186	166	148	234	214	342
	F	0.31	0.28	0.15	0.40	0.19	0.27	0.57	0.70	0.63	0.46
	H_E	0.84	0.85	0.92	0.80	0.90	0.88	0.61	0.46	0.58	0.71
	H_O	0.83	0.68	0.91	0.73	0.86	0.76	0.51	0.50	0.57	0.59
	PHWE	0.244	0.001	0.325	0.237	0.946	0.011	0.003	0.290	0.079	0.000
	Null	0.000	0.092	0.008	0.047	0.022	0.050	0.043	0.000	0.040	0.068

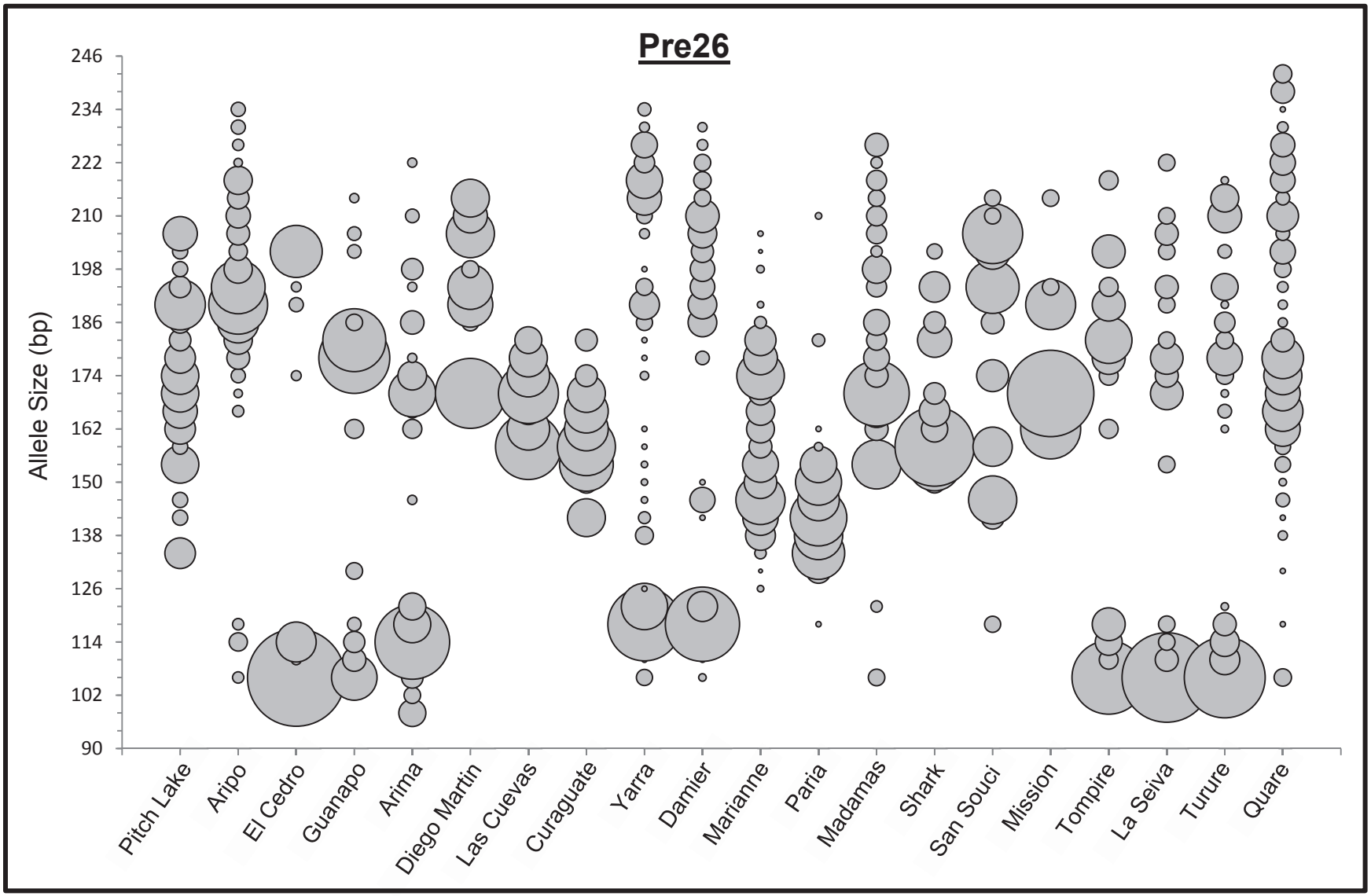
Population		Pre9	Pre13	Pre15	Pre26	Pret-27	Pret-28	Pret-38	Pret-46	Pret-80	G145
Yarra River Low Predation N=162	A	10	21	19	15	15	17	16	18	9	7
	A_E	6.0	9.3	6.0	5.9	6.6	8.0	5.3	7.1	5.1	4.1
	R	154-214	86-194	128-304	106-230	176-218	154-232	122-192	144-236	196-216	334-348
	S	182	154	140	118	214	188	152	188	202	334
	F	0.63	0.35	0.62	0.53	0.36	0.33	0.76	0.64	0.53	0.72
	H_E	0.58	0.83	0.58	0.65	0.77	0.81	0.41	0.58	0.64	0.46
	H_O	0.57	0.61	0.50	0.62	0.67	0.75	0.34	0.53	0.61	0.34
	PHWE	0.099	0.000	0.008	0.000	0.000	0.132	0.000	0.001	0.000	0.000
	Null	0.028	0.116	0.041	0.018	0.068	0.025	0.049	0.052	0.024	0.083
Yarra River High Predation N=86	A	15	17	30	19	20	27	11	18	9	8
	A_E	9.3	10.1	15.4	9.5	11.0	13.8	7.7	8.0	6.0	5.1
	R	162-226	102-194	132-336	106-234	174-216	146-240	120-192	156-230	196-214	332-346
	S	174	118	156	118	186	184	138	156	206	342
	F	0.21	0.18	0.09	0.38	0.31	0.16	0.32	0.46	0.40	0.35
	H_E	0.87	0.89	0.95	0.81	0.86	0.93	0.82	0.74	0.74	0.74
	H_O	0.84	0.72	0.92	0.78	0.73	0.88	0.66	0.51	0.66	0.60
	PHWE	0.005	0.037	0.023	0.332	0.007	0.001	0.000	0.000	0.014	0.002
	Null	0.028	0.089	0.024	0.000	0.075	0.028	0.092	0.123	0.070	0.084
Upper Yarra River N=75	A	7	13	17	12	16	21	7	7	6	7
	A_E	4.6	6.4	10.5	7.4	9.2	11.3	4.4	3.6	3.9	4.1
	R	174-230	102-214	140-332	118-234	178-234	158-234	122-174	154-198	202-214	334-348
	S	198	110	252,260	218	186	198,232	172	154	208	334
	F	0.43	0.64	0.18	0.33	0.35	0.18	0.55	0.85	0.39	0.67
	H_E	0.65	0.57	0.89	0.81	0.82	0.90	0.61	0.28	0.67	0.50
	H_O	0.63	0.27	0.81	0.70	0.81	0.75	0.51	0.21	0.62	0.31
	PHWE	0.210	0.000	0.005	0.000	0.057	0.004	0.094	0.000	0.901	0.000
	Null	0.010	0.199	0.047	0.048	0.011	0.068	0.044	0.040	0.022	0.140

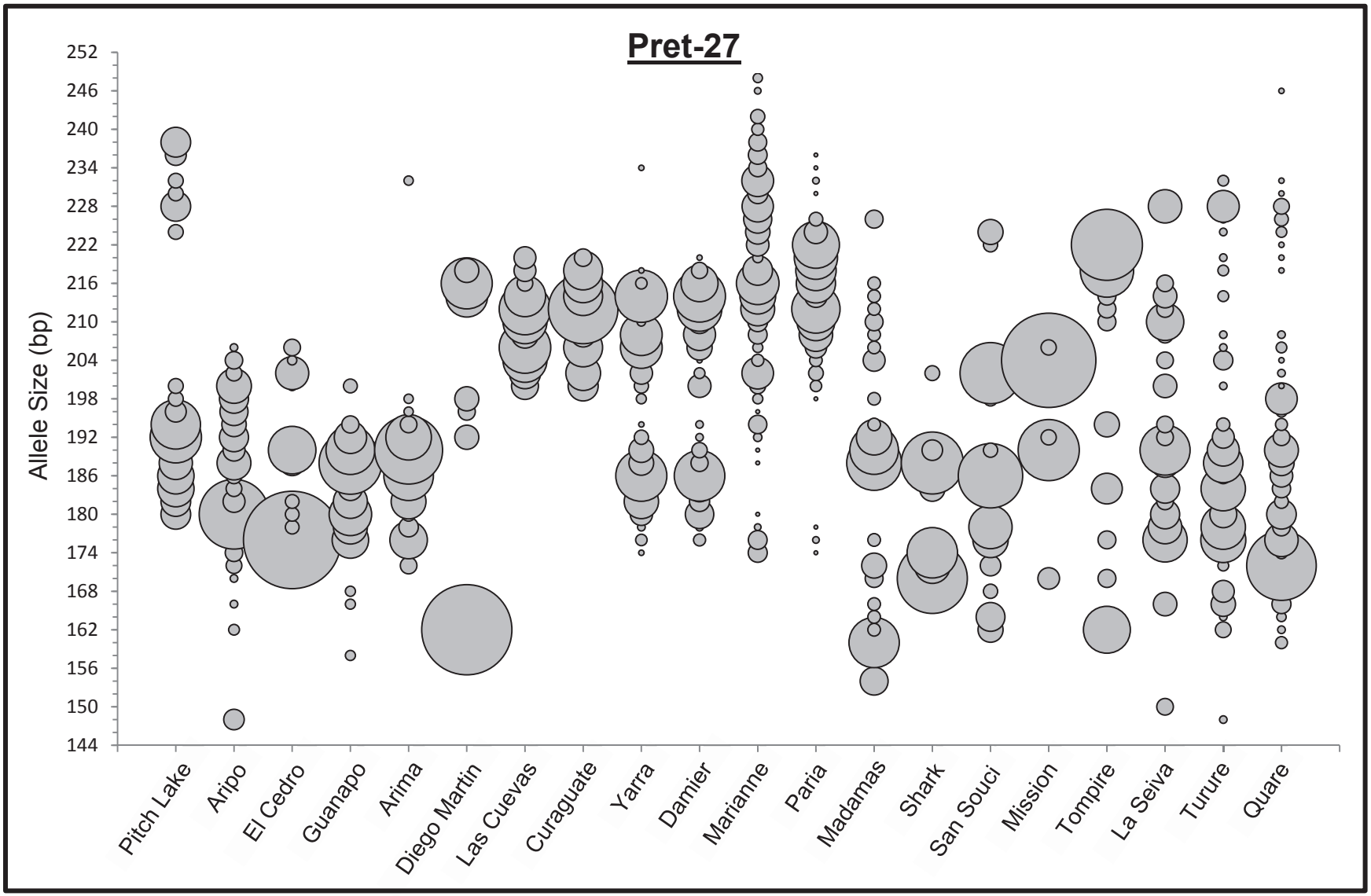
Appendix 5: Allele frequencies for each of ten microsatellite loci at 20 sites across Trinidad. Data from all sites and temporal replicates collected within each river was pooled in order to more accurately reflect overall frequencies.

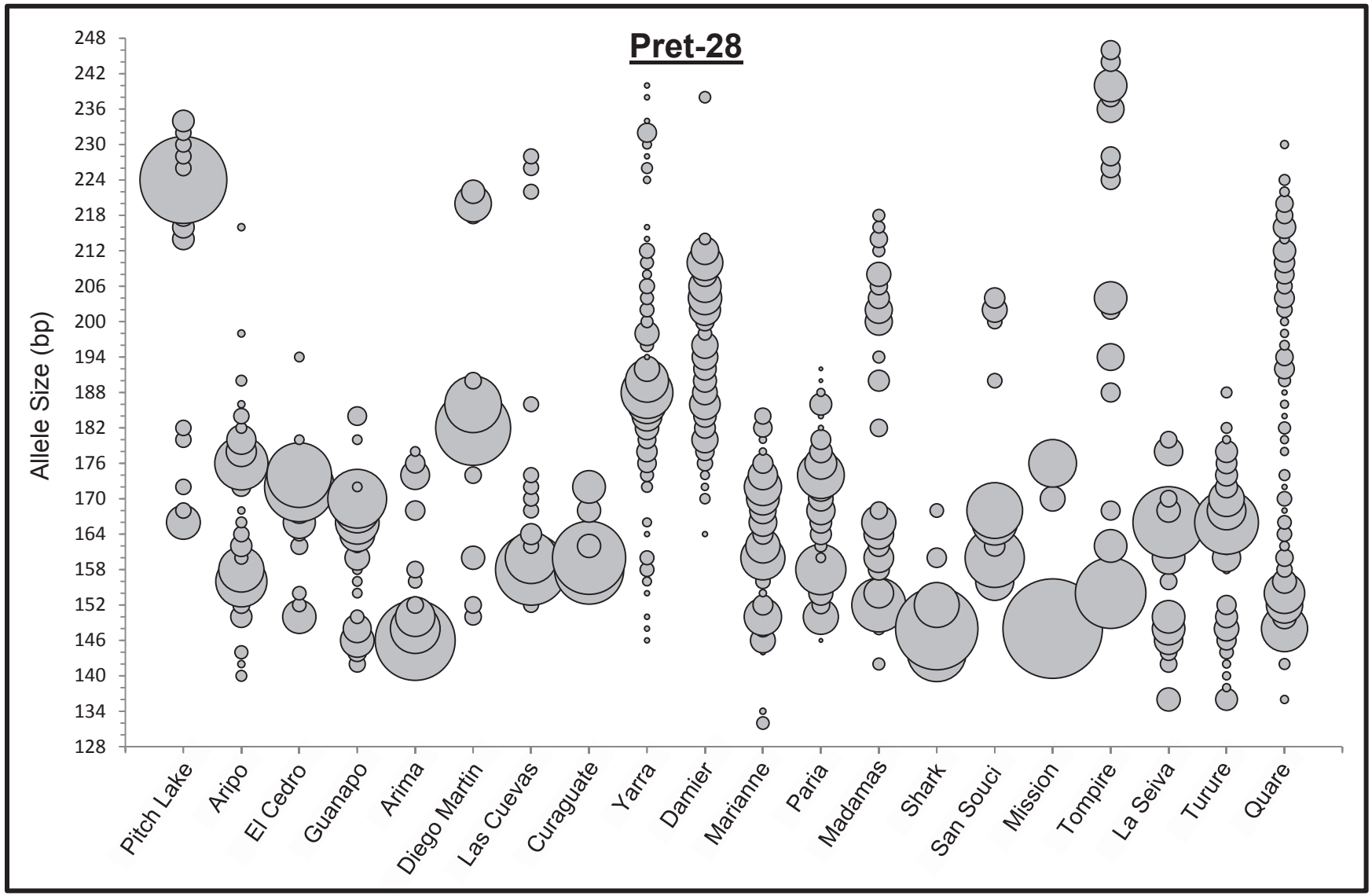


Pre13
Allele Size (bp)
356
344
332
320
308
296
284
272
260
248
236
224
212
200
188
176
164
152
140
128
116
104
92
80
68
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

Pre15
Allele Size (bp)
384
372
360
348
336
324
312
300
288
276
264
252
240
228
216
204
192
180
168
156
144
132
120
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

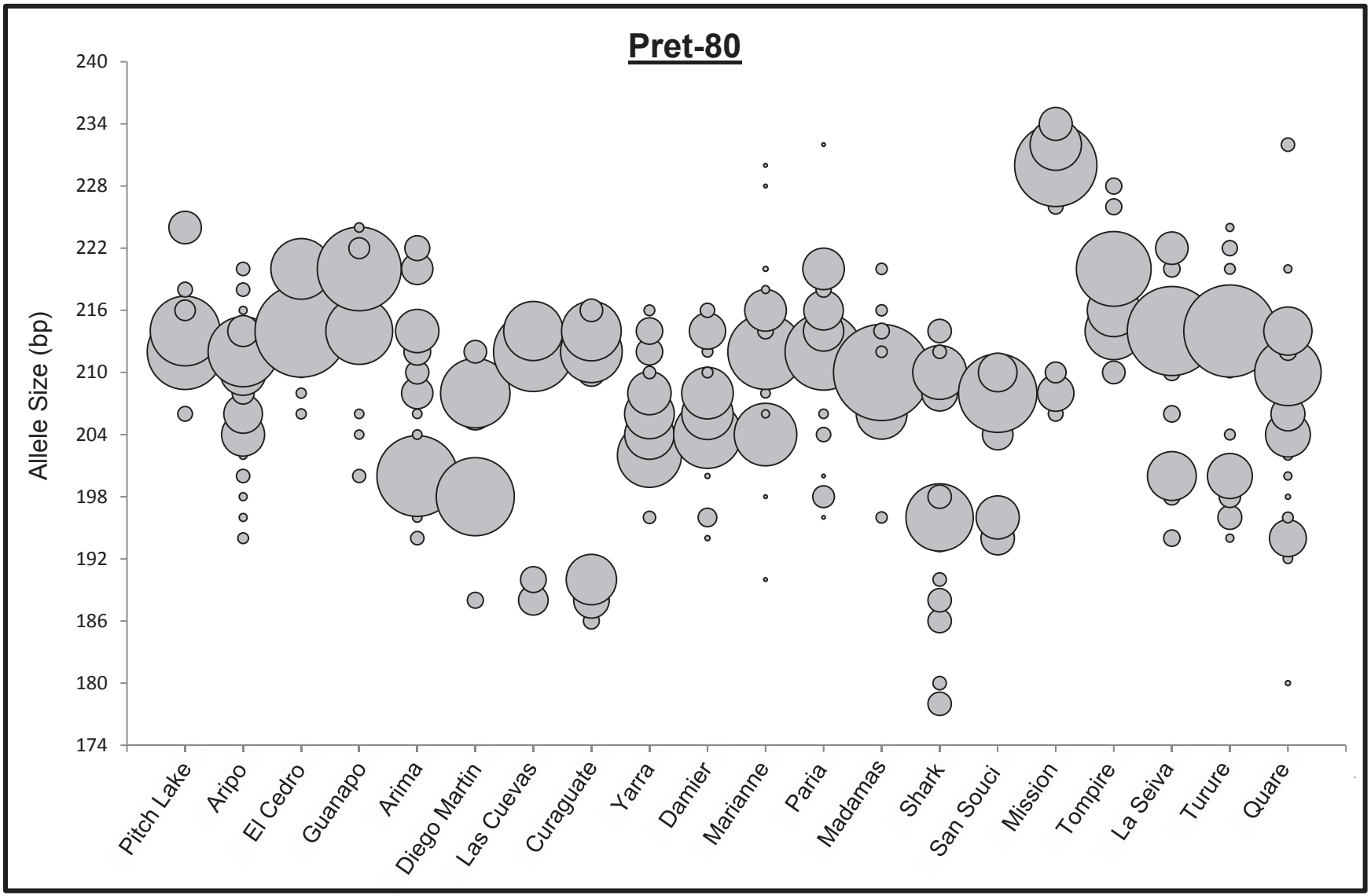

Pre26
Allele Size (bp)
246
234
222
210
198
186
174
162
150
138
126
114
102
90
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

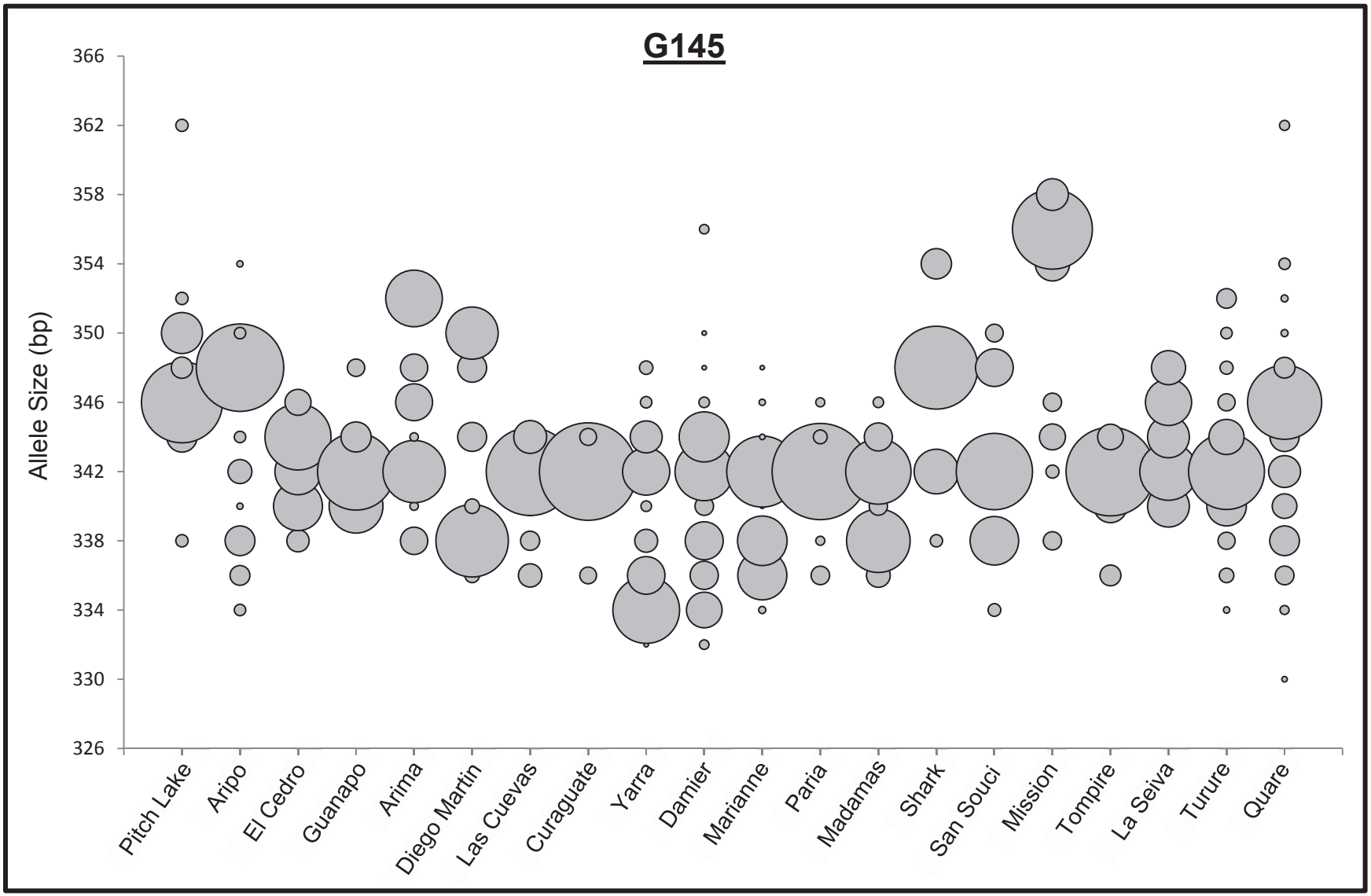

Pret-27
Allele Size (bp)
252
246
240
234
228
222
216
210
204
198
192
186
180
174
168
162
156
150
144
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare


Pret-28
Allele Size (bp)
248
242
236
230
224
218
212
206
200
194
188
182
176
170
164
158
152
146
140
134
128
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

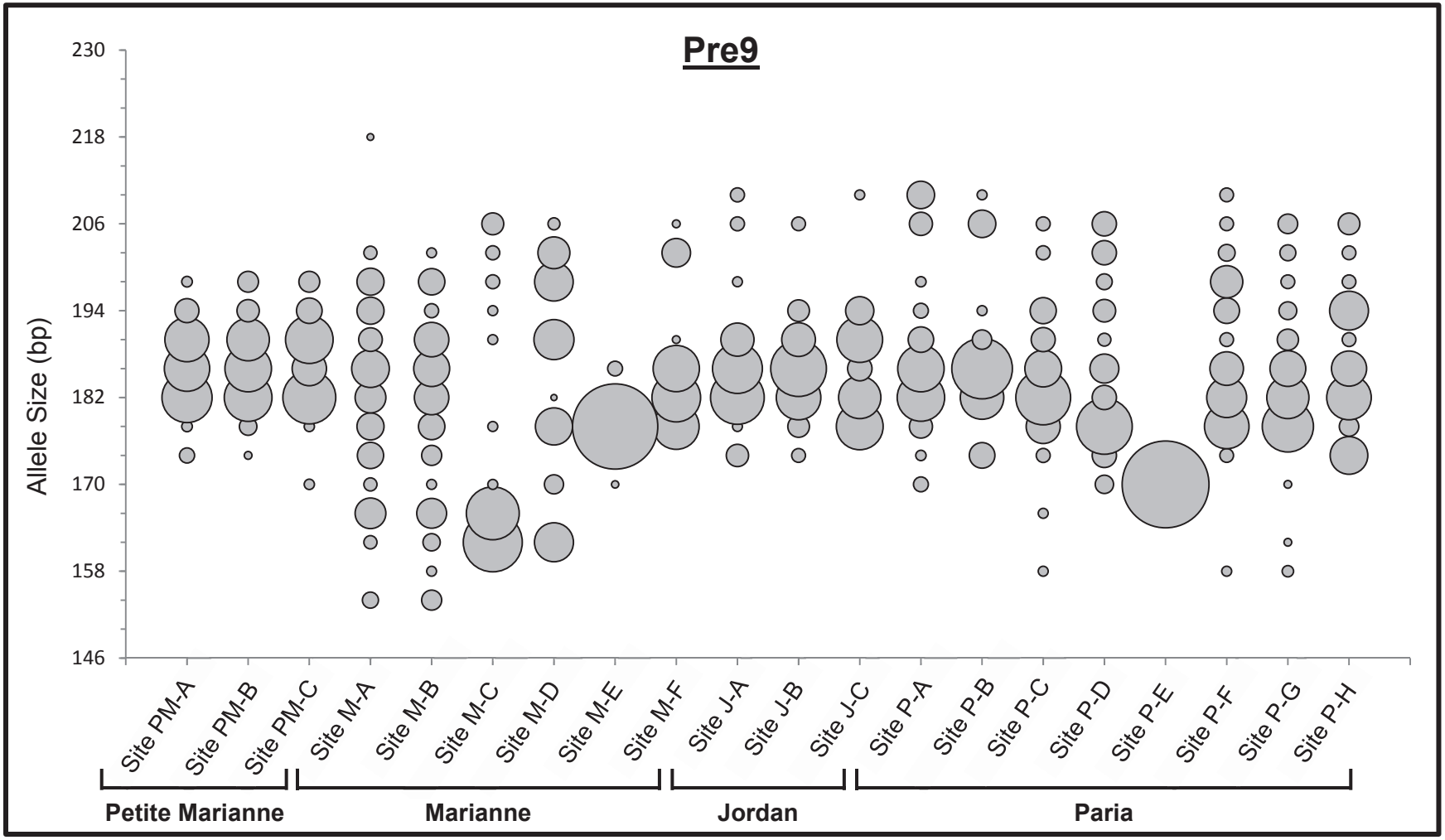
Pret-38
Allele Size (bp)
206
200
194
188
182
176
170
164
158
152
146
140
134
128
122
116
110
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

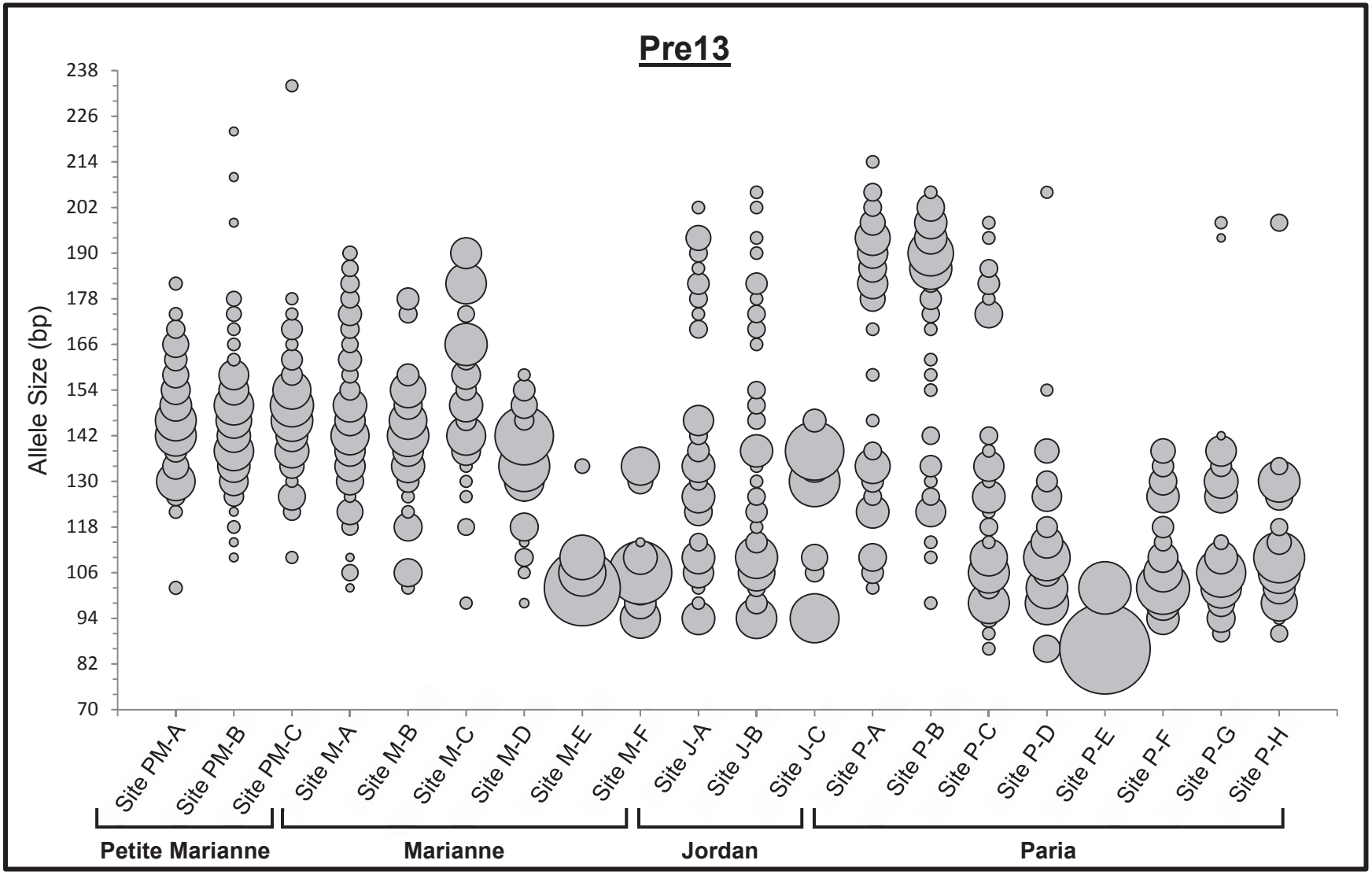
Pret-46
Allele Size (bp)
252
246
240
234
228
222
216
210
204
198
192
186
180
174
168
162
156
150
144
138
132
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare


Pret-80
Allele Size (bp)
240
234
228
222
216
210
204
198
192
186
180
174
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

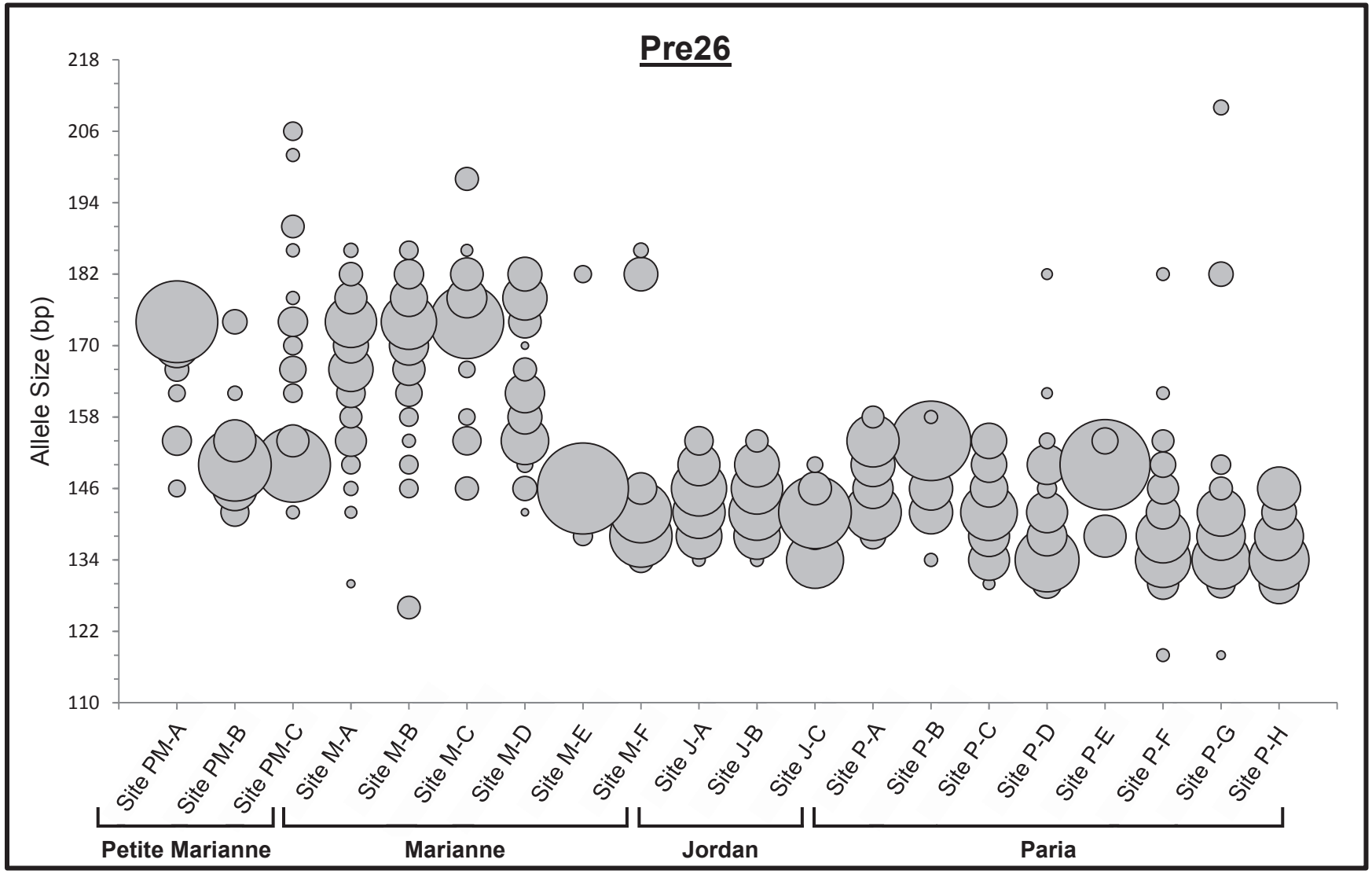

G145
Allele Size (bp)
366
362
358
354
350
346
342
338
334
330
326
Pitch Lake
Aripo
El Cedro
Guanapo
Arima
Diego Martin
Las Cuevas
Curaguate
Yarra
Damier
Marianne
Paria
Madamas
Shark
San Souci
Mission
Tompire
La Seiva
Turure
Quare

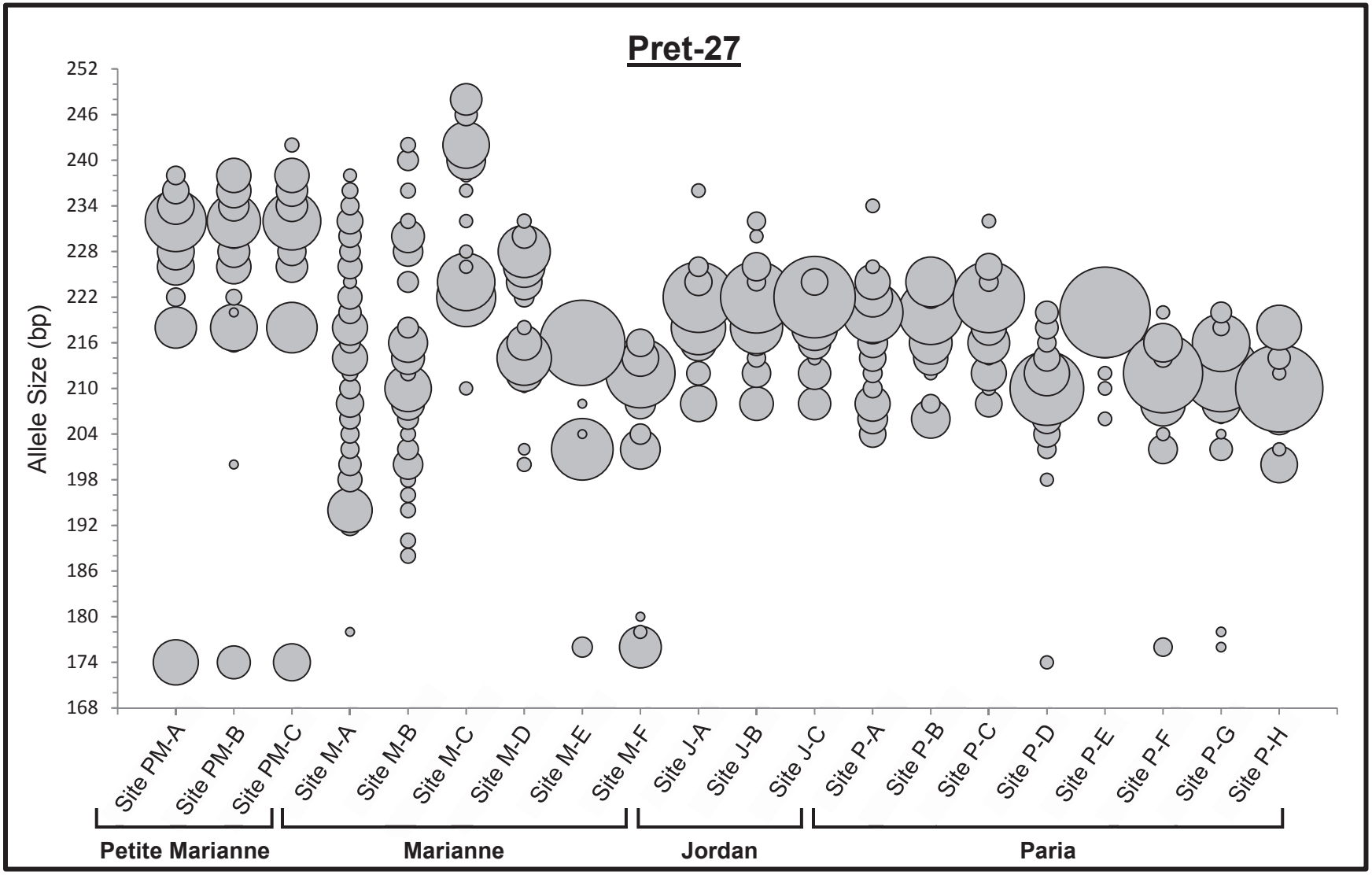
Appendix 6: Allele frequencies for each of ten microsatellite loci at 20 sites within the Marianne and Paria river watersheds on the north coast of Trinidad. At sites where temporal replicates were available data was pooled in order to more accurately reflect overall frequencies.

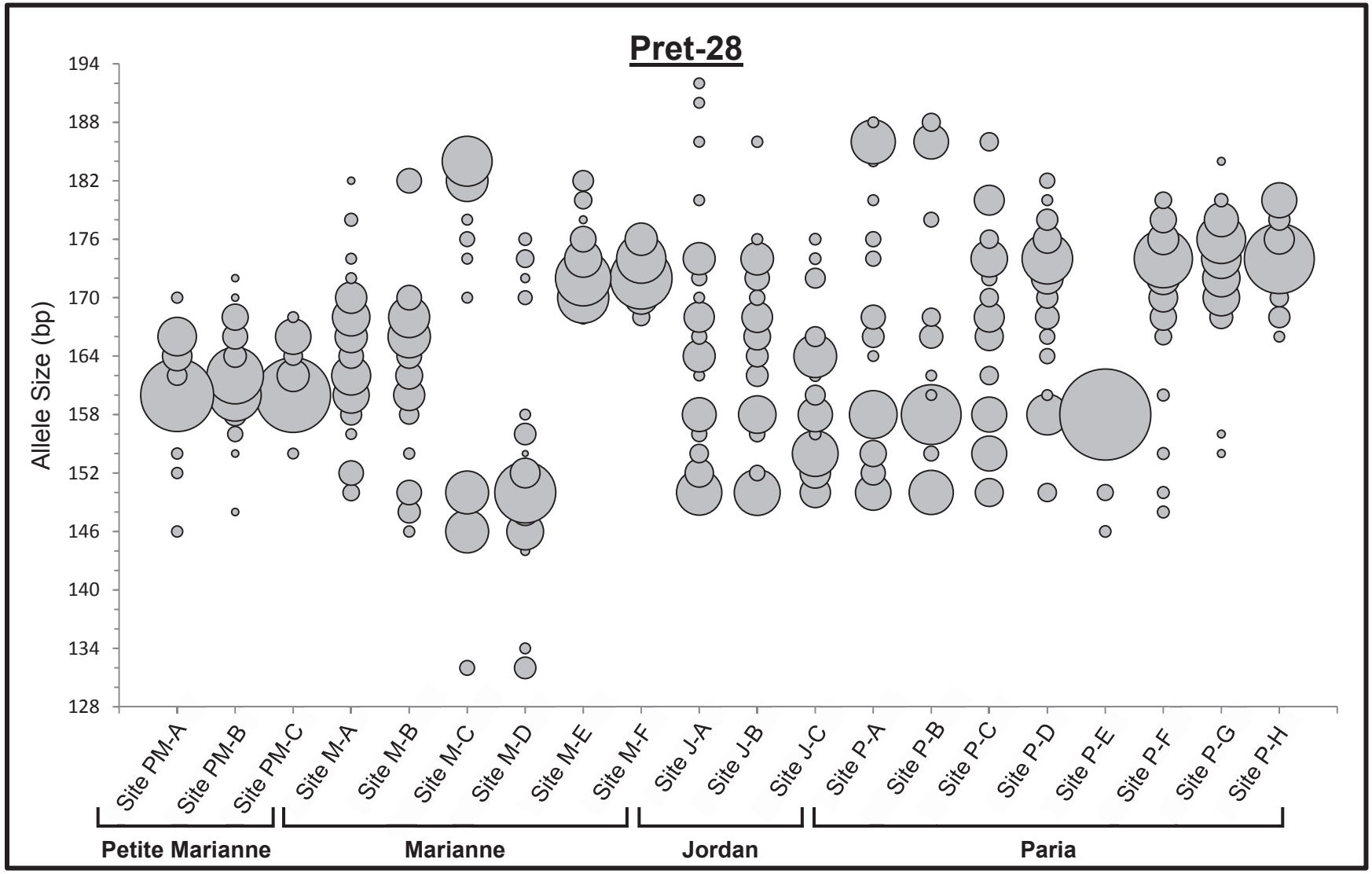


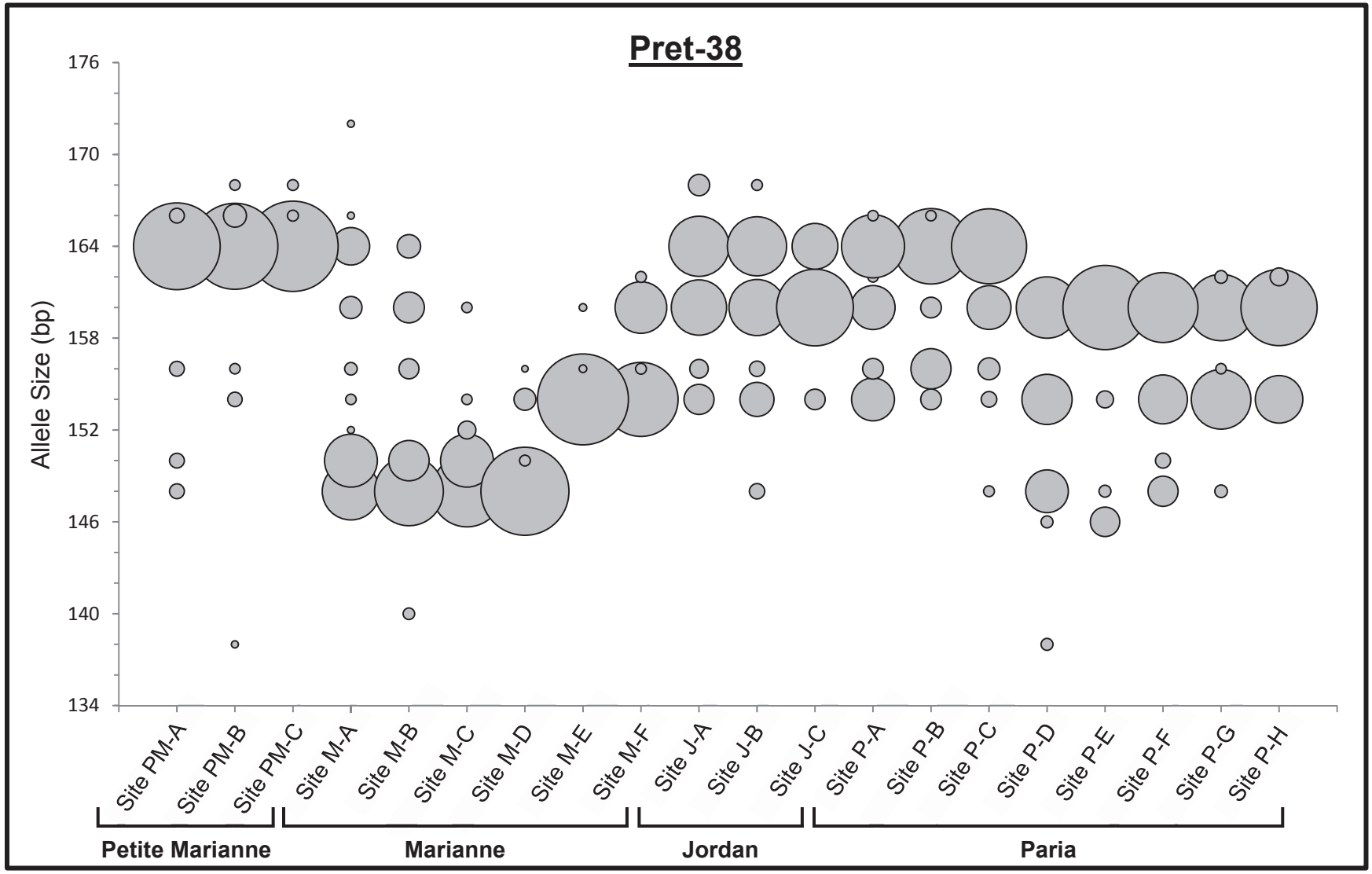

Pre13
Allele Size (bp)
238
226
214
202
190
178
166
154
142
130
118
106
94
82
70
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria

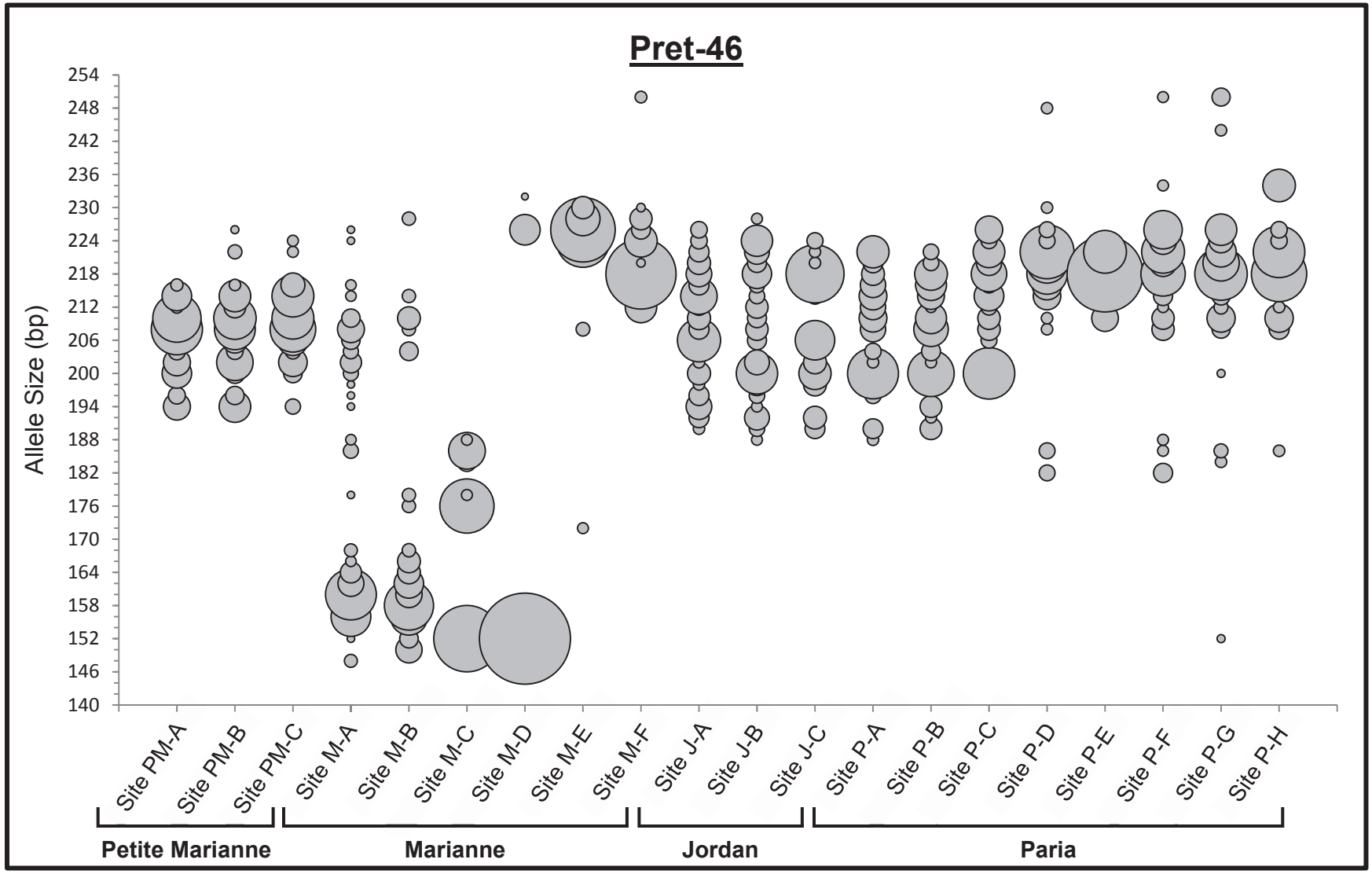
Pre15
Allele Size (bp)
376
364
352
340
328
316
304
292
280
268
256
244
232
220
208
196
184
172
160
148
136
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria

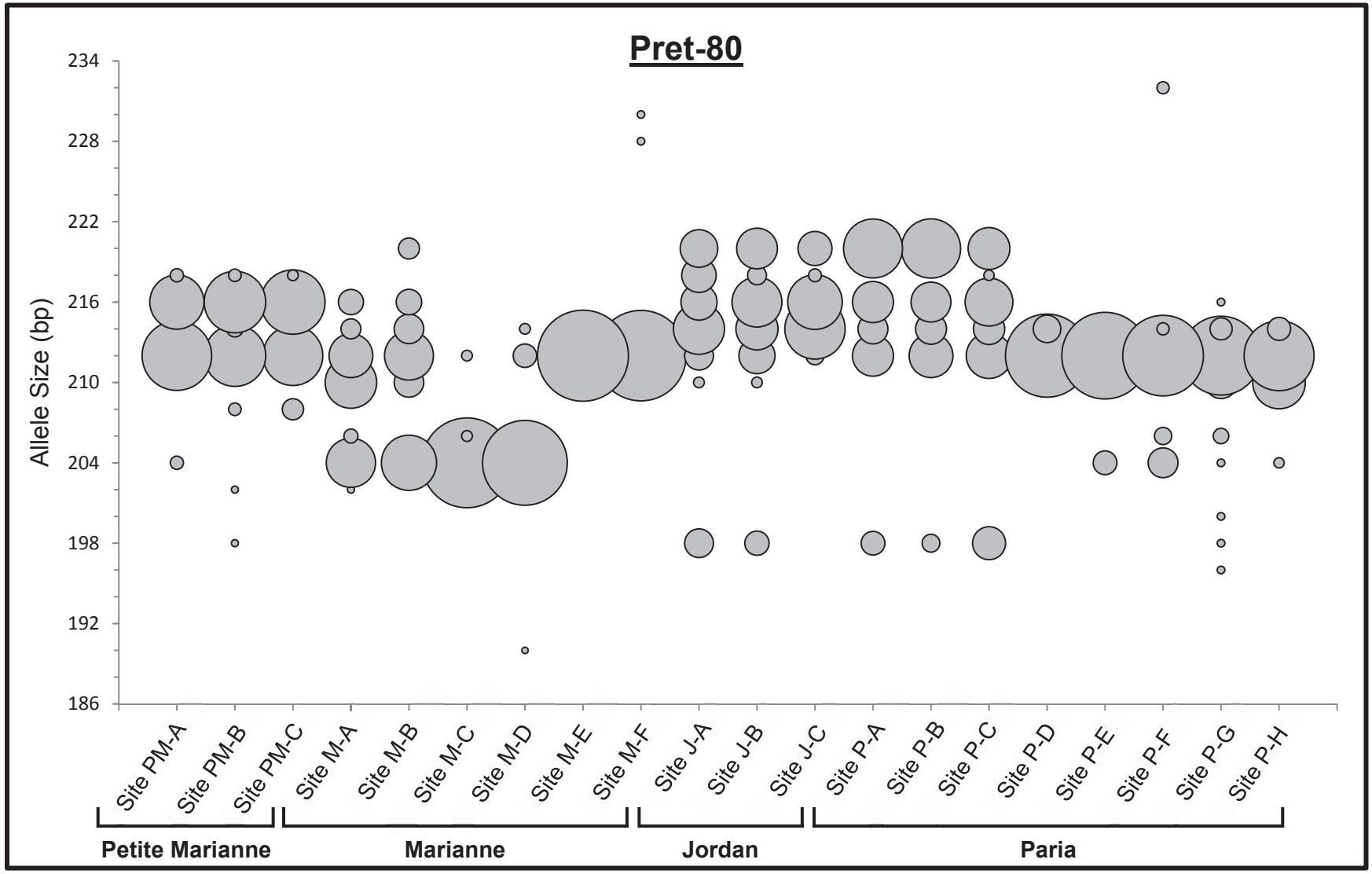

Pre26
Allele Size (bp)
218
206
194
182
170
158
146
134
122
110
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria

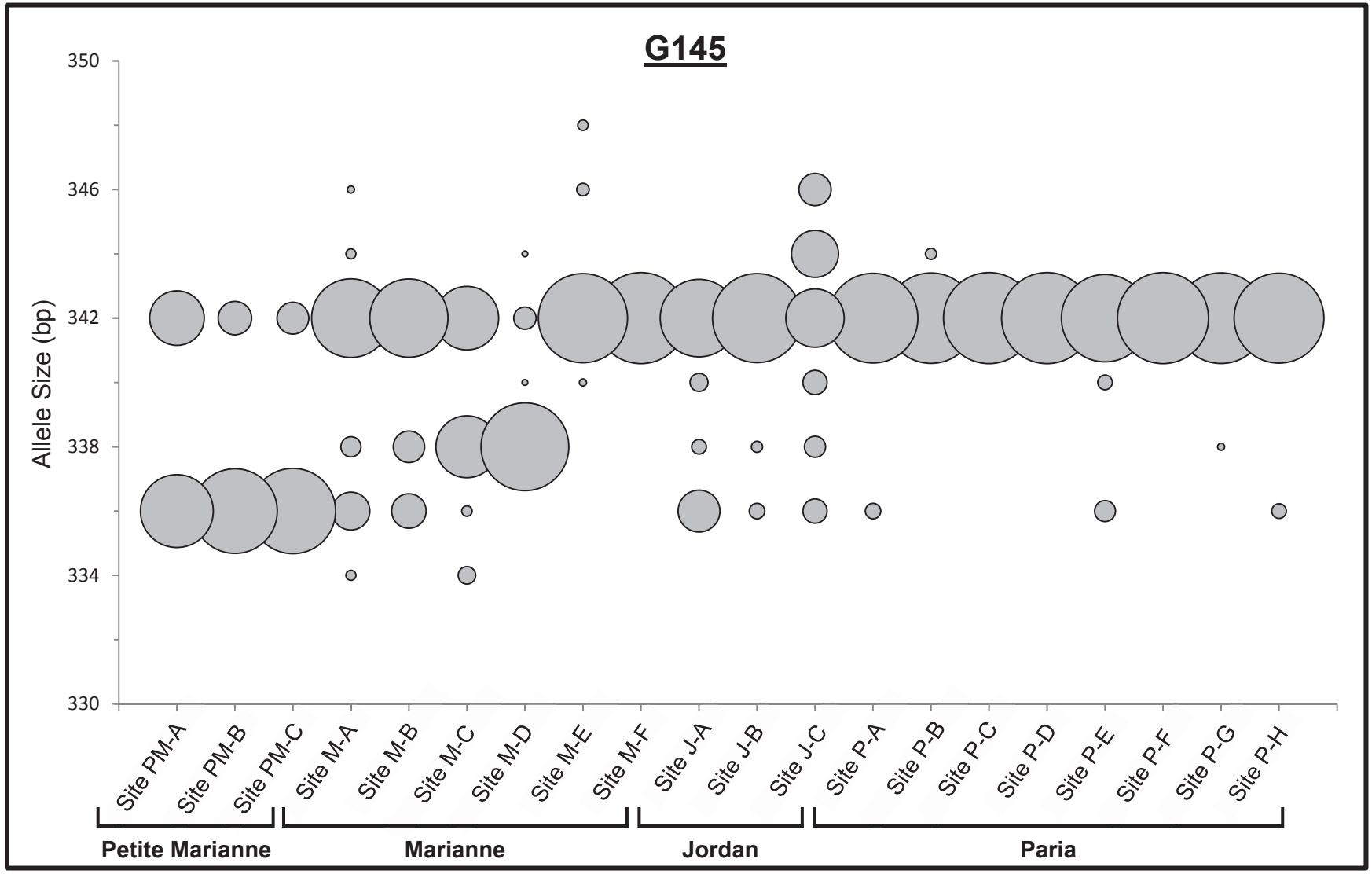

Pret-27
Allele Size (bp)
252
246
240
234
228
222
216
210
204
198
192
186
180
174
168
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria


Pret-28
Allele Size (bp)
194
188
182
176
170
164
158
152
146
140
134
128
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria


Pret-38
Allele Size (bp)
176
170
164
158
152
146
140
134
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria


Pret-46
Allele Size (bp)
254
248
242
236
230
224
218
212
206
200
194
188
182
176
170
164
158
152
146
140
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria


Pret-80
Allele Size (bp)
234
228
222
216
210
204
198
192
186
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria


G145
Allele Size (bp)
350
346
342
338
334
330
Site PM-A
Site PM-B
Site PM-C
Site M-A
Site M-B
Site M-C
Site M-D
Site M-E
Site M-F
Site J-A
Site J-B
Site J-C
Site P-A
Site P-B
Site P-C
Site P-D
Site P-E
Site P-F
Site P-G
Site P-H
Petite Marianne
Marianne
Jordan
Paria

Appendix 7: Matrix of estimated pairwise F_{ST} values for all sites and temporal replicates.

	Arima LP	Arima HP	Aripo LP	Aripo HP (2006)	Aripo HP (2010)	Curaguate	Damier LP (2005)	Damier LP (2009)	Damier LP (2010)	Damier HP (2005)	Damier HP (2009)	Damier HP (2010)	Diego Martin	El Cedro LP	El Cedro HP	Guanapo LP	Guanapo HP	La Seiva	Las Cuevas	Madamas 1	Madamas 2	Marianne A (2002)	Marianne A (2010)
Arima LP	0.000																						
Arima HP	0.297	0.000																					
Aripo LP	0.431	0.240	0.000																				
Aripo HP (2006)	0.314	0.132	0.132	0.000																			
Aripo HP (2010)	0.313	0.119	0.121	0.036	0.000																		
Curaguate	0.369	0.181	0.295	0.171	0.156	0.000																	
Damier LP (2005)	0.320	0.145	0.244	0.120	0.111	0.162	0.000																
Damier LP (2009)	0.296	0.149	0.243	0.116	0.112	0.157	0.018	0.000															
Damier LP (2010)	0.296	0.145	0.244	0.114	0.110	0.154	0.015	0.006	0.000														
Damier HP (2005)	0.346	0.141	0.251	0.116	0.101	0.163	0.017	0.034	0.020	0.000													
Damier HP (2009)	0.289	0.138	0.222	0.111	0.102	0.157	0.010	0.012	0.009	0.017	0.000												
Damier HP (2010)	0.297	0.126	0.225	0.099	0.090	0.154	0.019	0.026	0.015	0.019	0.009	0.000											
Diego Martin	0.398	0.244	0.325	0.191	0.197	0.233	0.194	0.194	0.179	0.203	0.179	0.172	0.000										
El Cedro LP	0.560	0.351	0.471	0.376	0.375	0.453	0.345	0.353	0.359	0.387	0.324	0.373	0.454	0.000									
El Cedro HP	0.376	0.139	0.310	0.198	0.191	0.231	0.189	0.194	0.195	0.191	0.178	0.188	0.282	0.224	0.000								
Guanapo LP	0.311	0.113	0.305	0.190	0.177	0.194	0.188	0.186	0.185	0.190	0.179	0.175	0.290	0.370	0.143	0.000							
Guanapo HP	0.340	0.175	0.341	0.235	0.223	0.273	0.241	0.233	0.234	0.247	0.225	0.222	0.343	0.442	0.215	0.091	0.000						
La Seiva	0.300	0.083	0.268	0.146	0.133	0.176	0.143	0.150	0.149	0.129	0.137	0.132	0.247	0.308	0.102	0.077	0.144	0.000					
Las Cuevas	0.335	0.147	0.252	0.140	0.128	0.033	0.140	0.135	0.133	0.138	0.135	0.132	0.219	0.400	0.203	0.177	0.244	0.153	0.000				
Madamas 1	0.323	0.136	0.273	0.141	0.133	0.152	0.152	0.146	0.142	0.151	0.145	0.125	0.229	0.416	0.213	0.167	0.229	0.151	0.137	0.000			
Madamas 2	0.386	0.193	0.311	0.177	0.169	0.236	0.190	0.187	0.179	0.192	0.179	0.166	0.247	0.460	0.265	0.252	0.304	0.214	0.203	0.055	0.000		
Marianne A (2002)	0.312	0.133	0.264	0.139	0.135	0.139	0.136	0.124	0.126	0.142	0.133	0.129	0.234	0.402	0.206	0.147	0.193	0.141	0.118	0.075	0.150	0.000	
Marianne A (2010)	0.286	0.113	0.235	0.123	0.124	0.109	0.125	0.112	0.115	0.132	0.120	0.113	0.221	0.386	0.194	0.132	0.180	0.126	0.091	0.081	0.158	0.024	0.000

	Arima LP	Arima HP	Aripo LP	Aripo HP (2006)	Aripo HP (2010)	Curaguate	Damier LP (2005)	Damier LP (2009)	Damier LP (2010)	Damier HP (2005)	Damier HP (2009)	Damier HP (2010)	Diego Martin	El Cedro LP	El Cedro HP	Guanapo LP	Guanapo HP	La Seiva	Las Cuevas	Madamas 1	Madamas 2	Marianne A (2002)	Marianne A (2010)
Marianne B	0.288	0.109	0.254	0.129	0.127	0.124	0.122	0.110	0.112	0.132	0.119	0.110	0.223	0.405	0.193	0.115	0.167	0.113	0.097	0.090	0.176	0.027	0.017
Marianne C	0.369	0.209	0.349	0.217	0.221	0.269	0.187	0.177	0.184	0.228	0.189	0.203	0.296	0.485	0.296	0.238	0.280	0.229	0.243	0.209	0.248	0.141	0.141
Marianne D (2002)	0.406	0.231	0.372	0.241	0.247	0.322	0.219	0.213	0.220	0.267	0.216	0.222	0.307	0.514	0.323	0.275	0.298	0.256	0.287	0.260	0.295	0.219	0.199
Marianne D (2008)	0.412	0.238	0.385	0.256	0.259	0.337	0.234	0.226	0.229	0.288	0.228	0.237	0.322	0.528	0.338	0.284	0.320	0.270	0.305	0.271	0.312	0.232	0.211
Marianne D (2010)	0.389	0.208	0.355	0.225	0.228	0.299	0.203	0.200	0.202	0.247	0.201	0.205	0.290	0.500	0.309	0.254	0.286	0.236	0.267	0.235	0.271	0.197	0.177
Marianne E (2002)	0.578	0.404	0.472	0.393	0.406	0.423	0.399	0.376	0.378	0.477	0.372	0.421	0.523	0.648	0.472	0.440	0.490	0.447	0.374	0.430	0.505	0.366	0.342
Marianne E (2010)	0.580	0.404	0.465	0.388	0.400	0.422	0.397	0.376	0.376	0.477	0.370	0.412	0.517	0.620	0.479	0.439	0.504	0.444	0.375	0.428	0.503	0.372	0.346
Marianne F (2002)	0.431	0.260	0.322	0.252	0.248	0.223	0.259	0.239	0.244	0.286	0.245	0.255	0.374	0.507	0.301	0.291	0.346	0.290	0.193	0.258	0.342	0.222	0.193
Marianne F (2010)	0.476	0.318	0.361	0.292	0.290	0.285	0.299	0.275	0.283	0.338	0.284	0.298	0.420	0.531	0.380	0.342	0.399	0.341	0.248	0.312	0.397	0.271	0.239
P. Marianne A	0.399	0.229	0.311	0.199	0.212	0.219	0.228	0.214	0.214	0.242	0.213	0.214	0.301	0.484	0.301	0.271	0.329	0.244	0.187	0.205	0.238	0.173	0.105
P. Marianne B (2002)	0.416	0.256	0.327	0.215	0.228	0.248	0.245	0.231	0.232	0.258	0.230	0.231	0.321	0.485	0.320	0.296	0.348	0.269	0.227	0.233	0.265	0.212	0.147
P. Marianne B (2010)	0.424	0.258	0.326	0.219	0.227	0.278	0.250	0.237	0.234	0.263	0.232	0.233	0.323	0.494	0.323	0.310	0.349	0.279	0.248	0.243	0.277	0.216	0.164
P. Marianne C	0.427	0.272	0.345	0.230	0.244	0.272	0.261	0.245	0.246	0.279	0.242	0.244	0.331	0.503	0.335	0.313	0.361	0.282	0.247	0.252	0.280	0.232	0.163
Mission	0.474	0.299	0.392	0.301	0.292	0.380	0.287	0.281	0.282	0.308	0.268	0.287	0.374	0.557	0.388	0.369	0.411	0.323	0.321	0.322	0.356	0.311	0.298
Paria A	0.341	0.174	0.286	0.176	0.165	0.131	0.168	0.153	0.152	0.175	0.160	0.157	0.269	0.424	0.216	0.181	0.235	0.189	0.121	0.135	0.219	0.125	0.101
Paria B	0.383	0.216	0.317	0.213	0.201	0.159	0.208	0.194	0.191	0.220	0.198	0.201	0.313	0.464	0.262	0.223	0.278	0.232	0.163	0.169	0.256	0.167	0.131
Paria C	0.343	0.176	0.294	0.185	0.173	0.143	0.182	0.164	0.164	0.190	0.173	0.168	0.280	0.431	0.224	0.193	0.251	0.193	0.133	0.152	0.224	0.128	0.107
Paria D	0.369	0.183	0.278	0.204	0.191	0.155	0.208	0.196	0.194	0.218	0.196	0.197	0.312	0.451	0.247	0.217	0.284	0.213	0.122	0.184	0.277	0.154	0.129
Paria E	0.587	0.429	0.486	0.400	0.385	0.405	0.401	0.379	0.385	0.480	0.372	0.419	0.514	0.644	0.470	0.424	0.512	0.444	0.355	0.401	0.491	0.369	0.339
Paria F	0.378	0.202	0.289	0.203	0.199	0.160	0.199	0.183	0.186	0.219	0.189	0.191	0.315	0.466	0.253	0.232	0.291	0.227	0.141	0.192	0.282	0.158	0.133
Paria G (2002)	0.387	0.216	0.293	0.209	0.207	0.171	0.212	0.195	0.196	0.228	0.201	0.200	0.321	0.476	0.269	0.247	0.298	0.241	0.153	0.197	0.284	0.167	0.149
Paria G (2010)	0.389	0.210	0.277	0.201	0.196	0.178	0.206	0.191	0.191	0.216	0.196	0.198	0.316	0.461	0.280	0.245	0.304	0.234	0.153	0.199	0.284	0.170	0.150
Paria H	0.409	0.237	0.327	0.229	0.230	0.218	0.235	0.217	0.220	0.254	0.222	0.224	0.345	0.487	0.272	0.267	0.328	0.255	0.184	0.203	0.296	0.184	0.175

	Marianne B	Marianne C	Marianne D (2002)	Marianne D (2008)	Marianne D (2010)	Marianne E (2002)	Marianne E (2010)	Marianne F (2002)	Marianne F (2010)	P. Marianne A	P. Marianne B (2002)	P. Marianne B (2010)	P. Marianne C	Mission	Paria A	Paria B	Paria C	Paria D	Paria E	Paria F	Paria G (2002)	Paria G (2010)	Paria H
Marianne B	0.000																						
Marianne C	0.118	0.000																					
Marianne D (2002)	0.178	0.147	0.000																				
Marianne D (2008)	0.187	0.156	0.012	0.000																			
Marianne D (2010)	0.152	0.131	0.024	0.015	0.000																		
Marianne E (2002)	0.374	0.482	0.527	0.538	0.508	0.000																	
Marianne E (2010)	0.374	0.480	0.529	0.536	0.505	0.164	0.000																
Marianne F (2002)	0.202	0.347	0.389	0.406	0.373	0.307	0.329	0.000															
Marianne F (2010)	0.256	0.397	0.435	0.454	0.418	0.385	0.304	0.149	0.000														
P. Marianne A	0.149	0.295	0.350	0.368	0.325	0.479	0.476	0.305	0.353	0.000													
P. Marianne B (2002)	0.196	0.335	0.363	0.378	0.342	0.494	0.488	0.337	0.380	0.058	0.000												
P. Marianne B (2010)	0.208	0.343	0.366	0.378	0.344	0.486	0.482	0.342	0.381	0.114	0.073	0.000											
P. Marianne C	0.214	0.352	0.379	0.396	0.359	0.517	0.513	0.361	0.404	0.054	**0.001**	0.072	0.000										
Mission	0.309	0.408	0.424	0.424	0.396	0.566	0.565	0.434	0.483	0.394	0.407	0.409	0.420	0.000									
Paria A	0.111	0.252	0.312	0.321	0.289	0.366	0.365	0.194	0.233	0.183	0.195	0.206	0.220	0.355	0.000								
Paria B	0.154	0.289	0.347	0.354	0.327	0.428	0.425	0.263	0.303	0.206	0.228	0.235	0.252	0.406	0.028	0.000							
Paria C	0.122	0.256	0.316	0.328	0.297	0.367	0.369	0.175	0.234	0.173	0.192	0.199	0.212	0.364	0.036	0.078	0.000						
Paria D	0.127	0.280	0.324	0.338	0.304	0.284	0.292	0.088	0.167	0.257	0.283	0.294	0.309	0.377	0.140	0.203	0.127	0.000					
Paria E	0.364	0.483	0.541	0.556	0.520	0.619	0.623	0.415	0.511	0.461	0.446	0.483	0.479	0.602	0.334	0.387	0.369	0.320	0.000				
Paria F	0.136	0.280	0.322	0.339	0.306	0.293	0.302	0.048	0.161	0.258	0.288	0.296	0.313	0.387	0.145	0.211	0.122	0.031	0.357	0.000			
Paria G (2002)	0.154	0.299	0.342	0.359	0.325	0.270	0.292	0.044	0.152	0.267	0.298	0.301	0.321	0.391	0.147	0.218	0.126	0.051	0.378	0.008	0.000		
Paria G (2010)	0.153	0.299	0.337	0.350	0.314	0.295	0.264	0.078	0.124	0.266	0.295	0.300	0.319	0.382	0.153	0.220	0.136	0.052	0.377	0.031	0.029	0.000	
Paria H	0.177	0.325	0.379	0.396	0.360	0.381	0.394	0.150	0.249	0.292	0.322	0.326	0.344	0.427	0.180	0.247	0.164	0.076	0.401	0.083	0.112	0.128	0.000

	Arima LP	Arima HP	Aripo LP	Aripo HP (2006)	Aripo HP (2010)	Curaguate	Damier LP (2005)	Damier LP (2009)	Damier LP (2010)	Damier HP (2005)	Damier HP (2009)	Damier HP (2010)	Diego Martin	El Cedro LP	El Cedro HP	Guanapo LP	Guanapo HP	La Seiva	Las Cuevas	Madamas 1	Madamas 2	Marianne A (2002)	Marianne A (2010)
Jordan A	0.325	0.152	0.271	0.151	0.147	0.137	0.153	0.140	0.137	0.152	0.145	0.139	0.249	0.397	0.199	0.177	0.231	0.163	0.127	0.131	0.202	0.111	0.099
Jordan B	0.341	0.163	0.280	0.170	0.162	0.135	0.169	0.155	0.154	0.172	0.161	0.155	0.272	0.408	0.209	0.181	0.239	0.177	0.131	0.140	0.221	0.114	0.102
Jordan C	0.369	0.186	0.310	0.185	0.180	0.197	0.180	0.179	0.170	0.173	0.173	0.169	0.272	0.408	0.238	0.223	0.277	0.185	0.181	0.177	0.232	0.169	0.158
Pitch Lake	0.350	0.135	0.243	0.140	0.134	0.198	0.152	0.157	0.151	0.143	0.141	0.137	0.224	0.384	0.210	0.209	0.256	0.139	0.161	0.177	0.209	0.158	0.137
Quare LP	0.377	0.145	0.277	0.155	0.150	0.228	0.165	0.168	0.164	0.154	0.159	0.148	0.261	0.417	0.232	0.232	0.272	0.169	0.201	0.144	0.162	0.163	0.160
Quare HP (2006)	0.286	0.084	0.215	0.089	0.092	0.142	0.108	0.108	0.104	0.100	0.102	0.089	0.185	0.325	0.151	0.142	0.191	0.094	0.114	0.071	0.108	0.091	0.086
Quare HP (2008)	0.320	0.116	0.232	0.109	0.115	0.179	0.134	0.135	0.127	0.124	0.124	0.108	0.195	0.363	0.192	0.192	0.231	0.137	0.149	0.088	0.100	0.122	0.117
Quare HP (2010)	0.290	0.107	0.197	0.080	0.080	0.142	0.111	0.101	0.096	0.104	0.099	0.088	0.179	0.366	0.181	0.163	0.205	0.125	0.109	0.087	0.116	0.095	0.082
Quare 3	0.300	0.098	0.219	0.102	0.103	0.157	0.120	0.120	0.114	0.106	0.111	0.101	0.191	0.338	0.176	0.171	0.211	0.118	0.130	0.093	0.110	0.112	0.105
Quare 4	0.376	0.173	0.290	0.161	0.167	0.199	0.174	0.169	0.163	0.169	0.162	0.160	0.214	0.429	0.249	0.243	0.286	0.189	0.187	0.178	0.194	0.186	0.174
San Souci	0.345	0.170	0.278	0.143	0.141	0.162	0.128	0.128	0.123	0.139	0.123	0.115	0.217	0.421	0.216	0.193	0.265	0.171	0.136	0.141	0.190	0.139	0.130
Shark	0.387	0.193	0.268	0.161	0.148	0.232	0.212	0.205	0.201	0.213	0.200	0.197	0.282	0.462	0.279	0.248	0.313	0.222	0.183	0.176	0.207	0.206	0.195
Tompire	0.372	0.178	0.320	0.194	0.187	0.187	0.175	0.172	0.169	0.174	0.169	0.169	0.282	0.402	0.177	0.175	0.241	0.166	0.170	0.184	0.246	0.160	0.158
Turure LP (2010)	0.353	0.159	0.340	0.223	0.212	0.207	0.201	0.201	0.206	0.202	0.191	0.203	0.314	0.348	0.143	0.113	0.200	0.050	0.194	0.208	0.282	0.179	0.171
Turure HP (2006)	0.290	0.089	0.265	0.153	0.136	0.159	0.137	0.144	0.143	0.126	0.131	0.133	0.246	0.283	0.102	0.065	0.154	0.014	0.136	0.144	0.214	0.132	0.121
Turure HP (2010)	0.295	0.083	0.267	0.152	0.138	0.166	0.154	0.157	0.154	0.143	0.143	0.139	0.246	0.317	0.110	0.077	0.121	0.024	0.149	0.153	0.217	0.135	0.119
Yarra LP (2006)	0.386	0.249	0.341	0.214	0.221	0.289	0.183	0.120	0.138	0.185	0.147	0.166	0.306	0.473	0.309	0.299	0.336	0.262	0.259	0.253	0.291	0.244	0.222
Yarra LP (2009)	0.409	0.277	0.364	0.246	0.255	0.313	0.226	0.153	0.175	0.227	0.192	0.214	0.341	0.488	0.340	0.320	0.359	0.292	0.281	0.283	0.328	0.268	0.248
Yarra LP (2010)	0.402	0.260	0.355	0.230	0.239	0.294	0.206	0.138	0.159	0.206	0.175	0.196	0.326	0.477	0.317	0.300	0.344	0.269	0.265	0.265	0.312	0.250	0.232
Yarra HP (2006)	0.310	0.138	0.239	0.116	0.102	0.160	0.033	0.032	0.029	0.022	0.012	0.017	0.190	0.363	0.187	0.185	0.240	0.139	0.134	0.146	0.186	0.142	0.125
Yarra HP (2009)	0.297	0.120	0.230	0.097	0.087	0.145	**0.038**	0.037	**0.026**	0.018	0.020	0.014	0.189	0.376	0.168	0.165	0.225	0.121	0.117	0.110	0.158	0.118	0.107
Yarra HP (2010)	0.317	0.160	0.249	0.127	0.122	0.176	0.058	0.056	0.053	0.061	0.043	0.044	0.201	0.386	0.208	0.195	0.247	0.163	0.145	0.152	0.193	0.150	0.138
Upper Yarra	0.364	0.207	0.286	0.189	0.185	0.242	0.142	0.128	0.130	0.137	0.128	0.120	0.262	0.383	0.244	0.248	0.296	0.217	0.211	0.212	0.253	0.217	0.201

	Marianne B	Marianne C	Marianne D (2002)	Marianne D (2008)	Marianne D (2010)	Marianne E (2002)	Marianne E (2010)	Marianne F (2002)	Marianne F (2010)	P. Marianne A	P. Marianne B (2002)	P. Marianne B (2010)	P. Marianne C	Mission	Paria A	Paria B	Paria C	Paria D	Paria E	Paria F	Paria G (2002)	Paria G (2010)	Paria H
Jordan A	0.102	0.236	0.291	0.301	0.270	0.371	0.369	0.189	0.237	0.160	0.169	0.169	0.189	0.342	0.036	0.086	0.032	0.145	0.348	0.136	0.141	0.149	0.161
Jordan B	0.107	0.246	0.308	0.321	0.291	0.365	0.362	0.177	0.224	0.180	0.191	0.197	0.217	0.359	0.037	0.086	0.026	0.130	0.345	0.126	0.131	0.139	0.156
Jordan C	0.163	0.277	0.320	0.331	0.295	0.435	0.430	0.259	0.306	0.227	0.239	0.239	0.254	0.342	0.134	0.204	0.115	0.191	0.406	0.189	0.193	0.177	0.215
Pitch Lake	0.150	0.245	0.275	0.290	0.253	0.393	0.390	0.259	0.300	0.214	0.241	0.239	0.252	0.313	0.197	0.241	0.211	0.208	0.424	0.213	0.218	0.213	0.250
Quare LP	0.172	0.259	0.306	0.316	0.277	0.495	0.492	0.332	0.382	0.259	0.280	0.282	0.298	0.325	0.224	0.258	0.240	0.273	0.485	0.277	0.280	0.276	0.298
Quare HP (2006)	0.093	0.188	0.223	0.233	0.202	0.370	0.368	0.236	0.278	0.171	0.193	0.200	0.208	0.264	0.138	0.175	0.148	0.182	0.351	0.183	0.187	0.185	0.197
Quare HP (2008)	0.132	0.218	0.247	0.259	0.225	0.414	0.410	0.274	0.321	0.188	0.209	0.212	0.222	0.273	0.169	0.204	0.178	0.220	0.393	0.220	0.222	0.218	0.234
Quare HP (2010)	0.092	0.188	0.228	0.241	0.203	0.395	0.391	0.235	0.282	0.156	0.182	0.188	0.194	0.281	0.130	0.165	0.146	0.185	0.370	0.185	0.189	0.188	0.207
Quare 3	0.116	0.198	0.239	0.248	0.215	0.393	0.390	0.257	0.300	0.177	0.200	0.203	0.214	0.256	0.152	0.183	0.161	0.207	0.381	0.207	0.212	0.208	0.226
Quare 4	0.180	0.246	0.290	0.300	0.266	0.476	0.473	0.334	0.378	0.264	0.279	0.282	0.293	0.338	0.228	0.269	0.240	0.278	0.465	0.276	0.283	0.280	0.303
San Souci	0.135	0.234	0.278	0.294	0.260	0.419	0.419	0.276	0.324	0.228	0.252	0.266	0.267	0.330	0.167	0.209	0.179	0.212	0.431	0.211	0.221	0.227	0.241
Shark	0.206	0.289	0.337	0.347	0.311	0.498	0.493	0.344	0.389	0.291	0.305	0.312	0.323	0.353	0.240	0.276	0.258	0.288	0.471	0.290	0.297	0.295	0.316
Tompire	0.158	0.269	0.336	0.350	0.311	0.462	0.461	0.292	0.348	0.268	0.291	0.295	0.309	0.372	0.155	0.208	0.159	0.223	0.443	0.230	0.238	0.237	0.260
Turure LP (2010)	0.166	0.290	0.335	0.347	0.314	0.452	0.461	0.313	0.369	0.297	0.322	0.340	0.340	0.391	0.221	0.264	0.221	0.242	0.456	0.256	0.264	0.269	0.285
Turure HP (2006)	0.108	0.229	0.265	0.279	0.246	0.410	0.406	0.262	0.310	0.235	0.260	0.269	0.274	0.326	0.167	0.209	0.170	0.192	0.400	0.205	0.219	0.212	0.234
Turure HP (2010)	0.108	0.219	0.244	0.255	0.219	0.426	0.421	0.274	0.324	0.246	0.268	0.264	0.282	0.331	0.185	0.228	0.189	0.200	0.424	0.214	0.229	0.224	0.248
Yarra LP (2006)	0.229	0.300	0.338	0.353	0.327	0.504	0.506	0.349	0.389	0.309	0.322	0.326	0.331	0.400	0.260	0.305	0.272	0.307	0.502	0.297	0.305	0.310	0.330
Yarra LP (2009)	0.256	0.333	0.362	0.378	0.352	0.505	0.506	0.369	0.401	0.336	0.350	0.355	0.359	0.423	0.285	0.324	0.295	0.327	0.504	0.322	0.328	0.328	0.349
Yarra LP (2010)	0.237	0.318	0.349	0.365	0.338	0.499	0.500	0.353	0.387	0.324	0.337	0.342	0.349	0.414	0.265	0.307	0.276	0.311	0.501	0.303	0.310	0.310	0.334
Yarra HP (2006)	0.125	0.212	0.251	0.265	0.233	0.425	0.419	0.260	0.306	0.228	0.248	0.253	0.263	0.288	0.166	0.211	0.176	0.202	0.421	0.197	0.210	0.203	0.231
Yarra HP (2009)	0.107	0.213	0.262	0.278	0.240	0.444	0.443	0.253	0.308	0.215	0.237	0.241	0.254	0.304	0.141	0.190	0.151	0.188	0.449	0.185	0.194	0.194	0.213
Yarra HP (2010)	0.139	0.214	0.260	0.272	0.242	0.420	0.417	0.275	0.318	0.236	0.256	0.260	0.268	0.292	0.177	0.221	0.189	0.215	0.421	0.214	0.225	0.222	0.244
Upper Yarra	0.209	0.294	0.321	0.338	0.311	0.440	0.438	0.328	0.367	0.290	0.303	0.310	0.316	0.356	0.240	0.277	0.250	0.268	0.442	0.273	0.287	0.282	0.292

	Jordan A	Jordan B	Jordan C	Pitch Lake	Quare LP	Quare HP (2006)	Quare HP (2008)	Quare HP (2010)	Quare 3	Quare 4	San Souci	Shark	Tompire	Turure LP (2010)	Turure HP (2006)	Turure HP (2010)	Yarra LP (2006)	Yarra LP (2009)	Yarra LP (2010)	Yarra HP (2006)	Yarra HP (2009)	Yarra HP (2010)	Upper Yarra
Jordan A	0.000																						
Jordan B	**0.006**	0.000																					
Jordan C	0.079	0.088	0.000																				
Pitch Lake	0.180	0.200	0.192	0.000																			
Quare LP	0.198	0.217	0.212	0.136	0.000																		
Quare HP (2006)	0.115	0.133	0.141	0.091	0.058	0.000																	
Quare HP (2008)	0.145	0.164	0.152	0.108	0.043	0.024	0.000																
Quare HP (2010)	0.113	0.133	0.151	0.098	0.060	0.030	0.025	0.000															
Quare 3	0.130	0.149	0.143	0.098	0.038	0.021	**0.004**	0.024	0.000														
Quare 4	0.210	0.229	0.222	0.157	0.108	0.087	0.067	0.081	0.060	0.000													
San Souci	0.161	0.172	0.212	0.182	0.205	0.126	0.144	0.108	0.136	0.202	0.000												
Shark	0.230	0.246	0.270	0.222	0.200	0.127	0.161	0.137	0.144	0.209	0.177	0.000											
Tompire	0.148	0.155	0.187	0.209	0.230	0.150	0.187	0.161	0.168	0.236	0.165	0.251	0.000										
Turure LP (2010)	0.202	0.206	0.241	0.221	0.263	0.158	0.221	0.198	0.200	0.271	0.220	0.291	0.205	0.000									
Turure HP (2006)	0.146	0.155	0.181	0.158	0.189	0.106	0.156	0.132	0.136	0.208	0.159	0.219	0.160	0.038	0.000								
Turure HP (2010)	0.160	0.172	0.191	0.154	0.184	0.106	0.151	0.131	0.127	0.198	0.170	0.224	0.165	0.084	0.040	0.000							
Yarra LP (2006)	0.244	0.268	0.282	0.241	0.265	0.203	0.225	0.196	0.212	0.270	0.263	0.304	0.292	0.327	0.256	0.261	0.000						
Yarra LP (2009)	0.271	0.291	0.308	0.276	0.303	0.235	0.261	0.231	0.248	0.307	0.296	0.332	0.319	0.348	0.283	0.289	0.032	0.000					
Yarra LP (2010)	0.250	0.269	0.290	0.260	0.285	0.217	0.243	0.215	0.231	0.290	0.278	0.318	0.296	0.326	0.261	0.266	0.027	**0.001**	0.000				
Yarra HP (2006)	0.150	0.166	0.180	0.146	0.174	0.105	0.130	0.105	0.117	0.170	0.126	0.209	0.176	0.195	0.128	0.149	0.162	0.218	0.201	0.000			
Yarra HP (2009)	0.122	0.141	0.164	0.132	0.147	0.077	0.102	0.079	0.092	0.160	0.115	0.188	0.155	0.188	0.113	0.132	0.158	0.203	0.184	**0.012**	0.000		
Yarra HP (2010)	0.165	0.179	0.203	0.163	0.188	0.115	0.138	0.116	0.127	0.185	0.117	0.203	0.183	0.223	0.158	0.166	0.178	0.224	0.207	0.044	0.036	0.000	
Upper Yarra	0.226	0.238	0.263	0.221	0.238	0.173	0.199	0.177	0.190	0.247	0.188	0.268	0.249	0.264	0.206	0.220	0.204	0.240	0.228	0.128	0.135	0.105	0.000

Appendix 8: Matrix of estimated pairwise F_{ST} values for all sites and temporal replicates in the Marianne and Paria Rivers.

F_{ST}	Marianne M-A (2002)	Marianne M-A (2010)	Marianne M-B	Marianne M-C	Marianne M-D (2002)	Marianne M-D (2008)	Marianne M-D (2010)	Marianne M-E (2002)	Marianne M-E (2010)	Marianne M-F (2002)	Marianne M-F (2010)	P. Mar. PM-A	P. Mar. PM-B (2002)	P. Mar. PM-B (2010)	P. Mar. PM-C	Paria P-A	Paria P-B	Paria P-C	Paria P-D	Paria P-E	Paria P-F	Paria P-G (2002)	Paria P-G (2010)	Paria P-H	Jordan J-A	Jordan J-B
Marianne M-A (2010)	0.02	0.00																								
Marianne M-B	0.03	0.02	0.00																							
Marianne M-C	0.14	0.14	0.12	0.00																						
Marianne M-D (2002)	0.22	0.20	0.18	0.15	0.00																					
Marianne M-D (2008)	0.23	0.21	0.19	0.16	0.01	0.00																				
Marianne M-D (2010)	0.20	0.18	0.15	0.13	0.02	0.01	0.00																			
Marianne M-E (2002)	0.37	0.34	0.37	0.48	0.53	0.54	0.51	0.00																		
Marianne M-E (2010)	0.37	0.35	0.37	0.48	0.53	0.54	0.51	0.16	0.00																	
Marianne M-F (2002)	0.22	0.19	0.20	0.35	0.39	0.41	0.37	0.31	0.33	0.00																
Marianne M-F (2010)	0.27	0.24	0.26	0.40	0.44	0.45	0.42	0.39	0.30	0.15	0.00															
P. Mar. PM-A	0.17	0.10	0.15	0.29	0.35	0.37	0.33	0.48	0.48	0.30	0.35	0.00														
P. Mar. PM-B (2002)	0.21	0.15	0.20	0.34	0.36	0.38	0.34	0.49	0.49	0.34	0.38	0.06	0.00													
P. Mar. PM-B (2010)	0.22	0.16	0.21	0.34	0.37	0.38	0.34	0.49	0.48	0.34	0.38	0.11	0.07	0.00												
P. Mar. PM-C	0.23	0.16	0.21	0.35	0.38	0.40	0.36	0.52	0.51	0.36	0.40	0.05	0.00	0.07	0.00											
Paria P-A	0.13	0.10	0.11	0.25	0.31	0.32	0.29	0.37	0.36	0.19	0.23	0.18	0.20	0.21	0.22	0.00										
Paria P-B	0.17	0.13	0.15	0.29	0.35	0.35	0.33	0.43	0.43	0.26	0.30	0.21	0.23	0.24	0.25	0.03	0.00									
Paria P-C	0.13	0.11	0.12	0.26	0.32	0.33	0.30	0.37	0.37	0.18	0.23	0.17	0.19	0.20	0.21	0.04	0.08	0.00								
Paria P-D	0.15	0.13	0.13	0.28	0.32	0.34	0.30	0.28	0.29	0.09	0.17	0.26	0.28	0.29	0.31	0.14	0.20	0.13	0.00							
Paria P-E	0.37	0.34	0.36	0.48	0.54	0.56	0.52	0.62	0.62	0.42	0.51	0.46	0.45	0.48	0.48	0.33	0.39	0.37	0.32	0.00						
Paria P-F	0.16	0.13	0.14	0.28	0.32	0.34	0.31	0.29	0.30	0.05	0.16	0.26	0.29	0.30	0.31	0.14	0.21	0.12	0.03	0.36	0.00					
Paria P-G (2002)	0.17	0.15	0.15	0.30	0.34	0.36	0.33	0.27	0.29	0.04	0.15	0.27	0.30	0.30	0.32	0.15	0.22	0.13	0.05	0.38	0.01	0.00				
Paria P-G (2010)	0.17	0.15	0.15	0.30	0.34	0.35	0.31	0.30	0.26	0.08	0.12	0.27	0.29	0.30	0.32	0.15	0.22	0.14	0.05	0.38	0.03	0.03	0.00			
Paria P-H	0.18	0.17	0.18	0.33	0.38	0.40	0.36	0.38	0.39	0.15	0.25	0.29	0.32	0.33	0.34	0.18	0.25	0.16	0.08	0.40	0.08	0.11	0.13	0.00		
Jordan J-A	0.11	0.10	0.10	0.24	0.29	0.30	0.27	0.37	0.37	0.19	0.24	0.16	0.17	0.17	0.19	0.04	0.09	0.03	0.14	0.35	0.14	0.14	0.15	0.16	0.00	
Jordan J-B	0.11	0.10	0.11	0.25	0.31	0.32	0.29	0.36	0.36	0.18	0.22	0.18	0.19	0.20	0.22	0.04	0.09	0.03	0.13	0.35	0.13	0.13	0.14	0.16	0.01	0.00
Jordan J-C	0.17	0.16	0.16	0.28	0.32	0.33	0.29	0.44	0.43	0.26	0.31	0.23	0.24	0.24	0.25	0.13	0.20	0.11	0.19	0.41	0.19	0.19	0.18	0.22	0.08	0.09