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1 **DEVELOPMENT AND VALIDATION OF AN OPEN ACCESS SNP ARRAY FOR**
2 **NILE TILAPIA (*Oreochromis niloticus*)**

3

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22 **A ~65K SNP ARRAY FOR NILE TILAPIA**

23 **Key words:** GIFT, Abbassa, aquaculture, Nile tilapia, SNP array

24

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42 **Abstract**

43 Tilapia are amongst the most important farmed fish species worldwide, and are
44 fundamental for the food security of many developing countries. Several genetically
45 improved Nile tilapia (*Oreochromis niloticus*) strains exist, such as the iconic
46 Genetically Improved Farmed Tilapia (GIFT), and breeding programmes typically
47 follow classical pedigree-based selection. The use of genome-wide single-nucleotide
48 polymorphism (SNP) data can enable an understanding of the genetic architecture of
49 economically important traits and the acceleration of genetic gain via genomic
50 selection. Due to the global importance and diversity of Nile tilapia, an open access
51 SNP array would be beneficial for aquaculture research and production. In the
52 current study, a ~65K SNP array was designed based on SNPs discovered from
53 whole-genome sequence data from a GIFT breeding nucleus population and the
54 overlap with SNP datasets from wild fish populations and several other farmed Nile
55 tilapia strains. The SNP array was applied to clearly distinguish between different
56 tilapia populations across Asia and Africa, with at least ~30,000 SNPs segregating in
57 each of the diverse population samples tested. It is anticipated that this SNP array
58 will be an enabling tool for population genetics and tilapia breeding research,
59 facilitating consistency and comparison of results across studies.

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INTRODUCTION

66 Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed freshwater fish
67 species in the world, with 4.2 million tonnes being produced in 2016 (FAO 2018).
68 Although this species is native to Africa, Nile tilapia aquaculture has been
69 successfully established in over fifty countries across Asia, Africa, and South
70 America (Eknath and Hulata 2009). The popularity of tilapias stem from their overall
71 ease of culture, which is largely based on their fast growth rate, robustness,
72 relatively short generation interval, and ability to adapt to diverse farming systems
73 and habitats (Ng and Romano 2013; Eknath *et al.* 1998), although see Jansen *et al.*
74 (2019) for discussion of recent disease outbreaks. These attributes make Nile tilapia
75 a suitable species for use in the diverse and often suboptimal farming systems of
76 many low and middle-income countries, where it represents an important source of
77 animal protein and social well-being (Ansah *et al.* 2014).

78 Several selective breeding programmes have been established for Nile tilapia (Neira
79 2010), among which a major success story is the development of the widely farmed
80 Genetically Improved Farmed Tilapia (GIFT) strain. The GIFT base population was
81 formed in the early 1990s and was composed of eight unrelated strains: four wild
82 populations from Africa (Egypt, Ghana, Kenya and Senegal) and four widely farmed
83 Asian strains (Israel, Singapore, Taiwan and Thailand) (Eknath *et al.* 1993). The
84 main breeding objective of the GIFT program was to improve growth rate, but other
85 relevant traits such as overall survival, resistance to diseases, and maturation rate
86 were also considered (Eknath and Acosta 1998; Tr o ng *et al.* 2013; Komen and
87 Tr o ng 2014). Breeding programs have achieved significant genetic gains for growth-
88 related traits in this species. For instance, after five generations of artificial selection
89 the GIFT strain showed on average a 67 % higher body weight at harvest compared

90 to the base population (Bentsen *et al.* 2017). Most of the genetic progress achieved
91 to date for tilapia was obtained through traditional pedigree-based approaches. The
92 use of genome-wide genetic markers to estimate breeding values for selection
93 candidates via genomic selection (Meuwissen *et al.* 2001; Sonesson and Meuwissen
94 2009) has the potential to increase genetic gain, particularly for traits that are difficult
95 or expensive to measure directly on the candidates. Therefore, the development and
96 application of high density genotyping platforms would be advantageous in
97 expediting genetic improvement in breeding programmes for Nile tilapia.

98 SNP arrays are powerful high-throughput genotyping tools that are increasingly
99 becoming available for aquaculture species including Atlantic salmon (*Salmo salar*)
100 (Houston *et al.* 2014; Yáñez *et al.* 2016), common carp (*Cyprinus carpio*) (Xu *et al.*
101 2014), rainbow trout (*Oncorhynchus mykiss*) (Palti *et al.* 2015), Pacific (*Crassostrea*
102 *gigas*) and European (*Ostrea edulis*) oysters (Lapegue *et al.* 2014; Qi *et al.* 2017;
103 Gutierrez *et al.* 2017), catfish (*Ictalurus punctatus* and *Ictalurus furcatus*) (Liu *et al.*
104 2014; Zeng *et al.* 2017;), Arctic charr (*Salvelinus alpinus*) (Nugent *et al.* 2019), tench
105 (*Tinca tinca*) (Kumar *et al.* 2019), and indeed Nile tilapia (Joshi *et al.* 2018; Yáñez *et*
106 *al.* 2020). Compared to other high-throughput genotyping methods, such as RAD-
107 Seq (Baird *et al.* 2008), SNP arrays have the advantage of increased genotyping
108 accuracy and SNP stability, as the same markers are interrogated each time
109 (Robledo *et al.* 2018a). These platforms have been used to study the genetic
110 architecture of diverse production traits such as growth (Tsai *et al.* 2015; Gutierrez *et*
111 *al.* 2018) and disease resistance (Tsai *et al.* 2016; Bangera *et al.* 2017; Robledo *et*
112 *al.* 2018b), and their utility for genomic prediction in several aquaculture species has
113 been clearly demonstrated (for a review see Zenger *et al.* (2019)).

114 The two Nile tilapia SNP arrays developed to date are both focused on the
115 broodstock strains of specific commercial breeding programmes. One of the
116 platforms was designed based on the analysis of the GenoMar Supreme Tilapia
117 (GST®) strain (Joshi *et al.* 2018), whereas the other platform derived from the
118 evaluation of two strains belonging to Aquacorporación Internacional (Costa Rica)
119 and an unspecified commercial strain from Brazil (Yáñez *et al.* 2020). These SNP
120 arrays have been shown to be highly effective in the discovery populations, and have
121 been used to generate high-density linkage maps and perform tests of genomic
122 selection (Joshi *et al.* 2019; Yoshida *et al.* 2019a). However, while all of these
123 commercial strains are related to the GIFT strain (which underpins a large proportion
124 of global tilapia aquaculture), their utility and performance in other farmed tilapia
125 strains, especially those inhabiting Asia and Africa, is unknown. To develop
126 platforms that are not exclusively informative in a focal strain, ideally additional SNP
127 panels derived from genetically diverse populations should be evaluated during the
128 SNP selection process (Montanari *et al.* 2019). This strategy would allow mitigating
129 ascertainment bias, and thus broadening the applicability of a SNP array.

130 The aim of this study was to develop a publicly available, open access ~65K SNP
131 array for Nile tilapia based on the widely cultured GIFT strain, but that also contains
132 informative markers in multiple tilapia strains across Asia and Africa. To achieve this,
133 a large SNP database was generated by whole genome Illumina sequencing of
134 pooled genomic DNA from 100 individuals from the WorldFish GIFT breeding
135 nucleus from Malaysia. These newly discovered markers were cross-referenced with
136 previously identified SNP panels in several populations, with the aim of prioritising
137 markers that are informative across strains. To test the performance of the SNP
138 array, nine Nile tilapia populations of different geographical origins and genetic

139 backgrounds (i.e. GIFT, GIFT-derived and non-GIFT strains / populations) were
140 genotyped. The broad utility and open-access availability of the array is anticipated
141 to benefit both the academic and commercial communities to advance genomic
142 studies in this species and support ongoing and emerging breeding programmes.

143

144

MATERIALS AND METHODS

145 **Animals, DNA extraction and sequencing**

146 One hundred Nile tilapia broodstock samples from the 15th generation of the core
147 GIFT Nile tilapia-breeding nucleus of WorldFish at the Aquaculture Extension Center
148 in Jitra (Kedah, Malaysia) were used for DNA sequencing for SNP discovery. Caudal
149 fin clips were sampled and preserved in absolute ethanol at -20° until shipment from
150 Malaysia to The Roslin Institute (University of Edinburgh, UK) for DNA extraction,
151 sequencing and genetic analysis.

152 Genomic DNA was isolated from the tilapia fin clips using a salt-based extraction
153 method (Aljanabi and Martinez 1997). The integrity of the DNA samples was
154 assessed by performing an agarose gel electrophoresis. DNA quality was also
155 evaluated by estimating the 280/260 and 230/280 ratios on a NanoDrop 1000 UV
156 spectrophotometer. The concentration of the DNA extractions was measured with
157 the Qubit dsDNA BR assay kit (Invitrogen, Life technologies). Samples were diluted
158 to 50 ng/ul and then combined in equimolar concentrations to generate two pools of
159 50 (different) individuals each. Library preparation and sequencing services were
160 provided by Edinburgh Genomics (University of Edinburgh, UK). DNA pools were
161 prepared for sequencing using a TruSeq PCR-free kit (Illumina, San Diego). The two

162 pools were then sequenced at a minimum 90X depth of coverage on an Illumina
163 HiSeq X platform with a 2x150 bp read length.

164

165 **SNP discovery in the GIFT strain**

166 The quality of the sequencing output was assessed using FastQC v.0.11.5 (Andrews
167 2010). Quality filtering and removal of residual adaptor sequences was conducted on
168 read pairs using Trimmomatic v.0.38 (Bolger *et al.* 2014). Specifically, Illumina
169 specific adaptors were trimmed from the reads, leading and trailing bases with a
170 Phred score less than 20 were removed, and reads were trimmed if the average
171 Phred score over four consecutive bases was less than 20. Only read pairs that had
172 a post-filtering-length longer than 36 bp were retained. Cleaned paired-end reads
173 were aligned to the *Oreochromis niloticus* genome assembly published by Conte *et*
174 *al.* (2017) (Genbank accession GCF_001858045.2) using BWA v0.7.17 (Li and
175 Durbin 2009). To minimise biased estimates of allele frequencies, PCR duplicates
176 were removed from the dataset using SAMtools v1.6 (Li *et al.* 2009). Variants were
177 called from the pools with the software Freebayes v1.0.2 (Garrison and Marth 2012
178 *preprint*) if (i) at least three reads supported the alternate allele or (ii) the SNP allele
179 frequency in the pool was above 0.02, whichever condition was met first. As a first
180 filtering step, only SNPs that had no interfering variants within less than 40 bp on
181 either side were retained. The resulting vcf file was then filtered to obtain a list of
182 high quality variants with vcfliib v1.0.0 (<https://github.com/vcflib/vcflib>); bi-allelic
183 SNPs meeting the following criteria were kept for further evaluation: (i) a minimum
184 coverage of 50X and maximum coverage of 150X, (ii) presence of supporting reads
185 on both strands, (iii) at least two reads balanced to each side of the site and (iv)

186 more than 90% of the observed alternate and reference alleles are supported by
187 properly paired reads. To enrich the platform for variants located on or nearby
188 genes, polymorphisms were annotated and classified using the software SnpEff v4.3
189 (Cingolani *et al.* 2012). This list of candidate SNPs were sent as 71-mer nucleotide
190 sequences to ThermoFisher for *in silico* probe scoring.

191

192 **Overlap between GIFT SNPs and other datasets**

193 In order to reduce ascertainment bias and increase the utility of the platform across
194 multiple strains, we prioritised markers that also segregated in other strains /
195 populations. The candidate GIFT SNP discovery panel was compared with four other
196 lists of variants. The first panel of variants used for comparison were identified in an
197 inter-generational sample of individuals of the Abbassa strain, a selectively bred Nile
198 tilapia strain from Egypt (Abbassa breeding panel: 6,163 SNPs) (Lind *et al.* 2017).
199 The second SNP panel corresponds to variants discovered in wild fish populations
200 from the region of Abbassa, Egypt (Abbassa wild panel: 6,749 SNPs). The third SNP
201 panel was obtained from a Nile tilapia stock that had been selected for growth for
202 over ten years in Kenya, and that was initially founded by individuals from several
203 populations from East Africa (Kenya breeding panel: 33,085 SNPs). The fourth panel
204 of variants derived from the joint analysis of farmed and wild fish populations from
205 Tanzania (Tanzania panel: 2,182 SNPs). In addition, and as a quality control check,
206 the candidate list of GIFT SNPs was cross-referenced against a panel of markers
207 identified in a sub-sample of the WorldFish GIFT population at Jitra, Malaysia
208 (Wageningen panel: 7,298 SNPs) (Van Bers *et al.* 2012).

209

210 **SNP selection**

211 The process of selecting the final panel of SNPs for inclusion on the Applied
212 Biosystems Axiom Tilapia Genotyping Array was as follows. First, SNPs that were
213 previously identified as being associated with phenotypic sex were included
214 (Palaiokostas *et al.* 2013, 2015) (Supplementary Table S1). Second, all SNPs that
215 were shared with at least one other SNP panel – either Abbassa breeding, Abbassa
216 wild, Kenya breeding, Tanzania or Wageningen – were considered as high priority
217 markers and included directly on the array. In addition, for each SNP that was
218 submitted for evaluation, ThermoFisher assigns a design score (p-converge value) to
219 both 35 bp probes flanking the variant. Probes with a high p-converge value indicate
220 an assay with a higher probability of SNP conversion. Based on their p-converge
221 value, probes can be classified as either ‘recommended’, ‘neutral’, ‘not
222 recommended’ or ‘not possible’. For downstream analysis, SNPs that had at least
223 one probe that was either ‘recommended’ or ‘neutral’ were retained. Next, SNPs
224 were filtered according to their minor allele frequency (MAF) by removing markers
225 with an average MAF (estimated from the two sequenced pools) < 0.05 or > 0.45 .
226 The latter MAF threshold was applied to avoid spurious SNPs resulting from
227 sequence differences between paralogues. Additional criteria for SNP selection
228 included filtering out *A/T* and *G/C* variants, as compared to other polymorphisms
229 they require twice as many assays on a ThermoFisher Axiom platform. From the
230 remaining list of high confidence SNPs identified in the discovery population,
231 polymorphisms located in exons were prioritized. To fill the remaining target of ~65K,
232 SNPs were selected from those located either within a gene or at most at a 1 kb
233 distance. The strategy of enriching for SNPs on genes was followed because they
234 are more likely to alter protein function, and therefore may have a larger effect on

235 phenotypes compared to variants occurring outside genes (Jorgenson & Witte 2006).
236 To obtain a uniform physical distribution across the Nile tilapia genome, all
237 chromosomes and 130 of the longest scaffolds were divided into 10-kb non-
238 overlapping windows, and the SNP with the highest MAF within each interval was
239 selected for inclusion in the platform. Finally, for 1-Mb regions exhibiting the lowest
240 number of markers, the SNP with the highest MAF within the region was included
241 manually.

242

243 **SNP array validation**

244 The ThermoFisher Axiom ~65K Nile tilapia SNP array designed in this study was
245 tested by genotyping nine Nile tilapia populations of different geographical locations
246 and genetic backgrounds (Table 1). The tested fish belonged to one wild population
247 from Egypt (Abbassa wild) and six genetically improved strains. The evaluated
248 strains were the (i) Genetically Improved Farmed Tilapia (GIFT) (Eknath and Acosta
249 1998; Eknath *et al.* 1993), (ii) Genetically Enhanced Tilapia-Excellent (GET-EXCEL)
250 (Tayamen 2004), (iii) Brackish water Enhanced Saline Tilapia (BEST) (Tayamen *et*
251 *al.* 2004), (iv) Freshwater Aquaculture Centre (FAC) selected Tilapia (FaST) (Bolivar
252 1998), and improved strains from (v) Kenya and (vi) Abbassa (Egypt). For each
253 representative strain, a single population was sampled, with the exception of the
254 GIFT strain, for which three populations from different countries were evaluated,
255 Malaysia (discovery population), Bangladesh and Philippines.

256 In total, 135 individuals, comprising 15 fish of balanced sex ratios in each population,
257 were genotyped by IndentiGEN (Ireland) using the Nile tilapia ~65K SNP array. To
258 perform a principal component analysis (PCA) on the genome-wide SNP data the

259 following SNPs and samples were retained using PLINK v1.9 (Chang *et al.* 2015): (i)
260 SNPs of the Poly High Resolution class (i.e. high quality markers with three well-
261 resolved genotype clusters) (ii) markers with a call rate > 0.95, (iii) individuals with a
262 call rate > 0.90, and (iv) one SNP of a pair showing high linkage disequilibrium ($r^2 >$
263 0.7). In addition, for individuals with greater than 80 % identity-by-state (IBS) with
264 another individual, only one was retained for further analysis. The structure of the
265 135 individuals genotyped with the SNP array was investigated using the R package
266 LEA (Frichot and François 2015), with the significance of the identified components
267 evaluated with Tracy-Widom statistics (Tracy and Widom 1994).

268

269 **Summary statistics of SNPs**

270 The levels of observed and expected heterozygosity (H_o , H_e) for each Nile tilapia
271 strain / population were calculated, and 95 % confidence intervals of H_o estimated
272 based on 1,000 bootstrap replicates. To evaluate the informativeness of the SNPs
273 on the array, the average MAF values per strain / population were calculated and
274 classified into five different categories: Common ($MAF > 0.3$); Intermediate ($0.3 >$
275 $MAF > 0.1$); Low ($0.1 > MAF > 0.05$); Rare ($MAF < 0.05$); and Fixed ($MAF = 0$).

276

277 **Linkage disequilibrium magnitude and decay**

278 To estimate linkage disequilibrium (LD) we used a version of the SNP dataset to
279 which all individual and SNP QC filters were applied (see SNP array validation
280 section), except the removal of markers based on pairwise LD. As a pairwise
281 measure of LD, r^2 (Hill and Robertson 1968) was chosen because it is most
282 frequently used in the context of association mapping (Ardlie *et al.* 2002). Moreover,

283 other LD metrics such as D' are highly affected by sample size (McRae *et al.* 2002)
284 and its use is not recommended when sample sizes are small. LD was estimated
285 separately for each strain / population as the inter-marker Pearson's squared
286 correlation coefficient r^2 corrected for relatedness (r^2_{vs}) using the package LDcorSV
287 v1.3.1 (Mangin *et al.* 2012) in R v 3.5.0 (R Core Team 2014). For comparison, two
288 MAF thresholds were applied to the data before measuring the extent of LD, MAF >
289 0.05 and MAF > 0.1. The average r^2 was calculated in 10-kb bins (pairwise distance
290 between SNPs) for each Nile tilapia chromosome. The LD decay was visualized
291 using the R package ggplot2 (Hadley 2009) by plotting the average r^2 within each bin
292 (across all chromosomes) against inter-marker distances, which extended from zero
293 up to 10 Mb.

294

295 **Ethics statement**

296 Data collection and sampling of the GIFT samples was performed as part of a non-
297 profit selective breeding program run by WorldFish. The animals from this breeding
298 population are managed in accordance with the Guiding Principles of the Animal
299 Care, Welfare and Ethics Policy of WorldFish. Tissue sampling was carried out in
300 accordance with the norms established by the Reporting *In Vivo* Experiments
301 (ARRIVE) guidelines.

302

303 **Data availability**

304 Raw sequence reads from the two pools analysed for SNP discovery have been
305 deposited in NCBI's Sequence Read Archive (SRA,
306 <https://www.ncbi.nlm.nih.gov/sra>) under accession number PRJNA520791. Genome

307 position and probes for all SNPs included in the ~65K SNP array are given in File
308 S1. Genome position and allele frequency of array SNPs discovered in the Pool-seq
309 data can be found in the European Variation Archive (EVA,
310 <https://www.ebi.ac.uk/eva/>) under accession number PRJEB38548. The tilapia SNP
311 array is available for commercial purchase from ThermoFisher (array number
312 551071, email: BioinformaticsServices@thermofisher.com).

313

314

RESULTS

SNP selection and array development

316 The pooled DNA sequencing resulted in 458M and 461M paired-end reads for the
317 two DNA pools. The alignment of the quality control filtered reads against the Nile
318 tilapia reference genome (Genbank accession GCF_001858045.2) led to the
319 discovery of ~20 million putative polymorphisms. Of the 1,166,652 bi-allelic SNPs
320 that remained after applying post-alignment quality control (QC) filters, 694,348 fell
321 within genes or in the neighbouring regions of genes (i.e. within <1 kb). After
322 additional filtering criteria related to allelic frequency thresholds (removal of SNPs
323 with average MAF < 0.05 or > 0.45) and the type of allele polymorphism (removal of
324 *A/T* and *G/C* variants), 351,188 SNPs were sent as 71-mer nucleotide sequences to
325 ThermoFisher for *in silico* probe scoring. From the list of scored SNP probes
326 provided by ThermoFisher, only those that were categorised as either
327 'recommended' or 'neutral' were selected.

328 The final ~65K SNP array contained (i) 7 sex determination markers, (ii) 6,883 SNPs
329 discovered in our population that overlap with SNP panels identified in other strains /
330 populations, (iii) 11,328 SNPs located in exons, and (iv) 47,239 SNPs occurring in

331 genes or within < 1 kb of genes. The latter set of SNPs were selected to be evenly
332 physically spaced along the 22 chromosomes (Supplementary Figure S1) and 130 of
333 the longest unplaced scaffolds of the Nile tilapia genome assembly.

334

335 **SNP array validation**

336 After QC of the genotyping data, seven, two and one fish were removed due low call
337 rate from the Abbassa wild, Abbassa strain and BEST population, respectively.

338 Therefore, 125 individual fish from across nine different strains / populations were
339 used to validate the SNP array (Table 1). The obtained raw intensity files were
340 imported to the Axiom Analysis Suite software v2.0.035 for quality control analysis
341 and genotype calling. Genotypes were called following the Best Practices Workflow
342 using the default settings for diploid organisms (Thermo Fisher Scientific Inc 2018).
343 The SNP probe sets were classified into one of the following six category classes
344 based on cluster properties and QC metrics: PolyHighResolution, NoMinorHom,
345 MonoHighRes, Off Target Variant (OTV), CallRateBelowThreshold, and Other. Of
346 the 65,450 SNPs assayed by the platform, 54,604 SNPs (83.4 %) were classified as
347 PolyHighResolution markers, the class with the highest quality probes and presence
348 of both the major and minor homozygous clusters. The number of SNPs that showed
349 a good cluster resolution but no evidence of individuals with minor homozygous
350 genotypes (NoMinorHom) was 2,122 (3.2 %). Only 374 SNPs (0.5 %) on the array
351 were monomorphic (MonoHighResolution). Among the SNPs that failed to provide
352 reliable genotypes, 194 SNPs (0.2 %) were OTV, 3,026 SNPs (4.6 %) had a SNP
353 call rate below the chosen threshold of 0.97 (CallRateBelowThreshold), and 5,130
354 (7.8 %) were not classified into any of the above categories (Other). After applying

355 standard QC filters, 54,310 (MAF > 0.05) and 49,429 (MAF > 0.1) SNPs and 125
356 individuals were retained for the assessment of LD decay; after the pruning of
357 markers based on LD, 42,460 SNPs remained for the estimation of general
358 population statistics and population structure.

359

360 **Minor allele frequency and genetic diversity in Nile tilapia populations / strains**

361 The average observed heterozygosity of the genotyped populations was 0.29, with
362 the GIFT strain from Malaysia (i.e. the primary discovery population) having the
363 highest value (0.35), and the Kenyan population the lowest (0.21) (Table 1). Overall,
364 the observed heterozygositites (H_o) were slightly higher than expected (H_e), and
365 showed a similar pattern across populations. The only exception was the Kenyan
366 strain, for which the H_o was lower than the H_e (0.21 vs 0.24).

367 The average MAF of all 42,460 successfully genotyped SNPs ranged from 0.23 to
368 0.26 across the six strains and the single wild population evaluated. The number of
369 informative markers (MAF > 0) in the array was highest for samples from GIFT and
370 GIFT-derived populations compared to populations with non-GIFT genetic
371 backgrounds (Figure 1). The primary discovery population had the greatest number
372 of informative markers, 40,930 SNPs (96 %). As expected, the populations
373 genetically closer to the GIFT discovery population from Malaysia had the second
374 and third highest numbers of informative markers – 40,743 (95 %) and 39,562 (93
375 %) informative SNPs in the GIFT stocks from Bangladesh and the Philippines,
376 respectively. Likewise, GIFT-derived strains exhibit similarly high levels of
377 informative SNPs, with 38,232 (90 %) markers segregating in the GET-EXCEL and
378 37,867 (89 %) in the BEST strain. The number of informative markers for the three

379 non-GIFT strains evaluated in this study were 30,631 (72 %) for the FaST strain,
380 31,061 (73 %) for the Kenyan domesticated line and 30,786 (72 %) for the Abbassa
381 strain. A large fraction of these informative SNPs co-segregate with the GIFT strain
382 (Figure 2). The average MAF for the markers that are common to all the different
383 representative strains evaluated (total = 19,815 SNPs) was similar and ranged from
384 0.26 to 0.28. The single wild population analysed, Abbassa-wild, exhibited the lowest
385 number of informative markers (28,421 SNPs; 66 %).

386

387 **Population structure**

388 The population stratification of the nine Nile tilapia strains / populations was
389 visualized using a PCA to reduce the dimensions of the genotype data (Figure 3).
390 The two first eigenvectors accounted for 22 % of the total variance. The first
391 dimension, which explains 13 % of the variance, mainly separates GIFT and GIFT-
392 derived populations from the Nile tilapia strains / populations of African origin
393 (Abbassa-strain, Abbassa-wild and Kenya). The second principal component
394 explains 9 % of the total variance and separates the strains / populations from Africa
395 into two clusters, one comprised of Nile tilapia individuals from Egypt (Abbassa-
396 strain and Abbassa wild) and the other comprising the Kenyan domestic line.
397 Additionally, this dimension also separates Asian GIFT, GIFT-derived and non-GIFT
398 strains into three distinct clusters represented by the (i) FaST strain, (ii) GIFT strains
399 from Malaysia, Philippines and Bangladesh, and (iii) non-GIFT strains, namely GET-
400 EXCEL and BEST. Three individuals of putative Kenyan origin did not group with the
401 Kenyan cluster (those with negative PC1 values in Figure 3).

402

403 **Linkage disequilibrium decay**

404 The overall average LD between marker pairs was relatively low and decayed as
405 physical distance increased. Similar patterns of LD decay were observed across Nile
406 tilapia populations for the two MAF thresholds applied to the data, although the MAF
407 filter of 0.1 resulted in higher magnitudes of r^2 (Figure 4). Two distinct patterns of LD
408 decay were observed across strains / populations. A first group – composed
409 exclusively by domestic lines (GIFT-Ma, GIFT-Ba, GIFT-Ph, GET-EXCEL, BEST,
410 FaST, Kenya and Abbassa-strain) – showed a moderate to low LD decay over short
411 and long-range distances. The average observed values of r^2 at the smallest inter-
412 marker distance evaluated (10 kb bin) was ~0.2 (MAF > 0.1 dataset). Within short-
413 range distances (< 100 kb), pairwise correlations experienced a 10 to 23% decrease
414 when LD between markers separated by ~10 kb was compared to pairs of loci
415 separated by ~ 100 kb. Considering long-range distances, the average r^2 dropped by
416 65 % from that estimated at 10 kb compared to 10,000 kb in GIFT-Ma (0.21 vs 0.08),
417 60 % in GIFT-Ba (0.18 vs 0.07), 61 % in GIFT-Ph (0.19 vs 0.07), 59 % in GET-
418 EXCEL (0.19 vs 0.08), 54 % in the BEST strain (0.19 vs 0.09), 72 % in FaST (0.27
419 vs 0.08), 54 % in the Kenyan strain (0.17 vs 0.08) and 52 % in the Abbassa-strain
420 (0.18 vs 0.09). By contrast, LD decayed much more slowly in the Abbassa-wild
421 population, as initial levels of LD persist over long inter-marker distances. The
422 reduction in r^2 between SNPs at 10 kb vs 10,000 kb distance apart from each other
423 was of only 22 % (0.19 vs 0.15) (Figure 4).

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DISCUSSION

428 The ~65K SNP array developed in this study is an open-access high-throughput
429 genotyping platform for Nile tilapia. A large majority of the SNPs on the platform
430 were of high quality and polymorphic – 87 % of the SNPs fell in either the
431 PolyHighResolution or NoMinorHom categories. This performance value lies in the
432 upper range of current aquaculture SNP arrays (e.g. 89 % for rainbow trout (Palti *et al.*
433 *et al.* 2015) and 77 % for the latest catfish array (Zeng *et al.* 2017)), demonstrating the
434 efficacy of our Pool-Seq strategy for robust SNP discovery at a fraction of the
435 sequencing effort of typical SNP chip designs.

436 Two published SNP arrays have been developed for Nile tilapia, each of ~58K SNPs
437 (Joshi *et al.* 2018; Yáñez *et al.* 2020). These platforms capture the genetic diversity
438 of specific improved lines, but their efficacy has only been demonstrated in the
439 GST® (i.e. GIFT line further improved through genomic tools) (Joshi *et al.* 2018) or
440 GIFT and GIFT-related strains from South America (Yáñez *et al.* 2020). In our array,
441 the bulk of SNPs were derived from a SNP discovery process performed on two
442 DNA pools of 100 fish of the core breeding nucleus of the WorldFish GIFT strain,
443 which underpins a large proportion of global tilapia production. However, to mitigate
444 ascertainment bias and widen the applicability of the platform, panels from previous
445 SNP discovery projects were cross-referenced and common SNPs were prioritised.
446 Yet, as expected, the number of informative SNPs decreases with increasing genetic
447 distance from the primary discovery population (e.g. ~63 % in non-GIFT strains;
448 Figure 2). Even though a small number of individuals (~15 per strain / population)
449 were genotyped with the array, there were at least ~30,000 SNPs segregating in
450 each of the population samples evaluated, and near 20,000 common SNPs
451 segregating in all non-GIFT strains tested, namely Abbassa, Kenya and FaST.

452 Therefore, it is proposed that this SNP array can serve as a common platform for
453 use by the tilapia genetics and breeding community to encourage cross-study
454 comparisons and meta-analyses of genomic datasets.

455 A principal component analysis demonstrated that our 65K SNP array distinguishes
456 the four major strains evaluated in this study (GIFT, Abbassa, Kenya and FaST),
457 indicating clear independent clusters based on the first two principal components.
458 While the purpose of this analysis was to test the utility of the SNP array to
459 distinguish populations, a few interesting observations were noted. First, individuals
460 from the Abbassa genetically improved strain clustered with wild fish from the same
461 region (i.e. Abbassa, Egypt). This pattern is consistent with a short period of artificial
462 selection that has not yet led to significant shifts in allele frequencies. Additionally,
463 the projection of the Kenyan cluster along a line in the PC plot may indicate the
464 recent admixture of two populations, as suggested for this dispersion pattern by
465 Patterson *et al.* (2006). On the other hand, GIFT and GIFT-derived strains form a
466 loose cluster that separates in dimension 2 of the PC plot but that is not clearly
467 maintained in dimensions 3 to 6 (Supplementary Figures S2-S3). This lack of
468 consistency likely indicates that the population structure of this cluster may not be
469 well represented by the first two PCs. As expected, there is a large degree of overlap
470 among the GIFT strains, most likely due to their common origin. The GIFT-derived
471 strains (GET-EXCEL and BEST) tend to co-cluster in the PC plot; as both strains
472 were developed in the Philippines (Tayamen 2004; Tayamen *et al.* 2004), this
473 concordance could reflect shared breeding goals and similar production systems and
474 breeding practices. Interestingly, although these GIFT-derived strains are the
475 product of selection programmes applied to base populations originating from
476 different strains, the PCA suggests they are genetically closer to the GIFT strain. For

477 instance, GET-EXCEL is a synthetic strain developed based on four parental lines:
478 the GIFT strain (8TH generation), the FaST strain (13TH generation), an Egyptian
479 strain (composed by animals sourced from eight locations in Egypt) and a Kenyan
480 strain (coming from stock collected in Lake Turkana) (Tayamen 2004). However, in
481 the PC plot GET-EXCEL individuals group with the BEST strain, closer to the GIFT
482 cluster, and more distant to any of the other genetic clusters they supposedly derive
483 from (i.e. Abbassa, Kenya and FaST). This observation may suggest that the GET-
484 EXCEL tilapia has a reduced Abbassa, Kenyan and FaST genetic component, which
485 could be explained by an unequal contribution of parental lines during the
486 establishment of the strain.

487 Linkage disequilibrium (LD) is the non-random association between the observed
488 frequencies of a particular combination of alleles (Wall and Pritchard 2003).

489 Adequate LD is critical for the implementation of GWAS studies and genomic
490 selection in breeding programmes. Both methods exploit the LD that exists between
491 markers and quantitative trait loci (QTL) or causative mutations (Flint-Garcia *et al.*
492 2003; Goddard and Hayes 2009). Hence, the magnitude and extent of LD decay
493 between genetic markers can be used to predict the marker density required for QTL
494 mapping. For all the evaluated Nile tilapia populations (GIFT, GIFT-derived and non-
495 GIFT), overall relatively low levels of LD ($r^2 \sim 0.2$) were accompanied by a moderate
496 to slow decay with increasing distance. Despite the small number of animals used to
497 assess LD decay (~ 15 individuals per strain / population), a similar pattern was found
498 to that reported by Yoshida *et al.* (2019b) for GIFT and GIFT-derived commercial
499 populations in South America. The weak correlation found between SNPs is
500 consistent with previous findings in GIFT strains (Xia *et al.* 2015; Yoshida *et al.*
501 2019b) and is comparatively lower than estimates obtained for other farmed fish

502 species such as Atlantic salmon (Barria *et al.* 2018; Kijas *et al.* 2017). Nevertheless,
503 it is worth noting that despite the relatively low levels of LD, the SNP density of the
504 array is in excess of requirements to obtain maximal genomic prediction accuracy in
505 the context of a typical sibling testing breeding programme in tilapia (Yoshida *et al.*
506 2019a), and indeed for the majority of aquaculture species tested to date (Houston *et*
507 *al.* 2020). Historical factors that affect effective population size (e.g. population
508 bottlenecks, admixture, selective breeding) may influence patterns of LD (Gaut and
509 Long 2003). Contrary to the expectation of domesticated lines showing longer LD
510 than wild populations (Gray *et al.* 2009; McRae *et al.* 2002), the single wild
511 population examined in this study (i.e. Abbassa wild) showed the slowest rate of
512 decrease and the highest LD at longer distances compared to all Nile tilapia strains
513 evaluated. Because it is possible that the sampled Abbassa population is not a good
514 representation of wild individuals (e.g. due to interbreeding with escapees) or LD
515 estimates are being biased by population structure (hypothesis that was not tested in
516 this study), additional wild populations should be evaluated. The general trend
517 observed across strains of overall low levels of r^2 suggests that patterns of LD in Nile
518 tilapia are complex and likely associated with particular features of the process of
519 domestication of this species (Xia *et al.* 2015).

520 At the mean inter-marker spacing on the SNP array (~16 kb), the average r^2 across
521 autosomes was 0.2. According to the simulations performed by Hu and Xu (2008),
522 an r^2 of at least 0.2 is required to achieve a power above 0.8 to detect a QTL for a
523 complex trait of low heritability ($h^2 \sim 0.05$). Although overall LD levels appear to be
524 low in Nile tilapia, our preliminary results suggest that this array provides sufficient
525 genomic resolution to capture association signals in different strains, and will

526 therefore contribute to expand genetic research in this species and effectively
527 support ongoing and future breeding programmes.

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CONCLUSION

530 A high quality Nile tilapia SNP array was created and validated in several strains.
531 The SNP array was built by prioritising markers that are evenly spaced across gene
532 entities and their local neighbourhood (within < 1 kb), thereby potentially increasing
533 the chance of detecting variants that alter gene expression and / or protein function.
534 The open-access nature of the SNP array together with demonstration of its utility
535 across multiple strains will facilitate its use in genetic research in this species. This
536 may include studies to assess the origin of farmed populations, to track introgression
537 of farmed genomes into the wild, and to understand the genetic architecture of traits
538 of interest. Further, this SNP array will contribute to the management of farmed
539 tilapia populations, and enable accelerated genetic gain and better control
540 inbreeding in breeding programmes via genomic selection.

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766

767 **Table 1.** Origin and observed (Ho) and expected (He) heterozygositites for the Nile tilapia populations
 768 used for the validation of the SNP array

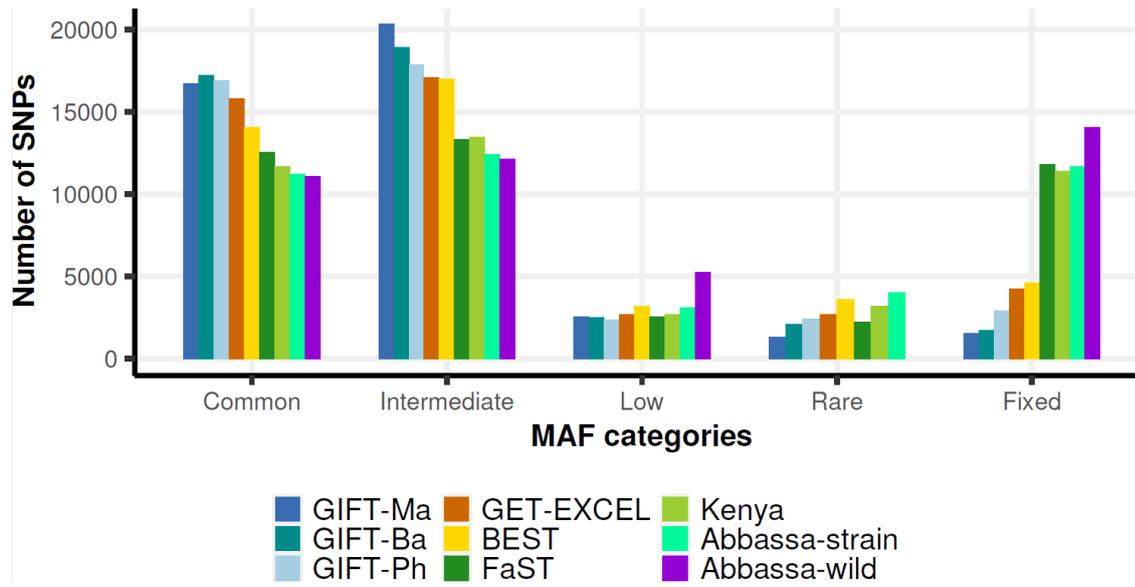
Population ID	Genetic background	Type	Origin	Number of samples passing QC filters	He	Ho	95% CI (Ho)
GIFT-Ma ^a	GIFT	Domesticated	Malaysia	15	0.337	0.350	0.348-0.352
GIFT-Ba	GIFT	Domesticated	Bangladesh	15	0.334	0.347	0.346-0.349
GIFT-Ph	GIFT	Domesticated	Philippines	15	0.322	0.328	0.327-0.330
GET-EXCEL	GIFT-derived	Domesticated	Philippines	15	0.304	0.325	0.323-0.327
BEST	GIFT-derived	Domesticated	Philippines	14	0.294	0.317	0.316-0.320
FaST	Non-GIFT	Domesticated	Philippines	15	0.243	0.252	0.250-0.254
Kenyan	Non-GIFT	Domesticated	Kenya	15	0.236	0.209	0.207-0.211
Abbassa strain	Non-GIFT	Domesticated	Egypt	13	0.229	0.239	0.237-0.241
Abbassa Wild	Non-GIFT	Wild	Egypt	8	0.220	0.258	0.259-0.264

769 ^adiscovery population

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Figure 1. MAF categories of SNPs from the ~65K SNP-chip across nine different Nile tilapia strains / populations.

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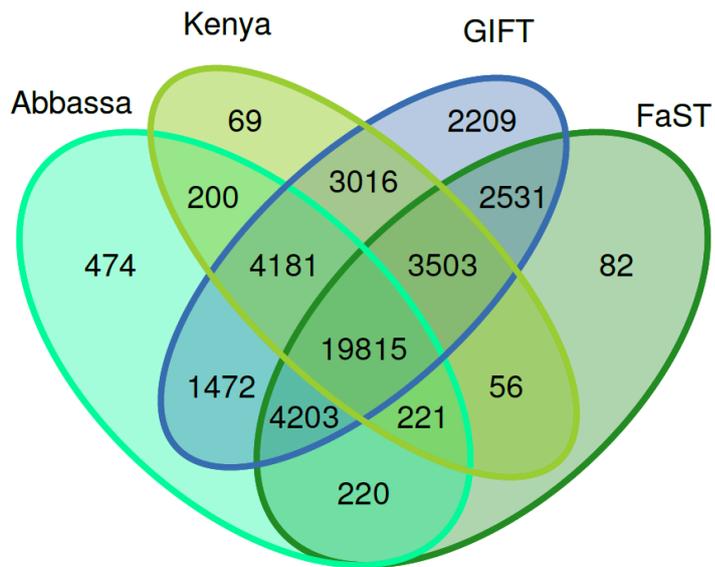
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790 **Figure 2. Number of informative SNPs (MAF>0) shared among the four distinct strains**
 791 **evaluated in this study: Abbassa, Kenya, GIFT and FaST.**

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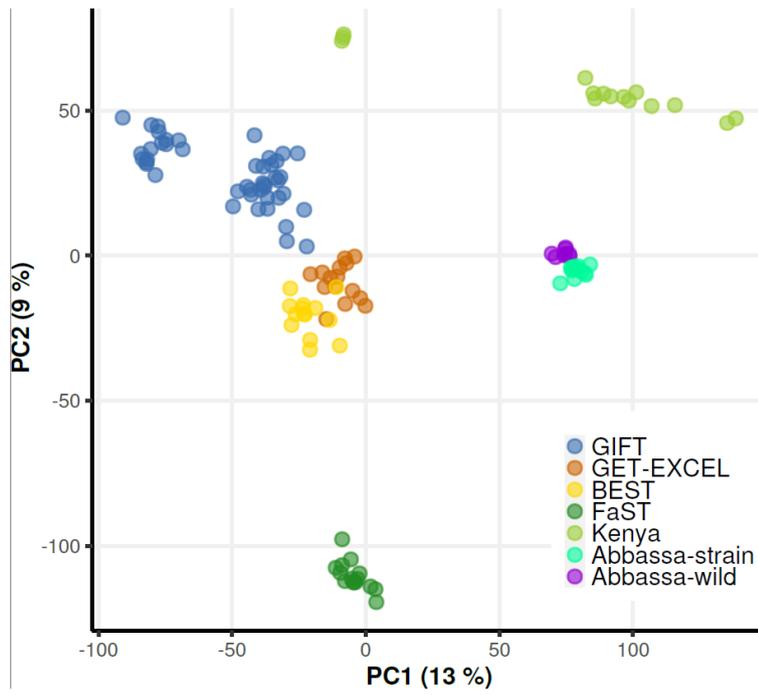
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803 **Figure 3. PCA representing the structure of nine different strains / populations used for the**
 804 **validation of the ~65k SNP array.** The total number of individuals (dots) is 125. Each dot is colour
 805 coded according to its origin, as shown in the legend at the bottom right corner of the plot.

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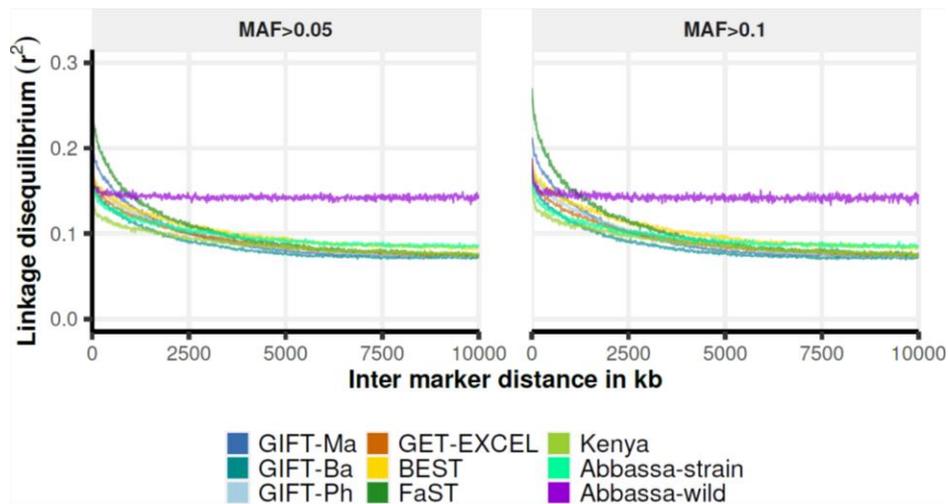
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818 **Figure 4. Linkage disequilibrium decay (r^2) over distance (in kb) among different Nile tilapia**

819 **strains / populations genotyped with the ~65K SNP array. LD decay after applying a MAF**

820 **threshold of 0.05 (left panel) and 0.1 (right panel).**

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