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

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14 **Abstract**

15 Hybridization is one of the major factors contributing to the emergence of highly successful parasites.
16 Hybrid vigor can play an important role in this process, but subsequent rounds of recombination in the
17 hybrid population may dilute its effects. Increased fitness of hybrids can, however, be frozen by asexual
18 reproduction. Here, we identify invasion of a "frozen hybrid" genotype in natural populations of
19 *Gyrodactylus turnbulli*, a facultatively sexual ectoparasitic flatworm that causes significant damage to
20 its fish host. We re-sequenced genomes of these parasites infecting guppies from six Trinidad and
21 Tobago populations, and found surprisingly high discrepancy in genome-wide nucleotide diversity
22 between islands. The elevated heterozygosity on Tobago is maintained by predominantly clonal
23 reproduction of hybrids formed from two diverged genomes. Hybridization has been followed by
24 spread of the hybrids across the island, implying a selective advantage compared to native genotypes.
25 Our results thus highlight that a single outcrossing event may be independently sufficient to cause
26 pathogen expansion.

27

28 **Keywords:** hybridization, clonal reproduction, heterosis, invasion, *Gyrodactylus*

29

30 **Introduction**

31 Hybridization refers to a successful mating between individuals from two populations, which are
32 distinguishable on the basis of one or more heritable characters and have divergent genomes (Arnold,
33 2004). It can be a potent evolutionary force, creating opportunities for adaptive evolution by increasing
34 genetic variation on which natural selection can act (Arnold, 1997, 2004; Oziolor et al., 2019; Rieseberg
35 et al., 2003; Taylor & Larson, 2019). The ecological and evolutionary consequences are potentially large
36 and may include significant effects on the evolutionary trajectories of pathogenic organisms
37 ('parasites' henceforth) (King, Stelkens, Webster, Smith, & Brockhurst, 2015). This role is likely to

38 increase in importance because human activities increase opportunities for hybridization by inducing
39 environmental changes and incidental translocations (Brooks & Hoberg, 2007; Cable et al., 2017; Patz,
40 Graczyk, Geller, & Vittor, 2000). Understanding the role of hybridization in parasite evolution is
41 therefore essential if we are to predict hot spots of disease risk and emergence (Laine, Barrès,
42 Numminen, & Siren, 2019).

43 Parasite strains of hybrid origin can gain an advantage because recombinants – individuals with
44 shuffled genomes - produced after hybridization may show enhanced phenotypic characteristics
45 compared to the parental generation, such as higher infectivity (Grigg, Bonnefoy, Hehl, Suzuki, &
46 Boothroyd, 2001), expanded host range (Detwiler & Criscione, 2010), increased transmission potential
47 (Volf et al., 2007) or increased tolerance to harsh environmental conditions (Laine et al., 2019).
48 Hybridization, however, is often associated with asexual reproduction, which may in the long term
49 preclude the initial benefits resulting from recombination between divergent genomes, since asexual
50 lineages tend to be less adaptable to environmental change compared to sexually reproducing lineages
51 (Neaves & Baumann, 2011).

52 Conversely, asexual reproduction may be selectively beneficial if clonal reproduction "freezes"
53 heterozygosity that confers short-term evolutionary advantage (Neiman, Sharbel, & Schwander, 2014).
54 Hybrids often show greater vigor, productivity, disease resistance, or overall increased Darwinian
55 fitness (Kingsbury, 2009). Two alternative hypotheses may explain the existence of hybrid vigor
56 (heterosis) (Lippman & Zamir, 2007). The overdominance hypothesis, attributes heterosis to superior
57 fitness of heterozygous genotypes over homozygous wild types. The dominance hypothesis assumes
58 that recessive deleterious mutations are to some extent masked in a heterozygous stage. Recent
59 theoretical work indicates that dominance-induced heterosis might play an important role in
60 evolution, especially if deleterious alleles are not effectively removed from populations as it is
61 expected when populations are small (Gilbert, Pouyet, Excoffier, & Peischl, 2020; Kim, Huber, &
62 Lohmueller, 2018; Zhao & Charlesworth, 2016). This mechanism may be particularly relevant to

63 parasite evolution. Many parasite populations are small or experience frequent bottlenecks, therefore
64 are prone to the accumulation of deleterious mutations (Lynch, Butcher, Bürger, & Gabriel, 1993), but
65 also to elevated homozygosity due to inbreeding. In such a case, one could expect higher fitness of
66 hybrid parasites. Successful parasite strains originating from a hybridization event have been
67 documented in several species, including *Batrachochytrium dendrobatidis* (see Farrer et al., 2011;
68 Greenspan et al., 2018), *Toxoplasma gondii* and *Sarcocystis neurona* (see Grigg et al., 2001; Wendte et
69 al., 2010). However, because in all these cases some degree of recombination occurred, the relative
70 role of heterozygosity versus recombination between highly divergent genomes remains unresolved.

71 Here, we report frozen genome-wide heterozygosity in wild *Gyrodactylus turnbulli*, a common
72 ectoparasite of the guppy (*Poecilia reticulata*) and well-known model species in epidemiological and
73 eco-evolutionary research (e.g. Houde & Torio, 1992; Jacquin et al., 2016; Phillips et al., 2018;
74 Reynolds, Arapi, & Cable, 2018; van Oosterhout, Harris, & Cable, 2003). Previous laboratory work
75 demonstrated that *G. turnbulli* from mixed strain infections often hybridize and have higher fitness
76 (more parasites per host, longer infection, but not increased host mortality), suggesting that in fact
77 hybrid individuals might have increased fitness, possibly due to heterosis or recombination (Schelkle,
78 Faria, Johnson, van Oosterhout, & Cable, 2012). However, the significance of hybridization in wild
79 populations is unknown (Xavier et al., 2015). We analyze thirty whole genomes of *G. turnbulli*
80 originated from six natural populations from Trinidad and Tobago. On Tobago, we identified a
81 widespread, recently formed “frozen hybrid” genotype, suggesting the potential importance of
82 heterosis in clonal invasions of *G. turnbulli* and highlighting that a single hybridization event can lead
83 to the emergence of highly successful parasites, despite suppressed recombination.

84

85 **Materials and Methods**

86 *Field collections*

87 During two expeditions (2017 and 2018), guppies (*Poecilia reticulata*) were sampled from six locations
88 on Trinidad and Tobago (Trinidad: Aripo River [ARI], Caura River [CAU], Lopinot River [LOP]; Tobago:
89 Dog River [DR], Health Center [HC], Spring Site [SPS]). All Trinidadian sites are located in the Caroni
90 drainage, while Tobagonian locations are not interconnected. Fish were transported to our field station
91 in Charlotteville, northeast Tobago, where each population was kept in a separate aquarium. Over four
92 weeks, guppies were subsequently screened for ectoparasites under a dissecting microscope. If
93 gyrodactylids were observed, a single worm was allowed to move to a naïve, anaesthetized fish,
94 obtained from *Gyrodactylus*-free mesocosms established by Phillips et al. (2018). The transfer was
95 closely monitored under the dissecting microscope to ensure movement of just a single worm. Fish
96 experimentally infected in this way were kept in separate tanks in order to establish strains from which
97 we could obtain enough DNA required to prepare libraries for sequencing. Fish were screened every
98 2-3 days to verify the presence of gyrodactylids and hosts were euthanized with an overdose of MS-
99 222 (tricaine methanesulfonate) 8-13 days post-infection. The number of worms was counted, samples
100 were preserved in 97% molecular grade ethanol and transported to Poland. Life expectancy for *G.*
101 *turnbulli* was estimated to 4.2 days (Scott, 1982). The first offspring is born approx. 1 day after the
102 birth of the parent, and subsequent offspring are born at 2-2.5 days intervals (Scott, 1982). We thus
103 expect that most individuals develop from the first or second births. The first-born always develops
104 clonally, while the second-born daughter originates from parthenogenesis (Bakke, Cable, & Harris,
105 2007; Cable & Harris, 2002). Even though it is unclear which mechanism restores the diploid
106 component during parthenogenesis (Cable & Harris, 2002), it has been suggested that heterozygosity
107 is maintained during that process, as reported for *G. salaris* (Kuusela et al., 2007). Third- and
108 subsequent born daughters may result from outcrossing, selfing, or parthenogenesis (Cable & Harris,
109 2002). Given our experimental design we expect that the majority of gyrodactylid individuals on each
110 experimentally infected fish developed from first or second births and should have preserved
111 founders' genomes. Samples with at least 10 worms were used for DNA extraction and for species

112 identification as described below. Parasites collected in 2017 and identified as *G. bullatarudis* were
113 reported in a separate study (Konczal et al., 2020), while this work focused solely on *G. turnbulli*.
114
115 *DNA extraction, libraries preparation and sequencing*
116 DNA was extracted from pools of individuals ('strain' henceforth) derived from a single worm collected
117 from a single experimentally infected fish using MagJET Genomic DNA kits (Thermo Scientific). DNA
118 concentration was measured using Qubit High Sensitivity reagents. *Gyrodactylus* were identified to
119 species using Sanger sequencing of a 262 bp fragment of the mitochondrial protein coding COII gene
120 (Xavier et al., 2015). Sequences were aligned together with records downloaded from the NCBI
121 Genbank, and a Neighbor Joining tree was reconstructed with MEGA X (Kumar, Stecher, Li, Knyaz, &
122 Tamura, 2018) to determine species. Thirty strains were selected for library preparation (Nextera DNA
123 Flex Library Prep Kit, Illumina) based on DNA quantity and population of origin. Subsets of strains were
124 sequenced in 2018 with HiSeq2500 (2 x 100 bp). All other strains were sequenced in 2019 on the
125 NovaSeq6000 platform in 2 x 150 bp mode (see Supplementary Table 1 for more details).
126
127 *SNP calling and population genomic analyses*
128 The quality of raw reads was assessed with Fastqc (Andrews & Babraham Bioinformatics, 2010). Reads
129 were then filtered with trimmomatic (version 0.36; Bolger, Lohse, & Usadel, 2014) and mapped using
130 bwa mem (version 0.7.10-r789) to the reference nuclear genome (Hahn et al., in prep.), which was
131 assembled from individuals from the *Gt3* laboratory strain kept at Cardiff University. Mapping results
132 was visualized with Qualimap (García-Alcalde et al., 2012) and duplicated reads were marked with
133 picardtools (version 2.18.5-6). SNPs were then called with samtools mpileup (version 1.6.0, options -R
134 -C50 -t DP, ADF, ADR). Based on inspection of the distributions of diagnostic statistics, we removed

135 SNPs with DP > 1200 or DP < 400 (for all samples together), with QUAL < 30, with MQ < 30 or in 5 bp
136 distance from identified indels.

137 We then used SNPs to calculate PCA with plink (version 1.90, Purcell et al., 2007) using only SNPs with
138 minor allele frequency greater than 0.05. The same dataset with additional sample representing
139 reference genotype (all genotypes encoded as reference homozygous) was used to calculate distance
140 matrix (in one minus identity-by-state units) with plink and to construct a Neighbor Joining tree using
141 the ape package in R. In addition, we ran PCA analyses with SNPs, that were previously pruned for
142 Linkage Disequilibrium. We first pruned SNPs with –indep-pairwise 100 10 0.1 option in plink, and then
143 used the same program to calculate PCA (D. H. Alexander, Novembre, & Lange, 2009).

144 Vcftools (version v0.1.12b; Danecek et al., 2011) was used to calculate nucleotide diversity (π) and
145 genetic divergence between populations (F_{ST}) in 25 kb non-overlapping windows, as well as inbreeding
146 coefficient (F_{IS}, option –het) per strain. To calculate F_{IS} per strain we considered only SNPs polymorphic
147 within population of origin. The fraction of heterozygous sites per genomic window was calculated
148 using a custom script and our estimates of genome wide heterozygosity per strain were inferred as the
149 average of these fractions across all genomic windows. For each strain we also calculated divergence
150 form the reference genome in 25 kb non-overlapping windows, by counting the number of non-
151 reference variants (alternative homozygous sites were counted as one and reference/alternative allele
152 heterozygous sites were counted as half) and dividing it by a window length. Windows shorter than
153 12.5 kb (ends of scaffolds and scaffolds shorter than that length) were excluded from all analyses.

154

155 *Mitochondrial genome assembly and mtDNA analyses*

156 To reconstruct phylogenetic relationships between strains we reconstructed the entire mitochondrial
157 haplotype for strain G126 using MITObim (*de novo* approach of Hahn, Bachmann, & Chevreux, 2013)
158 with COII fragment downloaded from NCBI (accession KP168411.1) as an initial bait. We then mapped
159 reads from all sequenced strains to that sequence using bwa mem with default parameters. Results

160 were inspected manually using IGV. Duplicates were marked with picardtools and SNPs were called
161 using mpileup and bcftools assuming haploid samples. SNPs with quality lower than phred 30 were
162 discarded from analyses. The nucleotide sequence for each strain was reconstructed with bcftools
163 consensus. Sequences were then aligned and used for phylogenetic analyses.

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

172

173 **Results**

174 A total of thirty *G. turnbulli* strains from three Trinidadian (Aripo, Caura and Lopinot Rivers) and three
175 Tobagonian populations (Dog River, Health Center and Spring Site; Figure 1A, details in Supplementary
176 Table 1) collected in two consecutive years were sequenced to an average coverage of 38.7 per strain.
177 Sequencing reads were then mapped to the reference nuclear genome and used for SNP calling. We
178 identified 776,881 single nucleotide- and 161,255 indel-polymorphisms. Principal Component Analyses
179 of SNP genotypes clearly separated Trinidad from Tobago, while differentiation between local
180 populations was present only in some cases (Figure 1B). In particular, strains from Health Center and
181 Dog River (Tobago) showed virtually no differentiation and, in contrast to the Trinidadian populations,
182 did not form monophyletic groups according to the populations of origin (Figure 1C). The Trinidadian
183 strains were more similar to the reference genome, obtained from a strain on commercial guppies,
184 *Gt3,* than strains collected from Tobago (Figure 1C). To remove the potential effect of linkage on

185 population differentiation, we pruned linked SNPs, but the general pattern remained the same
186 (Supplementary Figure 1). We calculated F_{ST} between Trinidad and Tobago populations in 25 kb non-
187 overlapping windows and found that 90% of the windows show F_{ST} values between 0.25 and 0.55
188 (mean F_{ST} = 0.42; Supplementary Figure 2). Pairwise F_{ST} values were higher among Trinidadian
189 populations (Supplementary Figures 3) than among populations from Tobago (Supplementary Figures
190 4). To resolve whether moderate F_{ST} between islands resulted from moderate diversification between
191 or from elevated levels of genetic variation within populations, we calculated nucleotide diversity
192 across Trinidad and Tobago strains separately. Nucleotide diversity on Tobago was 2.4 times greater
193 than that on Trinidad (0.0028 and 0.0012 respectively, Figure 1D). The difference does not result from
194 high differentiation among Tobagonian populations: nucleotide diversity was also higher within
195 Tobagonian populations, compared with those from Trinidad (Supplementary Figure 5).

196 Increased nucleotide diversity on Tobago was mainly driven by high heterozygosity of most individuals
197 (Figures 1D-E). Similarly, inbreeding coefficient, F_{IS}, was negative for most of the strains from Tobago,
198 suggesting over-representation of heterozygous positions (Supplementary Figure 6). In contrast, most
199 of the Trinidadian strains showed positive values of F_{IS}.

200 High heterozygosity was observed in all strains from the Tobago populations Health Center and Dog
201 River. The third Tobago population (Spring Site) showed a mixture of patterns. One Spring Site strain
202 (G334) had a low level of heterozygosity, reminiscent of the general pattern observed in Trinidadian
203 strains. Three other strains (G328, G367 and G368) showed bimodal distribution, with genomic regions
204 presenting either high or low level of heterozygosity. Finally, two Spring Site strains (G331 and G370)
205 contained only highly heterozygous regions, similar to the pattern observed in Health Center and Dog
206 River (Figure 1E).

207 The majority of positions found polymorphic among Tobagonian strains (325,473 out of 498,111) were
208 heterozygous in all Health Center and Dog River strains, as well as two (G331 and G370) Spring Site
209 strains. A total of 88% of these SNPs combined one allele from the reference genome and the

210 alternative allele found in the Tobagonian G334 strain (Figure 2). The genome-wide pattern of
211 divergence between re-sequenced strains and the reference genome demonstrated that strain G334
212 was the most divergent. Strains from Trinidad were consistently more closely related to the reference
213 genome (Figure 1C, Supplementary Figure 7). Based on these patterns we infer that highly
214 heterozygous Tobagonian strains consist of hybrids between two divergent haplotypes: one similar to
215 the reference sequence and the second similar to the G334 sequence. The same pattern with shared
216 heterozygous sites and high, genome-wide heterozygosity was observed in the Health Center and Dog
217 River strains from 2017 and 2018, indicating that the hybrid population is stable. This implies an
218 absence of recombination, as recombination would have fixed one of the two haplotypes in at least
219 some genomic regions. Auxiliary analysis comparing heterozygosity across all contigs to that of a
220 simulated, maximally heterozygous hybrid genome, created *in silico* from the reference sequence and
221 the G334 strain, confirmed that no such homozygous regions are observed in these populations
222 (Supplementary Figure 8). Furthermore, we compared genome wide nucleotide diversity of
223 Tobagonian strains collected in 2017 and 2018 and did not find evidence for a substantial difference
224 between time points (π_{2017} = 0.0018 vs π_{2018} = 0.0017). We also plotted relationships between
225 heterozygosity calculated in non-overlapping windows for each pair of strains and did not find any
226 indication for a reduction in heterozygosity in any strain (Supplementary Figure 9), suggesting no
227 ongoing recombination.

228 In contrast, divergent haplotypes have clearly recombined in the Spring Site population. Three
229 strains (G328, G367 and G368) show a mosaic pattern of the two haplotypes fixed in different genomic
230 locations, with other parts of the genome still remaining heterozygous (Figure 2, Supplementary
231 Figures 8 and 10). Signatures of sexual reproduction were also observed at least in some of the strains
232 from Trinidad. For example, SNPs localized in the scaffold scf7180000443747 (first sequence in the
233 reference genome) show admixture of another haplotype in strain G314 coming from Lopinot, and
234 another two strains in Lopinot are heterozygous in this region (Figure 2). Another strain from the same
235 population (G308) has negative F_{IS} (Supplementary Figure 6) and slightly elevated genome-wide

236 heterozygosity (Figure 1D), although the effect is weaker compared to strains from Tobago.
237 Nevertheless, heterozygous sites are on average rare in Trinidadian strains.

238 Clustering analyses (Figures 1B, 1C) and F_{ST} results (Supplementary Figure 4) based on nuclear SNPs
239 suggest low divergence between samples identified as hybrids. We explored it further by calculating
240 divergence in the haploid mitochondrial genomes. One Trinidadian strain (G126) was used for
241 mitochondrial genome assembly, and then reads from all other strains were mapped to that genome
242 for haplotype reconstruction. The resulting mt genome spans 14,159 bp. We found very little
243 divergence between strains identified as hybrids, compared to divergence amongst other samples
244 (Figure 3). Putative hybrid strains from Health Center, Dog River and Spring Site populations were more
245 closely related to Trinidadian strains than to remaining SPS strains, confirming introgression inferred
246 from genomic data. Within hybrids (Health Center, Dog River and two hybrid strains from Spring Site)
247 the mean divergence between populations was 0.02%. In contrast, the three other strains collected
248 from Spring Site had strongly divergent mitochondria - the nucleotide divergence between the two
249 Spring Site groups was 1.58%. To independently verify low divergence between Tobagonian hybrids
250 and strains from Trinidad we sequenced a 262 bp fragment of COII from 81 samples collected by us on
251 Trinidad and Tobago. The results confirmed little divergence between all Tobagonian samples except
252 for some derived from the Spring Site population (Supplementary Figure 11).

253 Lack of recombination in Tobago hampers identification of fast evolving regions in the genome,
254 because of strong linkage between sites. However, there was no evidence that the Trinidadian
255 populations experienced clonal expansion, and therefore rapidly evolving genes can be potentially
256 identified. For this reason, we tested for correlations between nucleotide diversity calculated in
257 windows separately for Tobagonian hybrid populations and for all Trinidadian strains. We used
258 nucleotide diversity calculated jointly for Health Center and Dog River strains as a proxy for the long-
259 term evolution of genomic regions (divergence between two haplotypes forming hybrids). Genomic
260 regions in which the nucleotide diversity calculated jointly for all strains from Trinidad deviates from

261 predictions based on this long-term evolutionary rate are therefore candidates for recent rapid
262 evolutionary change between years or populations. The divergence calculated in 25 kb windows was
263 highly correlated between Tobagonian hybrid populations and Trinidadian populations, while few
264 windows demonstrated elevated (according to overall trend) diversity in Trinidad (Figure 4A). One
265 scaffold (scf7180000445747; 19.4 kb long) showed extensive divergence between Trinidadian strains
266 collected in 2017 and 2018 (Figure 4B). It contains 7 protein coding genes, including putative
267 methylotransferase NSUN5, Endonuclease/exonuclease/phosphatase family domain-containing
268 protein 1, synaptogyrin and prohibitin.

269

270 **Discussion**

271 We examined whole genomes of thirty *G. turnbulli* strains, sampled from two Caribbean islands in two
272 consecutive years, providing insight into the evolution of their genomic diversity. On Tobago, parasite
273 populations tend to have much higher nucleotide diversity than those on Trinidad. This higher diversity
274 was caused by the presence of highly heterozygous strains in all studied Tobagonian populations (50-
275 100% of individuals per population). These strains shared most of the heterozygous loci, with a
276 repeatable pattern of having one reference-like allele and the other allele present in a homozygous
277 state only in one strain from Tobago (G334). This pattern led us to conclude that these highly
278 heterozygous strains comprise hybrids of two divergent lineages, one of which is close to a strain
279 isolated from ornamental guppies (Trinidad-like, used for the reference genome assembly), and the
280 second which is close to the Tobagonian G334 strain. In the Spring Site population (Tobago), we
281 observed two distinct mitochondrial lineages (diverged 145,000 years ago based on 13.1% divergence
282 per Mya (Kuusela et al., 2007), 95% HPD: 133,900 – 155,700). Most likely they represent mitochondrial
283 genomes originating from two parental populations that formed Tobagonian hybrids. The
284 mitochondrial divergence between these two lineages was much larger than between Tobagonian
285 hybrids and Trinidadian populations (7,600 years, 95% HPD: 5,200 – 9,800 years), demonstrating that
286 hybrids inherited mitochondria from Trinidad-like ancestors. Initial hybridization must have been

287 followed by reproduction without recombination, otherwise recombination would have produced
288 both homozygous and heterozygous genotypes at least in some of the genomic regions (as observed
289 in two strains at Spring Site). In contrast, in most Tobagonian strains, heterozygosity was elevated
290 genome-wide (Figure 1D) and heterozygous genotypes were very similar for all samples (Figure 2),
291 suggesting that these genomes represent "frozen hybrids". Indeed, our analyses imply that no
292 recombination occurred since the hybrids were formed. If recombination happened before expansion
293 of the hybrid genotype, some regions with loss of heterozygosity would be found and shared for all HC
294 and DR strains. We did not find any evidence for loss of heterozygosity in HC nor DR (Supplementary
295 Figure 8). In contrast to HC and DR, patterns suggesting recombination were obvious in some strains
296 from the Spring Site population. Strains G328, G367 and G368 showed non-identical patterns of
297 heterozygosity across the nuclear genome, consistent with recent independent outcrossing/selfing
298 events followed by recombination. Two of these three Spring Site strains (G328, G368) have a
299 mitochondrial genome significantly divergent from other hybrids, suggesting that subsequent rounds
300 of outcrossing occurred between hybrid and non-hybrid individuals, the latter carrying the divergent
301 mtDNA. On Trinidad, a single individual showed slightly elevated heterozygosity, possibly as a result of
302 sexual reproduction between two moderately diverged strains (G308, Figure 1D, Supplementary Figure
303 6), and indeed another individual from the same population showed a pattern consistent with being a
304 subsequent generation recombinant (G312). Thus, in contrast to a set of pure hybrid populations we
305 found on Tobago (Dog River and Health Centre), recombination was not completely absent in other
306 populations both on Tobago (Spring Site) and on Trinidad. This evidence is consistent with the data
307 from laboratory cross infections with *G. turnbulli,* which demonstrated that in experimental infections
308 with two strains, 4-11% of offspring were produced by outcrossing of the two strains (Schelkle et al.,
309 2012).

310 Not only sexual reproduction, but also most forms of parthenogenesis involve meiotic recombination,
311 which is expected to lead to loss of heterozygosity spanning large genomic regions. However,
312 automictic parthenogenesis (automixis) with central fusion can freeze heterozygosity in spite of

313 recombination (Engelstädter, 2017; Jaron, Bast, Ranallo-Benavidez, Robinson-Rechavi, & Schwander,
314 2018). Under automixis with central fusion the probability of recombination increases with distance
315 from the centromere, and particularly regions distal to the centromere are affected if recombination
316 does occur. While we were not able to identify centromeres/telomeres in the reference genome of *G.*
317 *turnbulli*, we did not find any evidence for large genomic regions with loss of heterozygosity
318 (Supplementary Figures 8 and 9). Thus, either Tobagonian hybrids (i) reproduce only mitotically, (ii)
319 recombination is strongly suppressed during automictic parthenogenesis, or (iii) recombinants are
320 removed by selection leading to clonal-like evolution. Our data suggest that the inferred, clonally-like
321 reproducing hybrids spread relatively recently across Tobago, as indicated by high similarity of their
322 mitochondrial genomes, corresponding to 2,700 (95% HPD: 1,500 – 4,000) years of divergence (Figure
323 3). The divergence time is based on the indirectly derived substitution rate in a single mitochondrial
324 locus of *Gyrodactylus salaris* (13.1% per Mya; Kuusela et al., 2007). Other indirect estimates suggest
325 lower (5.1% per Mya; Hahn et al., 2015) or higher substitution rates (up to 20.3% per Mya; Meinilä et
326 al., 2004) in *Gyrodactylus* species, and therefore divergence dating should be taken with caution.
327 Nevertheless, low nucleotide divergence between mitochondrial genomes indicates a recent origin of
328 the hybrids, and their widespread occurrence across Tobago provides evidence for their evolutionary
329 success. Hybrid genomes that were most widespread on Tobago did not show any signs of
330 recombination, and apparently remained stable, as would be expected under predominantly clonal
331 evolution.

332 What evolutionary forces are responsible for the apparent success of clonal hybrids on Tobago?
333 Tibayrenc and Ayala (2012, 2017) proposed that clonal evolution is a prominent feature of parasitic
334 linages and that selection has favoured reduced recombination as a strategy to minimize the disruption
335 of favourable gene combinations. Their hypothesis considers the total set of reproductive strategies
336 used by pathogens to escape 'recombination load'. In contrast, the 'epidemic clonality model' (Smith,
337 Smith, O'Rourke, & Spratt, 1993) advocates for the occurrence of occasional bouts of clonally
338 reproduced strains in otherwise recombining species. Ephemeral clones are frequently replaced by

339 other genotype combinations, maintaining a dominant role of sexual reproduction in evolution.
340 Complex patterns of outcrossing events in *G. turnbulli* populations seem to be consistent with an
341 'epidemic clonality model' of evolution.

342 In the case of *G. turnbulli* a bout of clonality is associated with hybridization between two, relatively
343 divergent strains, confirming that hybridization of parasites can lead to the emergence of highly
344 successful, and potentially virulent strains. The potential to increase the fitness of *G. turnbulli* by
345 combining different genomes was suggested in an earlier report of laboratory experiments, in which
346 infection with two inbred stains led to higher parasite load compared to single strain infections
347 (Schelkle et al., 2012). However, the genomic divergence between these strains was unknown and, as
348 discussed by the authors, this result could be due to competition between clones leading to the
349 evolution of increased virulence (Schelkle et al., 2012).

350 Human activity, migrations and climate change shift the geographic distribution of many species
351 (Brooks & Hoberg, 2007; Cable et al., 2017; Lafferty, 2009), which increases the probability of
352 hybridization followed by adaptive evolution. This could be the case for *G. turnbulli.* Guppies are
353 commonly kept in aquaria, and *G. turnbulli* are regularly reported in aquarium populations (Maceda-
354 Veiga & Cable, 2019) from where they can potentially be released to nature. Alternatively, hybrids
355 might have appeared naturally. While source populations of individuals that formed the hybrid are
356 unknown, our approximate dating of divergence of mtDNA between Trinidad and Tobago is consistent
357 with the end of the last glacial maximum, when a land bridge that probably existed between the islands
358 during glaciation-related sea level decline was disrupted (Alexander, Taylor, Sze-Tsun Wu, & Breden,
359 2006; Lambeck, 2004). This land bridge, while present, might have facilitated migration of guppies
360 between the islands, and possibly other fish species capable of spreading *G. turnbulli* (see Cable et al.,
361 2013; King & Cable, 2007).

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

364 variation, but in that case in populations from Trinidad (Konczal et al., 2020). Hybridization between
365 two diverged lineages and its subsequent recombination resulted in a mosaic genome composition of
366 *G. bullatarudis*. Around half of the genome originates from one or another lineage. High divergence
367 between recombined lineages and relatively little divergence between local populations led us to
368 conclude that recent hybridization was followed by rapid expansion of a recombinant *G. bulllatarudis*
369 strain. Similar patterns of successful admixed hybrid genotypes have been reported for many other
370 parasites, including schistosomes (Kincaid-Smith et al., 2019; Platt et al., 2019), *Trypanosoma* (see
371 Tihon et al., 2017) and *Leishmania* (see Rogers et al., 2014) species, as well as Dutch elm disease
372 pathogens (Brasier, 2001; Hessenauer et al., 2020) and other fungal pathogens (Mixão & Gabaldón,
373 2018) highlighting the role of recombination between divergent genomes in parasite evolution. Our
374 analysis of *G. turnbulli* genomes indicates, that unlike its congener *G. bullatarudis,* natural expansion
375 occurred without prior recombination. This scenario is similar to suggestions for *Gyrodactylus salaris*
376 a significant pathogen of Atlantic salmon. The Baltic-salmon specific *G. salaris* was proposed to have
377 originated from a single hybridization event between two clades, followed by the clonal reproduction
378 of 'frozen hybrids', however this suggestion was based on analyses of a single anonymous nuclear
379 marker (Kuusela et al., 2007). Therefore, not only clonal expansion of hybrids, but also linkage to lethal
380 variants for both alleles (balanced lethal system), or epistatic interactions could potentially explain the
381 pattern observed in *G. salaris*. Our genome-wide data suggest that clonal expansion of 'frozen hybrids'
382 can indeed take place amongst gyrodactylids.

383 Similar observations in other pathogens suggest that 'frozen hybrids' might have a significant
384 evolutionary advantage. An interesting example comes from a common fungal parasite of amphibians,
385 *Batrachochytrium dendrobatidis,* in which asexually reproducing hybrids have higher virulence than
386 both parental strains in some host species (Farrer et al., 2011; Greenspan et al., 2018). That hybrid is
387 highly heterozygous and widespread. Most likely contact between previously genetically isolated
388 allopatric populations of the fungus facilitated hybridization, resulting in generation, spread and
389 invasion of the hypervirulent strain (Farrer et al., 2011). However, the strain is not completely clonal

390 and diversification can proceed by either mitotic or sexual recombination. Similarly, in *Toxoplasma*
391 *gondii* and *Sarcocystis neurona* outcrossing can precede a disease outbreak (Grigg et al., 2001; Wendte
392 et al., 2010), but subsequent selfing events were responsible for epidemic expansions, and, as pointed
393 out by the authors, it is not clear whether an out-cross is independently sufficient to cause an epidemic
394 (Wendte et al., 2010). Our data demonstrates that a hybridization event has produced genotypes with
395 an apparent advantage compared to non-hybrid strains, potentially via masking of recessive,
396 deleterious mutations and/or allowing a widening of the ecological niche, and importantly, that the
397 genomic diversity gained through this initial hybridization appears to have been largely conserved
398 ('frozen') by suppression of- and/or selection against recombination.

399 Parasites may be particularly prone to accumulation of deleterious mutations in their genomes
400 (Criscione & Blouin, 2005). In general, the rate of accumulation depends primarily on the effective
401 population size. In small populations, random genetic drift can overpower selection making it easier
402 for deleterious mutations, particularly those with small effects, to become fixed (Kimura, Maruyama,
403 & Crow, 1963). This can cause mutational meltdown leading to population extinction (Lynch & Gabriel,
404 1990). Several features of macroparasite life cycles can act in concert to reduce effective population
405 sizes and increase the risk of such meltdown (Criscione & Blouin, 2005). In particular, frequent
406 bottlenecks associated with colonization/recolonization of new hosts can significantly reduce the
407 efficiency of selection in removing mildly deleterious mutations from populations. Additionally,
408 observations of *G. turnbulli* in the wild suggest that census population sizes are often small and
409 fluctuating over time (Stephenson, van Oosterhout, Mohammed, & Cable, 2015), which is consistent
410 with the low heterozygosity and generally positive inbreeding coefficient in the non-hybrid populations
411 that we investigated (Figure 1E, Supplementary Figure 6). If this leads to homozygosity for a number
412 of recessive or partially recessive mutations, hybridization between divergent strains carrying those
413 mutations at different genomic locations would result in hybrid vigor. Alternatively, the advantage of
414 hybridization can be due to alleles carried by heterozygotes being adapted to different, and a wider
415 range of environments. In the case of parasites, divergent alleles may each allow infection of different

416 host species or genomes, allowing heterozygotes to infect a wider range of hosts. For example,
417 laboratory experiments on tapeworms in sticklebacks showed that two genetic lines were each only
418 able to infect a single host species, whereas their hybrids could infect both species (Henrich, Benesh,
419 & Kalbe, 2013). In the case of *G. turnbulli,* however, hybridization occurred among two strains infecting
420 the same host species.

421 Clonal expansion of hybrids is likely facilitated by facultative sexual reproduction of *Gyrodactylus*
422 species. In such systems, switching between facultative and obligate clonal reproduction does not
423 appear to be evolutionarily constrained and can easily evolve when favoured by selection (Neiman et
424 al., 2014). In the case of *G. turnbulli,* however, recombination is not completely suppressed, because
425 it was observed in one of the hybrid populations on Tobago (Spring Site), where it might have occurred
426 between hybrid and non-hybrid individuals. The fact that recombinant individuals are rare on Tobago
427 might result either from rarity of recombination events, or from effective removal of recombinants
428 from the population by selection. The disadvantage of recombinants may stem from restoring
429 homozygotes for recessive deleterious mutations. Discrimination between these two possibilities
430 ideally requires direct estimates of recombination rate, possibly based on changes in genotype
431 frequencies over time in both hybrid and non-hybrid populations (Becheler et al., 2017).

432 Recombination can, at least to some degree, enable independent evolution of different genomic
433 regions, allowing identification of genomic regions with elevated genomic variation between the two
434 sampling years, possibly driven by selection. We therefore searched for such regions in Trinidad
435 populations and found that one genomic region showed an extreme pattern of genetic diversity, with
436 several interesting genes previously associated with modulating lifespan, stress resistance
437 (Methylotrasferase NSUN5; Schosserer et al., 2015) and host-parasite interactions (Prohibitin; Jain et
438 al., 2010) in other taxa. Future work including more individuals and experimental validation is needed
439 to confirm involvement of these genes in coevolutionary dynamics.

440 Concluding, our population genomic dataset of *G. turnbulli* allowed us to infer that hybridization
441 followed by clonal reproduction contributed to evolutionary success of these parasites on Tobago. It
442 is likely that the evolutionary success stems from heterotic effects, which are frozen in hybrids.
443 Benefits of such freezing may only be temporary, as the lack of recombination may hinder the
444 pathogen in keeping up in the evolutionary arms-race with the host's immune system. That
445 recombination seemed to be maintained in Trinidadian populations suggests that it may be favoured
446 in the long run as well. It would be interesting to see if after initial success, recombination would again
447 be favoured, as might have been the case with *G. bullatarudis* (see Konczal et al. 2020). Our results
448 highlight that a single outcrossing event may be sufficient to cause pathogen expansion, thus
449 emphasizing that such processes can result in emergence of invasive pathogens, some of which may
450 be of public health and conservation concern.

451

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461

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667 **Data accessibility**

668 The raw sequences are available as FASTQ files in the GeneBank (BioProject accession no.

669 PRJNA603444).

670

671 **Author Contributions**

672 M.K. and J.R. designed research; M.K., K.J.P. and R.S.M. collected samples; C.H. provided reference
673 genome; M.K. analysed data with suggestions from J.R., J.C. and C.H.; MK drafted manuscript, J.R.
674 and J.C. contributed to manuscript writing. All authors corrected manuscript and approved the final
675 version.

676 **Figure captions**

677

678 **Figure 1. Patterns of genetic variation in *Gyrodactylus turnbulli* populations from Trinidad and**
679 **Tobago. A:** Map of Trinidad and Tobago with sampling sites. **B:** Principal Component Analyses of *G.*
680 *turnbulli* strains. PCA based on 673,104 SNPs which passed filtering criteria. SNPs were identified within
681 30 isolates from three Trinidadian and three Tobagonian populations. **C:** Neighbor Joining tree (Saitou
682 and Nei 1987) constructed using distance matrix, with one minus identity-by-state units, calculated
683 based on 673,104 SNPs. Populations are represented by different colours. The reference sequence
684 (obtained from a strain on commercial guppies) is shown in black. **D:** Distribution of nucleotide
685 diversity (π) calculated across the genome in 25 kb non-overlapping windows. **E:** Distribution of
686 heterozygosity calculated in 25 kb non-overlapping windows for each strain collected from Trinidad
687 (white background) and Tobago (shaded background) populations.

688

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

694

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

700

701 **Figure 4. Genetic variation in genomic region with elevated nucleotide diversity in populations from**
702 **Trinidad. A:** Correlation between nucleotide diversity calculated jointly for Health Center and Dog
703 River populations and nucleotide diversity calculated jointly for all strains from Trinidad. Black line
704 represents linear regression ($y = 0.00 + 0.38*x$). Scaffold scf7180000443747 is shown in red. **B:**
705 Graphical representation of genotypes in polymorphic sites of *Gyrodactylus turnbulli* localized in
706 scaffold scf7180000443747 (707 SNPs). Genotypes identified as reference homozygotes are shown in
707 green, alternative homozygotes in red, heterozygous in yellow. Strains collected in 2017 and 2018 are
708 shown in grey and black respectively, and those collected from different local populations are
709 separated by horizontal grey lines.

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