Expansion of frozen hybrids in guppy ectoparasite, *Gyrodactylus turnbulli*

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Abstract

Hybridization is one of the major factors contributing to the emergence of highly successful parasites. Hybrid vigor can play an important role in this process, but its contribution to the evolutionary success of parasites has not been demonstrated. Here, we identify an invasion of a "frozen hybrid" genotype in natural populations of *Gyrodactylus turnbulli*, ectoparasitic flatworms with facultative sexual reproduction and significant impact on cultured fish. We re-sequenced genomes of these parasites infecting guppies from six Trinidad and Tobago populations, and found surprisingly high discrepancy in genome-wide nucleotide diversity between islands. The elevated heterozygosity on Tobago is maintained by predominantly clonal reproduction of hybrids formed from two diverged genomes. Hybridization has been followed by spread of the hybrids across the island, implying a selective advantage compared to native genotypes. Our results highlight the potential consequences of heterosis in the epidemiology of parasite hybrids and its impact on population genomic diversity.

Introduction

Hybridization can be a potent evolutionary force, creating opportunities for adaptive evolution by increasing genetic variation on which natural selection can act (Arnold, 1997; Oziolor et al., 2019; Rieseberg et al., 2003; Taylor and Larson, 2019). The ecological and evolutionary consequences are potentially large, and not just for the hybrid organism itself: such hybridization may, for example, exert strong influence on the evolution of pathogenic organisms ('parasites' henceforth) (King et al., 2015). This role is likely to increase in importance because human activities increase opportunities for hybridization by inducing environmental changes and incidental translocations (Brooks and Hoberg, 2007; Cable et al., 2017; Patz et al., 2000). Understanding the role of hybridization in parasite evolution is therefore essential if we are to predict hot spots of disease risk and emergence (Laine et al., 2019).

Parasite strains of hybrid origin can gain an advantage because recombinants produced after hybridization may show enhanced phenotypic characteristics compared to the parental generation, such as higher infectivity (Grigg et al., 2001), expanded host range (Detwiler and Criscione, 2010), increased transmission potential (Volf et al., 2007) or increased tolerance to harsh environmental conditions (Laine et al., 2019). The probability of hybridization increases when species co-infect the same host, and such systems may be considered hotspots for studying its phenotypic effects. However, hybridization is often associated with asexual reproduction, precluding benefits resulting from recombination between divergent genomes. Asexual reproduction is thought to be enforced by Bateson-Dobzhansky-Muller incompatibilities accumulated in parental linages (Neaves and Baumann,

2011). The genomes of parental species, divergent enough to disrupt meiosis in hybrids, yet not diverged enough to seriously affect hybrid viability, could drive asexual reproduction of hybrids (Janko et al., 2018; Moritz et al., 1989). In fact, many asexually reproducing organisms have originated from hybridization events (Neaves and Baumann, 2011).

Conversely, asexual reproduction may be selectively beneficial if clonal reproduction "freezes" heterozygosity that confers short-term evolutionary advantage (Neiman et al., 2014). Hybrids often show greater vigor, productivity, disease resistance, or overall increased Darwinian fitness (Kingsbury, 2009). Two alternative hypotheses may explain the existence of hybrid vigor (heterosis) (Lippman and Zamir, 2007). The overdominance hypothesis, attributes heterosis to superior fitness of heterozygous genotypes over homozygous wild types. The dominance hypothesis assumes that recessive deleterious mutations are to some extent masked in a heterozygous stage. Recent theoretical work indicates that dominance-induced heterosis might play an important role in evolution, especially if deleterious alleles are not removed effectively from populations as is expected when populations are small (Gilbert et al., 2020; Kim et al., 2018; Zhao and Charlesworth, 2016). This mechanism may be particularly relevant to parasite evolution. Many parasite populations are small or experience frequent bottlenecks, therefore they are prone to accumulation of deleterious mutations (Lynch et al., 1993), but also to elevated homozygosity due to inbreeding. In such case, one could expect higher fitness of hybrid parasites. Clonal reproduction can then preserve heterosis in so called 'frozen hybrids' and facilitate expansion of successful parasites. However, a case of successful frozen hybrid parasites has not, to our knowledge, been demonstrated.

Here, we report genomic analysis of the guppy parasite, *Gyrodactylus turnbulli*, which allowed us to infer the expansion of a predominantly clonal hybrid strain in a species otherwise reported to regularly reproduce sexually (Schelkle et al., 2012). Interactions between gyrodactylids and guppies have been the subject of intensive eco-evolutionary research focusing on coevolution between host and parasites (e.g. Houde & Torio, 1992; Jacquin et al., 2016; Phillips et al., 2018; Reynolds, Arapi, & Cable, 2018; van Oosterhout, Harris, & Cable, 2003), while the effect of different reproductive strategies (Cable and Harris, 2002) on population structure and dynamics of infections in the parasite wild populations are poorly understand (Xavier et al., 2015). Our earlier work on another monogenean guppy parasite, *G. bullatarudis*, identified the potential of recombination between divergent strains in the emergence of invasive parasites (Konczal et al., in press). In contrast, the results reported here indicate the importance of heterosis in rapid clonal invasions of *G. turnbulli* and highlight that hybridization can lead to the emergence of highly successful parasites, despite suppressing recombination.

85 **Results**

86 A total of 30 *G. turnbulli* samples from three Trinidadian (Aripo, Caura and Lopinot Rivers) and three
87 Tobagonian populations (Dog River, Health Center and Spring Site; Figure 1A, details in
88 Supplementary Table 1) collected in two consecutive years were sequenced to an average coverage
89 of 38.7 per sample. Sequencing reads were then mapped to the reference genome and used for SNP
90 calling. We identified 776,881 single nucleotide- and 161,255 indel-polymorphisms. Principal
91 Component Analyses of SNP genotypes clearly separated Trinidad and Tobago populations, while
92 differentiation between local rivers was present only in some cases (Figure 1B). In particular, samples
93 from Health Center and Dog River (Tobago) showed virtually no differentiation and, in contrast to the
94 Trinidadian populations, do not form monophyletic groups according to the populations (Figure 1C).
95 The Trinidadian samples are also more similar to the reference genome, obtained from a strain on
96 commercial guppies, *Gt3,* than samples collected from Tobago (Figure 1C). To remove the potential
97 effect of linkage on population differentiation, we pruned linked SNPs, but the general pattern
98 remained the same (Supplementary Figure 1). Furthermore, a similar pattern was observed with
99 ADMIXTURE analyses: the most likely number of genetic groups is two, with pronounced distinction
100 between the Trinidad and Tobago samples (Supplementary Figure 2). We calculated F_{ST} between
101 Trinidad and Tobago populations in 25 kb non-overlapping windows and found that 90% of the
102 windows show F_{ST} values between 0.25 and 0.55 (mean F_{ST} = 0.42; Supplementary Figure 3). Pairwise
103 F_{ST} values were higher among Trinidadian populations (Supplementary Figures 4) than among
104 populations from Tobago (Supplementary Figures 5). To resolve whether moderate F_{ST} between
105 islands resulted from moderate diversification or from elevated level of genetic variation within
106 groups, we calculated nucleotide diversity across Trinidad and Tobago samples separately.
107 Nucleotide diversity on Tobago was 2.4 times greater than that for Trinidad (0.0028 and 0.0012
108 respectively, Figure 1D). The difference does not result from high differentiation among Tobagonian
109 populations: nucleotide diversity was also higher within Tobagonian populations, compared with
110 those from Trinidad (Supplementary Figure 6).

111 Increased nucleotide diversity on Tobago was mainly driven by high heterozygosity of most
112 individuals (Figures 1D/E). Similarly, inbreeding coefficient, F_{IS}, was negative for most of the samples
113 from Tobago, suggesting over-representation of heterozygous positions (Supplementary Figure 7). In
114 contrast, most of the Trinidadian samples showed positive values of F_{IS}.

115 High heterozygosity was observed in all samples from two populations on Tobago (Health Center and
116 Dog River), whereas the third population (Spring Site) showed a mixture of patterns. One Spring Site
117 sample (G334) had low level of heterozygosity, reminiscent of the general pattern observed in
118 Trinidadian samples. Three other samples (G328, G367 and G368) showed bimodal distribution, with

119 genomic regions presenting either high or low level of heterozygosity. Finally, two Spring Site
120 samples (G331 and G370) contained only highly heterozygous regions, similar to the pattern
121 observed in Health Center and Dog River (Figure 1E).

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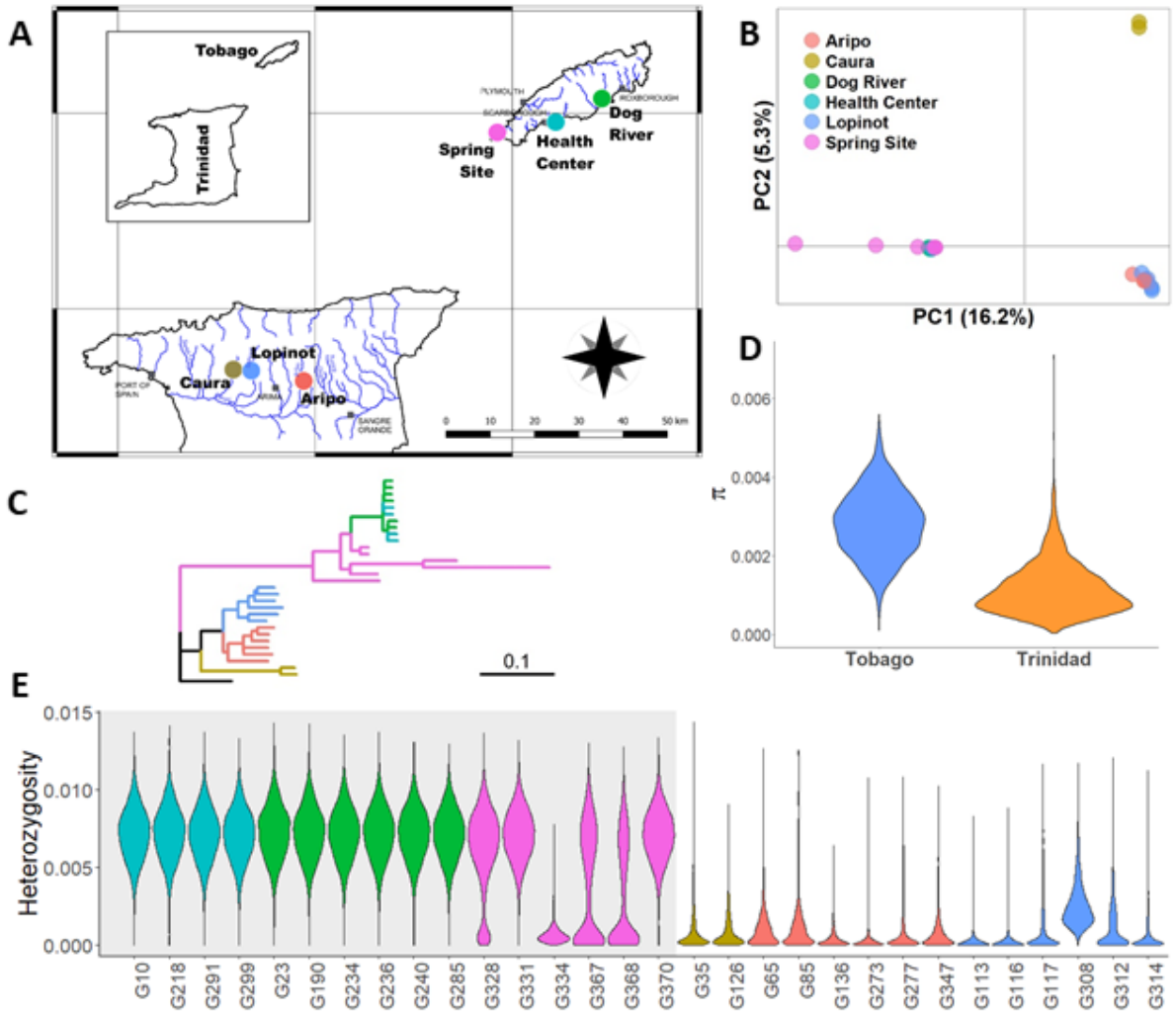

123

124 **Figure 1 Patterns of genetic variation in *Gyrodactylus turnbulli* populations from Trinidad and**
125 **Tobago. A:** Map of Trinidad and Tobago with sampling sites. **B:** Principal Component Analyses of *G.*
126 *turnbulli* samples. PCA based on 673,104 SNPs which passed filtering criteria. SNPs were identified
127 within 30 isolates from three Trinidadian and three Tobagonian populations. **C:** Neighbor Joining tree
128 (Saitou and Nei 1987) constructed using distance matrix, with one minus identity-by-state units,
129 calculated based on 673,104 SNPs. Populations are represented by different colours. The reference
130 sequence (obtained from a strain on commercial guppies) is shown in black. **D:** Distribution of
131 nucleotide diversity (π) calculated across the genome in 25 kb non-overlapping windows. **E:**
132 Distribution of heterozygosity calculated in 25 kb non-overlapping windows for each sample
133 collected from Trinidad (white background) and Tobago (shaded background) populations.

The majority of polymorphic Tobagonian SNPs (325,473 out of 498,111) were heterozygous in Health Center, Dog River,and two (G331 and G370) Spring Site samples, and 88% of these SNPs contained an allele from the reference genome and its alternative found in the Tobagonian G334 sample (Figure 2). Genome-wide pattern of divergence between re-sequenced samples and the reference genome demonstrated that G334 sample is the most divergent, while all samples from Trinidad are more closely related to the reference genome (Figure 1C, Supplementary Figure 8). Based on these patterns we infer that highly heterozygous Tobagonian samples are hybrids between two divergent haplotypes: one similar to the reference sequence and the second similar to the G334 sequence. The same pattern with shared heterozygous sites and high, genome-wide heterozygosity was observed in the Health Center and Dog River samples from 2017 and 2018, indicating that the hybrid population is stable, and implying an absence of sexual reproduction (otherwise recombination would have fixed one of the two haplotypes in at least some genomic regions). In contrast, divergent haplotypes have clearly recombined in the Spring Site population. Three samples (G328, G367 and G368) show a mosaic pattern of the two haplotypes fixed in different genomic locations, with other parts of the genome still remaining heterozygous (Figure 2, Supplementary Figure 9).

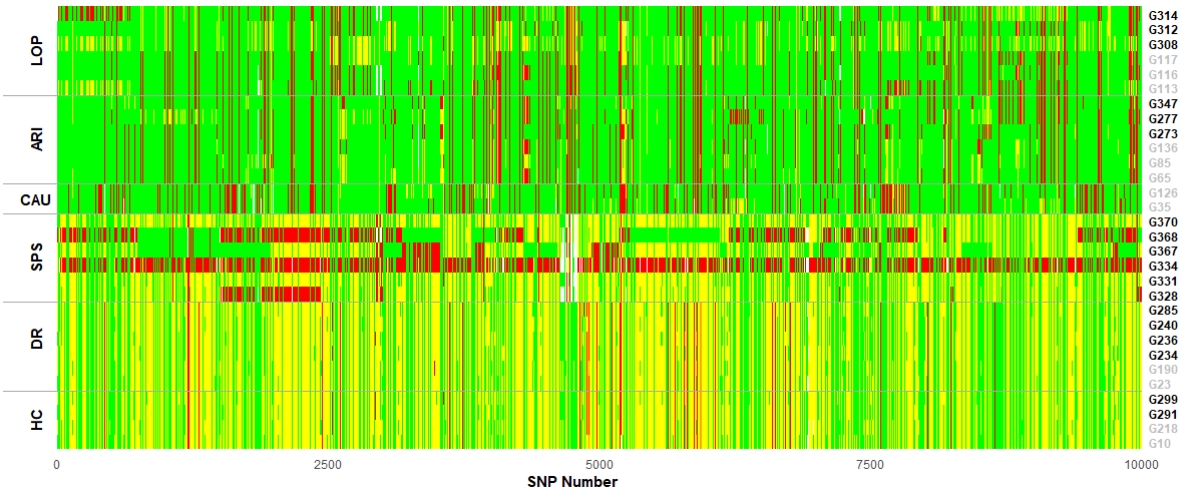

Figure 2. Graphical representation of genotypes in *Gyrodactylus turnbulli* samples. Genotypes identified as reference homozygotes are shown in green, alternative homozygotes in red, heterozygous in yellow. Samples collected in 2017 and 2018 are labeled in grey and black respectively, and those collected from different local populations are separated by horizontal grey lines. A subset of polymorphic sites (10,000 SNPs) from the longest scaffolds is shown.

158 Signatures of sexual reproduction are also observed at least in some of the samples from Trinidad.
159 For example, SNPs localized in the first scaffold show admixture of another haplotype in sample G314
160 coming from Lopinot, and another two samples in Lopinot are heterozygous in this region (Figure 2).
161 Another sample from the same population (G308) has negative F_{IS} (Supplementary Figure 7) and
162 slightly elevated genome-wide heterozygosity (Figure 1D), although the effect is weaker compared to
163 samples from Tobago. Nevertheless, heterozygous sites are on average rare in Trinidadian samples.

164 Clustering analyses (Figure 1B, 1C) and F_{ST} results (Supplementary Figure 5) suggest low divergence
165 between samples identified as hybrids. We explored it further by calculating divergence in the
166 haploid mitochondrial genomes. One Trinidadian sample (G126) was used for mitochondrial genome
167 assembly, and then reads from all other samples were mapped to that genome for haplotype
168 reconstruction. The resulting mt genome spans 14,159 bp. We found very little divergence between
169 samples identified as hybrids, compared to divergence amongst other samples (Figure 3,
170 Supplementary Figure 10). Putative hybrid samples from Health Center, Dog River and Spring Site
171 populations were found more closely related to Trinidadian samples than to remaining SPS samples,
172 confirming introgression inferred from genomic data. Within hybrids (Health Center, Dog River two
173 hybrid samples from Spring Site) the mean divergence between populations was 0.02%. In contrast,
174 three other samples collected from Spring Site had strongly divergent mitochondria - the nucleotide
175 divergence between the two Spring Site groups was 1.58%. To independently verify low divergence
176 between Tobagonian hybrids from Tobago and samples from Trinidad we sequenced a 262 bp
177 fragment of COII from 81 samples collected by us on Trinidad and Tobago. The results confirmed
178 little divergence between all Tobagonian samples except for some derived from the Spring Site
179 population (Supplementary Figure 11).

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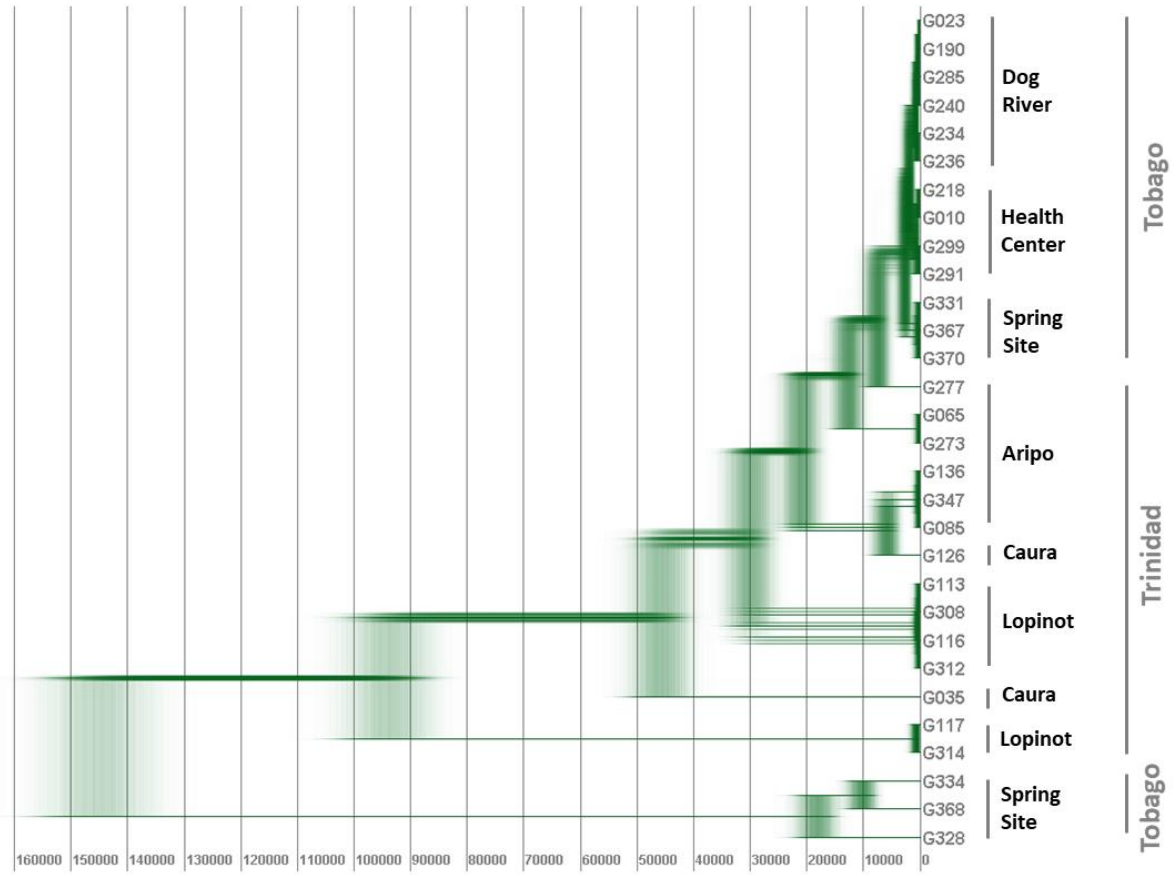

Figure 3. Phylogenetic relationship between *Gyrodactylus turnbulli* samples, reconstructed based on mitochondrial genome sequences (14,159 bp). The trees were derived via a Bayesian phylogenetic method, using 10,000,000 generations of MCMC samples, with HKY substitution model and strict clock model, assuming 13.1% divergence per million years. The estimated time before present (in years) for tree nodes is shown at the bottom of the tree.

We compared genome wide nucleotide diversity of Tobagonian samples collected in 2017 and 2018 and did not find evidence for a substantial difference between time points (π_{2017} = 0.0018 vs π_{2018} = 0.0017). We also plotted relationships between heterozygosity calculated in non-overlapping windows for each pair of samples and did not find any indication for reduction in heterozygosity in any sample (Supplementary Figure 12). Little variation between years in both nucleotide diversity and heterozygosity demonstrate that the populations from Tobago reproduce mostly clonally.

The mostly clonal reproduction in Tobago hampers identification of fast evolving regions in the genome, because of strong linkage between sites. However, there was no evidence that the Trinidadian populations experienced clonal expansion, and therefore rapidly evolving genes can be potentially identified. For this reason, we tested for correlations between nucleotide diversity

198 calculated in windows separately for Tobagonian hybrid populations and for all Trinidadian samples.
199 We used nucleotide diversity calculated jointly for Health Center and Dog River samples as a proxy
200 for the long-term evolution of genomic regions (divergence between two haplotypes forming
201 hybrids). Regions in which the nucleotide diversity calculated jointly for all samples from Trinidad
202 deviates from predictions based on this long-term evolutionary rate are therefore candidates for
203 recent rapid evolutionary change between years or populations. The divergence calculated in 25 kb
204 windows were highly correlated between Tobagonian hybrid populations and Trinidadian
205 populations, while few windows demonstrated elevated (according to overall trend) diversity in
206 Trinidad (Figure 4A). One scaffold (scf7180000445747; 19.4 kb long) showed extensive divergence
207 between Trinidadian samples collected in 2017 and 2018 (Figure 4B). It contains 7 protein coding
208 genes, including putative methylotransferase NSUN5, Endonuclease/exonuclease/phosphatase
209 family domain-containing protein 1, synaptogyrin and prohibitin.

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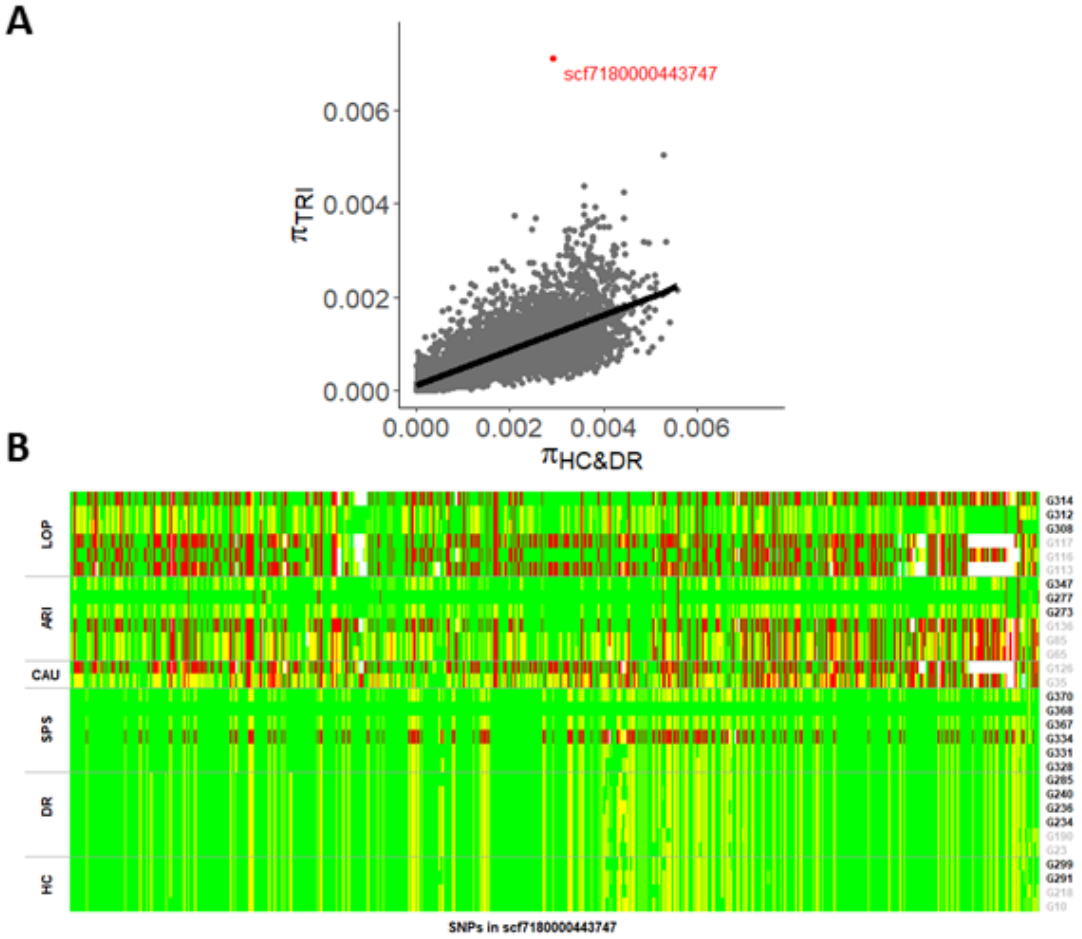

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212 **Figure 4 Genetic variation in genomic region with elevated nucleotide diversity in populations from**
213 **Trinidad. A:** Correlation between nucleotide diversity calculated jointly for Health Center and Dog

River populations and nucleotide diversity calculated jointly for all samples from Trinidad. Black line represents linear regression (y = 0.00 + 0.38*x). Scaffold scf7180000443747 is shown in red. **B:** Graphical representation of genotypes in polymorphic sites of *Gyrodactylus turnbulli* localized in scaffold scf7180000443747 (707 SNPs). Genotypes identified as reference homozygotes are shown in green, alternative homozygotes in red, heterozygous in yellow. Samples collected in 2017 and 2018 are shown in grey and black respectively, and those collected from different local populations are separated by horizontal grey lines.

Discussion

We examined whole genomes of 30 *G. turnbulli* parasites, sampled from two Caribbean islands in two consecutive years, providing insight into the evolution of their genomic diversity. On Tobago, parasite populations tend to have much higher nucleotide diversity than those on Trinidad. This higher diversity was caused by the presence of highly heterozygous individuals in all studied Tobagonian populations. These individuals shared most of the heterozygous loci, with a repeatable pattern of having one reference-like allele and the other allele present in a homozygous state only in one individual from Tobago (G334 sample). This pattern led us to conclude that these highly heterozygous individuals are hybrids of two divergent lineages, one of which is close to a strain isolated from ornamental guppies (Trinidad-like, used for the reference genome assembly), and the second which is close to the Tobagonian G334 sample, suggesting that these two lineages originated from different islands. In the G334 population of origin (Tobago, Spring Site) two considerably diverged mitochondrial genomes (diverged 145,000 years ago based on 13.1% divergence per Mya, (Kuusela et al., 2007), 95% HPD: 133,900 – 155,700) were observed. Most likely they represent mitochondrial genomes originating from two parental populations that formed Tobagonian hybrids. The mitochondrial divergence between these two lineages was much larger than between Tobagonian hybrids and Trinidadian populations (7,600 years, 95% HPD: 5,200 – 9,800 years), demonstrating that hybrids inherited mitochondria from Trinidad-like ancestors. Initial hybridization must have been followed by predominantly clonal reproduction, as otherwise recombination would produce both homozygous and heterozygous genotypes at least in some of the genomic regions. This was not the case: heterozygosity was elevated genome-wide (Figure 1D) and heterozygous genotypes were the same for all samples (Figure 2). Genetic divergence between putative hybrids as inferred based on whole mitochondrial genomes was low. The mitochondrial divergence corresponds to 2,700 (95% HPD: 1,500 – 4,000) years of divergence between these samples (Figure 3), indicating that clonally reproducing hybrids spread relatively recently across Tobago. The divergence times are

247 based on the indirectly derived substitution rate in a single mitochondrial locus of *Gyrodactylus*
248 *salaris* (13.1% per Mya; Kuusela et al., 2007), and other indirect estimates suggest lower (5.1% per
249 Mya; Hahn et al., 2015) or higher divergence rate (up to 20.3% per Mya; Meinilä et al., 2004) in
250 *Gyrodactylus* species, and therefore divergence dating should be taken with caution. Nevertheless,
251 little nucleotide divergence between mitochondrial genomes indicates a recent origin of the hybrids,
252 and their widespread occurrence across Tobago provides the evidence for their evolutionary success.
253 Hybrid genomes that were most widespread on Tobago did not show any signs of recombination,
254 and apparently remained stable. This suggests that the selective advantage of the hybrids might have
255 arose due to heterosis. Thus, our results suggest that hybrid vigor can have dramatic consequences
256 for the evolution of natural parasite populations.

257 Hybridization of parasites can lead to the emergence of highly invasive, virulent strains and therefore
258 is an emerging public health and conservation concern (King et al., 2015). Human activity, migrations
259 and climate change shift the geographic distribution of many species (Brooks and Hoberg, 2007;
260 Cable et al., 2017; Lafferty, 2009), which increases the probability of contact between distinct
261 populations, potentially leading to hybridization followed by adaptive evolution. Parasites are often
262 transported with their hosts, and even if hosts do not contribute to genetic pools of local
263 populations, hitchhiked parasites can do so. This could be the case for *G. turnbulli*, a common guppy
264 parasite. *Gyrodactylus* species have no free-living stage and are transmitted primarily through direct
265 physical contact between hosts. Our approximate dating of divergence of mtDNA between hybrids
266 from Trinidad and Tobago is consistent with the end of the last glacial maximum, when a land bridge
267 that probably existed between the islands during glaciation-related sea level decline was disrupted
268 again (Alexander et al., 2006; Lambeck, 2004). This might have facilitated migration of guppies
269 between the islands, and possibly other fish species capable of spreading *G. turnbulli* (Cable et al.,
270 2013; King and Cable, 2007). Alternatively, human activity could increase the potential for
271 hybridization between divergent gyrodactylid strains. Guppies are commonly kept in aquaria, and *G.*
272 *turnbulli* are regularly reported in aquarium populations (Maceda-Veiga and Cable, 2019) from where
273 they can potentially be released to nature. The potential to increase virulence of *G. turnbulli* by
274 combining different genomes was suggested in an earlier report of laboratory experiments, in which
275 infection with two inbred stains led to higher parasite load compared to single strain infections
276 (Schelkle et al., 2012). However, the genomic divergence between these strains was unknown and, as
277 discussed by the authors, this result could be due to competition between clones leading to
278 evolution of increased virulence (Schelkle et al., 2012).

279 Our recent study on the natural populations of another monogenean parasite infecting guppies
280 (*Gyrodactylus bullatarudis*) suggested that recent hybridization has also played a predominant role in

281 shaping genetic variation, but in that case in populations from Trinidad (Konczal et al., in press).
282 Hybridization between two diverged linages and its subsequent recombination resulted in a mosaic
283 genome composition of *G. bullatarudis*. Around half of the genome originates from one or another
284 lineage. High divergence between recombined lineages and relatively little divergence between local
285 populations led us to conclude that recent hybridization was followed by rapid expansion of a
286 recombinant *G. bulllatarudis* strain. Similar patterns of successful admixed hybrid genotypes have
287 been reported for many other parasites, including schistosomes (Kincaid-Smith et al., 2019; Platt et
288 al., 2019), *Trypanosoma* (see Tihon et al., 2017) and *Leishmania* (see Rogers et al., 2014) species,
289 highlighting the role of recombination between divergent genomes in parasite evolution. Our
290 analysis of *G. turnbulli* genomes indicates, that unlike its congener *G. bullatarudis,* natural expansion
291 occurred without prior recombination, highlighting that invasive hybrid parasites can arise without
292 recombination. So far, there has been little evidence for clonal expansion of hybrid parasites. An
293 interesting suggestion comes from *Gyrodactylus salaris* – a significant pathogen of Atlantic salmon.
294 The Baltic-salmon specific *G. salaris* could have originated from a single hybridization event between
295 two clades, followed by the clonal reproduction and frozen heterozygosity, however this suggestion
296 is based on analyses of single nuclear marker (Kuusela et al., 2007). Indirect observations in other
297 systems also suggest that non-recombined hybrids might have significant evolutionary advantage.
298 Hybridization between schistosomes - parasites of great medical and veterinary importance - results
299 in higher fecundity, faster maturation time, and wider intermediate host range, although this
300 evidence is based only on laboratory experiments (Wright and Ross, 1980). Another potential
301 example of non-recombined hybrid advantage occurs in a common fungal parasite of amphibians,
302 *Batrachochytrium dendrobatidis,* in which asexually reproducing hybrids have higher virulence than
303 both parental strains in some host species (Greenspan et al., 2018). However, no clear evidence for
304 expansion of these asexually reproducing hybrids has yet emerged.

305 Heterosis arising from hybridization between species or strains is known for traits other than
306 pathogen invasiveness, and is a widespread phenomenon in plant and animal species (Lippman and
307 Zamir, 2007). For example, spontaneous heterosis has been demonstrated in European water frog
308 hybrids, increasing survival and growth rate in F1 hybrids. These frog hybrids reproduce asexually,
309 freezing heterozygosity, which may contribute to their ecological success (Hotz et al., 1999). Another
310 example, freshwater snails, has been used to demonstrate that hybrid vigor can be more important
311 than an increase in genetic variance in the evolution of invasiveness (Facon et al., 2005). Our results
312 highlight that heterosis could influence epidemiological dynamics. In such case, heterosis may then
313 interplay with other factors influencing parasite distribution, including for example

314 metapopulational structure (Tadiri et al., 2018), host density (Johnson et al., 2011) and host
315 immunological gene repertoire (Phillips et al., 2018).

316 Parasites may be particularly prone to accumulation of deleterious mutations in their genomes
317 (Criscione and Blouin, 2005). In general, the rate of accumulation depends primarily on the effective
318 population size. In small populations, random genetic drift can overpower selection making it easier
319 for deleterious mutations, particularly those of small effects, to become fixed (Kimura et al., 1963).
320 This can cause mutational melt-down leading to population extinction (Lynch and Gabriel, 1990).
321 Several features of macroparasite life cycles can act in concert to reduce effective population sizes
322 and increase the risk of such melt-down (Criscione and Blouin, 2005). In particular, frequent
323 bottlenecks associated with colonization/recolonization of new hosts can significantly reduce the
324 efficiency of selection in removing mildly deleterious mutations from populations. Additionally,
325 observations of *G. turnbulli* in the wild suggest that census population sizes are often small and
326 fluctuating over time (Stephenson et al., 2015). If this leads to homozygosity for a number of
327 recessive or partially recessive mutations, hybridization between divergent strains carrying those
328 mutations at different genomic locations would result in hybrid vigor.

329 Alternatively, the advantage of hybridization can be due to alleles carried by heterozygotes being
330 adapted to different, and a wider range of, environments. In the case of parasites, divergent alleles
331 may each allow infection of different host species or genomes, allowing heterozygotes to infect a
332 wider range of hosts. For example, laboratory experiments on tapeworms in sticklebacks showed
333 that two genetic lines were only able to infect a single respective host species, whereas their hybrids
334 could infect both species (Henrich et al., 2013). In the case of *G. turnbulli,* however, hybridization
335 occurred among two strains infecting the same host species.

336 Clonal expansion of hybrids is likely facilitated by facultative sexual reproduction of *Gyrodactylus*
337 species. The first generation is always produced clonally, whereas the following can be produced
338 sexually or asexually (Cable and Harris, 2002). In such systems, switching between facultative and
339 obligate clonal reproduction does not appear to be evolutionarily constrained and can easily evolve
340 when favored by selection (Neiman et al., 2014). However, though it has been proposed that
341 hybridization is the main mechanism responsible for evolution of asexual reproduction, its causality
342 and underlying mechanisms are poorly understood (Janko et al., 2018; Neiman et al., 2014; Schön et
343 al., 2009; Xu et al., 2013). Mortiz et al. (1989) suggested that reproducing asexually results from the
344 accumulation of incompatibilities between hybridizing species that disrupts cellular regulation of
345 sexual reproduction. In fact, the majority of species which recently evolved asexual reproduction are
346 of hybrid origin (Neaves and Baumann, 2011). In the case of *G. turnbulli,* however, recombination is

347 not completely suppressed, because it was observed in one of the hybrid populations on Tobago
348 (Spring Site). The fact that recombinant individuals are rare on Tobago might result either from rarity
349 of recombination events, or from effective removal of recombinants from the population by
350 selection. The disadvantage of recombinants may stem from restoring homozygotes for recessive
351 deleterious mutations. Discrimination between these two possibilities ideally requires direct
352 estimates of recombination rate, possibly based on changes in genotype frequencies over time in
353 both hybrid and non-hybrid populations (Becheler et al., 2017).

354 Unlike the Tobagonian hybrids, the patterns observed in the Trinidadian *G. turnbulli* populations
355 suggest that recombination might be more common there. On Trinidad, only a single individual
356 showed slightly elevated heterozygosity, possibly as a result of sexual reproduction between two
357 moderately diverged strains (G308, Fig 1D, Supplementary Figure 7), and indeed another individual
358 from the same population showed a pattern consistent with being a subsequent generation
359 recombinant (G312). All other Lopinot and Aripo individuals had low genome-wide heterozygosity
360 and positive inbreeding coefficients (Supplementary Figure 7). This suggests relatively common
361 sexual reproduction because long term clonal reproduction would have led to accumulation of
362 different mutations on homologous chromosomes, resulting in the excess of heterozygosity, i.e.
363 negative inbreeding coefficients (Meselson effect; Birky, 1996; Stoeckel and Masson, 2014).

364 Recombination on Trinidad could, at least to some degree, have enabled independent evolution of
365 different genomic regions, allowing identification of genomic regions with elevated genomic
366 variation between the two sampling years, possibly driven by selection. We found that one genomic
367 region showed an extreme pattern of genetic diversity, with several interesting genes previously
368 associated with modulating organismal lifespan, stress resistance (Methylotrasferase NSUN5;
369 Schosserer et al., 2015) and host-parasite interactions (Prohibitin; Jain et al., 2010). Future work
370 including more individuals and experimental validation is needed to confirm involvement of these
371 genes in coevolutionary dynamics.

372 Concluding, our population genomic dataset of *G. turnbulli* allowed us to infer that hybridization
373 followed by clonal reproduction contributed to evolutionary success of these parasites on Tobago. It
374 is likely that the evolutionary success stems from heterotic effects, which are frozen in hybrids.
375 Benefits of such freezing may only be temporary, as the lack of recombination may hinder the
376 pathogen in keeping up in the evolutionary arms-race with the host's immune system. That
377 recombination seemed to be maintained in Trinidadian populations suggesting that it may be
378 favoured in the long run in *G. turnbulli,* as well. Hybrids on Tobago appear to have persisted for a
379 while, so it would be interesting to see if after initial success, recombination would again be favored,

380 as might have been the case with *G. bullatarudis* (see Konczal et al., in press). Our results thus
381 highlight that both heterosis and increased evolutionary potential associated with recombination
382 between divergent genome, can play important role in the evolution of invasive parasites, possibly at
383 different stages following hybridization.

384

385 **Materials and Methods**

386 *Field collections*

387 During two expeditions (2017 and 2018), guppies (*Poecilia reticulata*) were sampled from six
388 locations on Trinidad and Tobago (Trinidad: Aripo River [ARI], Caura River [CAU], Lopinot River [LOP];
389 Tobago: Dog River [DR], Health Center [HC], Spring Site [SPS]). Fish were transported to our field
390 station in Charlotteville, northeast Tobago, where each population was kept in separate aquarium.
391 Over four weeks, guppies were subsequently screened for ectoparasites under a dissecting
392 microscope. If gyrodactylids were observed, a single worm was allowed to move to a naïve,
393 anesthetized fish, with transfer closely monitored under the microscope to ensure movement of just
394 a single worm. Experimentally infected fish were then screened every 2-3 days for the presence of
395 gyrodactylids. Approximately 8-13 days post-infection, hosts were euthanized with an overdose of
396 MS-222, the number of worms was counted, and samples were preserved in 97% molecular grade
397 ethanol and transported to Poland. Samples with at least 10 worms were used for DNA extraction
398 and species identification as described below. Parasites collected in 2017 and identified as *G.*
399 *bullatarudis* were reported in a separate study (Konczal et al., in press), while this work focused
400 solely on *G. turnbulli*.

401

402 *DNA extraction, libraries preparation and sequencing*

403 DNA was extracted from each pool of individuals derived from a single worm (sample) with a MagJET
404 Genomic DNA kits (Thermo Scientific). DNA concentration was measured using Qubit High Sensitivity
405 reagents. *Gyrodactylus* were identified to species using Sanger sequencing of a 262 bp fragment of
406 the mitochondrial protein coding COII gene (Xavier et al., 2015). Sequences were aligned together
407 with records downloaded from the NCBI Genbank, and a Neighbor Joining tree was reconstructed
408 with MEGA X (Kumar et al., 2018) to determine species. Thirty samples were selected for library
409 preparation (Nextera DNA Flex Library Prep Kit, Illumina) based on DNA quantity and population of
410 origin. Subsets of samples were sequenced in 2018 with HiSeq2500 (2 x 100 bp). All other samples

411 were sequenced in 2019 on the NovaSeq6000 machine in 2 x 150 bp mode (see Supplementary Table
412 1 for more details).

413

414 *SNP calling and population genomic analyses*

415 Quality of raw reads was assessed with Fastqc (Andrews and Babraham Bioinformatics, 2010). Reads
416 were then filtered with trimmomatic (version 0.36; Bolger, Lohse, & Usadel, 2014) and mapped to
417 the reference genome (Hahn et al., in prep.) with bwa mem (version 0.7.10-r789). Mapping results
418 was visualized with Qualimap (García-Alcalde et al., 2012) and duplicated reads were marked with
419 picardtools (version 2.18.5-6). SNPs were then called with samtools mpileup (version 1.6.0, options -
420 R -C50 -t DP, ADF, ADR). Based on inspection of the distributions of diagnostic statistics, we removed
421 SNPs with DP > 1200 or DP < 400 (for all samples together), with QUAL < 30, with MQ < 30 or in 5 bp
422 distance from identified indels.

423 We then used SNPs to calculate PCA with plink (version 1.90, Purcell et al., 2007) using only SNPs
424 with minor allele frequency greater than 0.05. The same dataset with additional sample representing
425 reference genotype (all genotypes encoded as reference homozygous) was used to calculate distance
426 matrix (in one minus identity-by-state units) with plink and to construct Neighbor Joining tree with
427 ape package in R. In addition, we ran PCA and Admixture analyses with SNPs, that were previously
428 pruned for Linkage Disequilibrium. We first pruned SNPs with –indep-pairwise 100 10 0.1 option in
429 plink, and then used the same program to calculate PCA. Admixture (version 1.3.0; Alexander et al.,
430 2009) analysis was performed to determine the number of ancestral populations (K) from one to ten.

431 Vcftools was used to calculate nucleotide diversity (π) and genetic divergence between populations
432 (F_{ST}) in 25 kb non-overlapping windows, as well as inbreeding coefficient (F_{IS}, option –het) per sample
433 (version v0.1.12b; Danecek et al., 2011). To calculate F_{IS} per sample we considered only SNPs
434 polymorphic within population of origin. Custom script calculating fraction of sites being
435 heterozygous in given genomic window was used to estimate genome wide heterozygosity per
436 sample. For each sample we calculated also divergence form the reference genome in 25 kb non-
437 overlapping windows, by counting number of non-reference variants (alternative homozygous sites
438 were counted as one and reference/alternative allele heterozygous sites were counted as half) and
439 divide it by a window length. Windows shorter than 12.5 kb (ends of scaffolds and scaffolds shorter
440 than that length) were excluded from all analyses.

441

442 *Mitochondrial genome assembly and mtDNA analyses*

To reconstruct phylogenetic relationships between samples we reconstructed the entire mitochondrial haplotype for sample G126 using MITObim (*de novo* approach of Hahn, Bachmann, & Chevreux, 2013) and COII fragment downloaded from NCBI (accession KP168411.1) as an initial bait. The resulting sequence spanned 14,159 bp. We then mapped reads from all sequenced samples to that sequence using bwa mem with default parameters. Samples were inspected manually using IGV. Duplicates were marked with picardtools and SNPs were called using mpileup and bcftools assuming haploid samples. SNPs with quality lower than phred 30 were discarded from analyses. The nucleotide sequence for each sample was reconstructed with bcftools consensus. Sequences were then merged and used for phylogenetic analyses.

The evolutionary relationship between samples was inferred using ML with a GTR model, implemented in MEGA X Software (Kumar et al., 2018). The same software and model were used to calculate mean evolutionary distances between groups. The BEAST2 (v2.6.1; Bouckaert et al., 2014) was then used to perform Bayesian phylogenetic analysis of mitochondrial genomes and to estimate time to most recent common ancestor (TMRCA). TMRCAs of nodes of interest were estimated based on 10,000,000 generations, logging every 1,000 steps, with the first 20% generations discarded as a burn-in. We used a Yule Model as prior, HKY substitution model, and strict clock model. Divergence time estimates were based on 13.1% divergence per million years estimated for *G. salaris* (see Kuusela et al., 2007).

Data accessibility

The raw sequences will be available as FASTQ files in the GeneBank (BioProject accession no. PRJNA603444).

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474

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