

1 **Inbreeding depression in an outbred nine-spined stickleback (*Pungitius pungitius*) population**

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13

14 **Abstract**

15 Inbreeding depression refers to the reduced fitness of offspring produced by related individuals and is
16 expected to be rare in large outbred populations. When it occurs, marked fitness loss is possible as large
17 populations can carry large loads of recessive harmful mutations which are normally sheltered at the
18 heterozygous state. Using experimental cross data and genome-wide identity-by-descent (IBD)
19 relationships from an outbred marine nine-spined stickleback (*Pungitius pungitius*) population, we
20 documented a significant decrease in offspring survival probability with increasing parental IBD
21 sharing associated with an average inbreeding load (B) of 15.896. Interestingly, we found that this
22 relationship was also underlined by a positive effect of paternal inbreeding coefficient on offspring
23 survival, suggesting that certain combinations of parental inbreeding and genetic relatedness among
24 mates may promote offspring survival. Apart from demonstrating substantial inbreeding load in an
25 outbred population, the results also highlight the potential caveat associated with artificial establishment
26 of families in experimental studies: wild founder individuals are often - and perhaps mistakenly -
27 assumed to be unrelated.

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33 **Keywords:** genetic load, IBD, heritability, inbreeding, *Pungitius pungitius*, survival

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35 INTRODUCTION

36 Mating between close relatives often reduces the fitness of offspring through morphological,
37 physiological or reproductive defects, a phenomenon known as inbreeding depression [1]. Inbreeding
38 depression may lead in the most extreme case to offspring unviability and eventually to population
39 extinction [2]. Quantitative and population genomic approaches to study inbreeding depression have
40 become increasingly important as the environmental pressures imposed by global changes are predicted
41 to increase habitat fragmentation, reduce population sizes and consequently increase the probability of
42 inbreeding in the wild [3-5].

43

44 The magnitude of inbreeding depression depends on the interplay between the effective population size
45 (N_e), the rate at which new mutations arise in the population and the efficiency of selection at
46 eliminating harmful mutations [6]. Populations with large N_e are large mutational targets but natural
47 selection is more efficient against deleterious mutations in large than in small populations [7,8]. As a
48 result, most mutations segregating in populations with high N_e are recessive, mildly deleterious, and
49 can subsist in the population in the heterozygous state [9]. This in turn means that the cost of inbreeding
50 in large populations is expected to be high, as it increases the chance of recessive mutations to be
51 expressed as lethal homozygotes [10]. Conversely in small populations, and particularly those confined
52 to population isolates with no or limited migration, the probability of mating with genetically related
53 individuals is drastically increased. Nonetheless, the cost of inbreeding in such populations should be
54 reduced, as deleterious mutations would be rapidly purged due to homozygotes lethality [6, 11, 12].

55

56 These theoretical expectations have been investigated in a variety of wild model species across different
57 taxa [13, 1]. In a comprehensive empirical study of the link between N_e and genetic load in the water
58 flea *Daphnia magna*, Lohr & Haag [14] reported a clear positive relationship between the load of
59 segregating recessive mutations (expressed through inbreeding depression) and N_e . Similarly, empirical
60 work comparing large and small populations of *Arabidopsis thaliana* have shown that small-sized
61 populations carry large genetic loads due to the fixation of deleterious mutations (*i.e.*, drift load; [10]).

62 To date, theoretical expectations regarding the relationship between N_e , genetic load and inbreeding
63 depression have been verified in a number of terrestrial systems (*e.g.*, [15-19])

64

65 In marine organisms, studies of genetic load are still rare [20] and have been often focused on specific
66 groups (*e.g.*, Oysters; [21-23]). The magnitude of inbreeding depression in fish species in particular
67 seem to have been under-studied with the exception of classical models (*e.g.*, guppies, [24, 25]; three-
68 spined sticklebacks, [26, 27]; zebrafish, [28]) or species of economic interest (*e.g.*, salmonids, [29, 30]).
69 Yet, demographic and genetic parameters specific to marine organisms make them an opportune model
70 to test theoretical predictions of inbreeding depression in the wild. Indeed, while several marine species
71 are characterised by large N_e , high dispersal and low genetic differentiation among populations [31-34],
72 it has been long recognized that population structure in the sea may be more prevalent than previously
73 thought [35-39] and that such structure can have major implications for evolutionary responses to
74 selection [40, 41]. In this context, population genomics tools can shed light on the fine-scale relatedness
75 patterns occurring in structured populations, and advance our understanding of inbreeding depression
76 in wild outbred populations [42].

77

78 The main objective of this study was to investigate whether cryptic relatedness estimated from genome-
79 wide identity-by-descent (IBD) relationships among wild-collected parents had an effect on offspring
80 survival in a large crossing experiment of the nine-spined stickleback (*Pungitius pungitius*).
81 Specifically, we tested if relatedness among a set of wild parental fish used to produce F_1 -generation
82 sibs through artificial mating led to inbreeding depression in offspring viability. Our results show that
83 genetic similarity among parents can yield substantial reduction in offspring survival even when the
84 level of relatedness is low.

85

86 MATERIAL AND METHODS

87 *Sampling and rearing*

88 The fish used in this study came from a large crossing design used in previous studies [43]. Wild
89 parental fish were caught from a marine population in the Baltic Sea in Finland (60°13'N, 25°11'E) and

90 brought to the aquarium facility of the University of Helsinki. The crossing scheme consisted of a
91 maternal half-sib design where 46 females were crossed to two different males resulting in a total of 86
92 half-sib/full-sib families (3 females were mated to a single male). Parental fish we crossed artificially
93 following standard *in vitro* split-clutch approach for sticklebacks [44]. Eggs were stripped from gravid
94 females by gently squeezing their abdomen and placed in two different petri dishes. Males were over-
95 anesthetized using tricaine methanesulfonate (MS222) in order to dissect their testes, which were
96 subsequently minced in the dish containing the eggs from their corresponding females. Fertilization was
97 performed by mixing the eggs and sperm using a sterile plastic pipette and was ensured by looking for
98 signs of post-fertilization membranes under a binocular microscope. Following egg hatching and after
99 larvae started feeding, each half-sib family was thinned to approximately 25 F₁ offspring per family and
100 transferred to two large aquaria. Individuals were placed at random to ensure that half of each family
101 was represented in each aquarium. Offspring were mass-reared and their parental identity were
102 subsequently recovered from genotype data as explained in [43] (and see below). Offspring survival
103 was scored as the number of F₁ fish alive at the end of the experiment.

104

105 *Sequencing and genotyping*

106 Parental fish were whole-genome sequenced (WGS) on an Illumina HiSeq platform (BGI, Hong-Kong)
107 at 5-10X sequencing coverage and offspring were sequenced using the Diversity Arrays Technology
108 (DarTseq technology, Pty Ltd, Australia). Fastq files were mapped to the contig assembly of the *P.*
109 *pungitius* reference genome [45] using bwa-mem [46] and then sorted bams for each file were obtained
110 using SAMtools [46]. We obtained genotype likelihoods using Lep-MAP3 [47]. The linkage mapping
111 and the pedigree construction were conducted using Lep-MAP3. Offspring were assigned to parents
112 using the Lep-MAP3's IBD module by calculating the Mendelian genotypic error rate for each
113 combination of parents and offspring and by taking all combinations with error rate < 10% (average
114 error rate was 5.5%). Finally, offspring sex was obtained by comparing the sequencing coverage on X
115 and Y specific regions using SAMtools depth. The final genomic dataset consisted of 49,493 biallelic
116 SNPs.

117

118 *Estimation of genomic inbreeding and relatedness*

119 We estimated the genomic inbreeding coefficient (F_G) of all dams and sires from SNP data using the -
120 *-ibc* option implemented in the GCTA software (v.1.93.2, [48]). This metric provides a measure of
121 inbreeding that is unbiased, as homozygote genotypes are scaled by their allele frequencies [48].
122 Second, we estimated the pairwise relatedness between each pair of parents by computing the Genomic
123 Relationship Matrix (GRM) in GCTA using the *--make-grm* function. GCTA uses the allelic correlation
124 coefficient (see [48] p. 76 and [49]) to construct the pairwise matrix of IBD coefficients among all
125 individuals.

126 To ensure that our results were not influenced by the specific approaches used to estimate F_G and IBD
127 in GCTA, we replicated all analyses based on alternative estimates using the software PLINK (v1.90,
128 [50]). We used the *--het* option in PLINK to obtain individual inbreeding coefficients and the *--genome*
129 option to estimate the proportion of genome shared IBD between all pairs of parents. Inbreeding
130 coefficients estimated with PLINK correspond to excess homozygosity and deviation from Hardy-
131 Weinberg equilibrium. In PLINK, the GRM is constructed following the approach of VanRaden [51]
132 and based on the probability of IBD computed from identity-by-state values. We chose these two
133 software as they provide two alternative approaches to the estimation of each parameter of interest, and
134 have previously been shown to improve detection of inbreeding depression using genomic data [52]
135 and particularly in population with large N_e [53]. For the sake of comparison, we follow the
136 nomenclature of other studies (*e.g.*, [42, 52, 54, 55]) and hereafter refer to GCTA estimates of
137 inbreeding coefficient as F_{UNI} and PLINK estimates as F_{HOM} . Similarly, estimates of relatedness using
138 GCTA and PLINK are referred to IBD_{YANG} and IBD_{VR} , respectively. Finally, to account for the possible
139 effects of SNP filtering on the estimation of inbreeding and relatedness coefficients, all analyses were
140 ran using estimates of F_G and IBD from data pruned for minimum allele frequencies ($MAF < 0.05$) and
141 linkage disequilibrium ($LD > 0.8$) using the R package SNPrelate [56] or using unpruned data. Prior to
142 all analyses, we removed all markers present on *P. pungitius* sex chromosome (Chromosome 12, [57])
143 to retain only autosomal SNPs.

144

145 *Statistical analyses*

146 We ran generalized linear mixed models (GLMMs) to investigate the effect of parental relatedness and
147 parental inbreeding on offspring survival using the *lme4* package (v.1.1-27.1; [58]) in R (v.4.1.1; [59]).
148 Offspring survival was modelled as a binomial response variable using the *glmer* function in *lme4* using
149 a logit link function. Fixed effect terms included parental relatedness (IBD; the proportion of genome
150 shared IBD between each pair of parents), paternal inbreeding coefficient (F_{SIRE}), maternal inbreeding
151 coefficient (F_{DAM}) and their two-way interactions. To account for the used maternal half-sib design,
152 dam identity was used as a random effect in all models. Preliminary investigations of the data indicated
153 that the inclusion of the dam random effect led to a change in the sign of the effect of parental relatedness
154 on offspring survival, indicating distinct effects of within-family relatedness (relatedness among the
155 triad of parents of each half-sib family) and between-family relatedness (relatedness across dams) on
156 offspring survival. To account for these distinct effects, we used a technique of within-subject centering
157 [60] by calculating the mean relatedness (mR) value of each half-sib family and added it as a fixed effect
158 in our GLMMs. Similarly, we estimated the component of relatedness within half-sib families by
159 normalizing individual values as $wR = IBD - mR$, for each pair of parents. Model selection was performed
160 based on the Akaike Information Criterion corrected for small sample size (AICc; which balances model
161 fit and complexity, [61]), by using the *dredge* functions in the R package *MuMin* (v.1.43.17, [62]).
162 Collinearity among the predictor variables was assessed using the variance inflation factor (VIF)
163 analysis by using the *vif* function in the R package *car* (v.3.0-11, [63]). Our final model had the
164 following syntax:

165

$$\begin{aligned} 166 \quad y = mR + wR + F.\text{sire} + F.\text{dam} + (F.\text{dam}:F.\text{sire}) + (F.\text{dam}:wR) + (F.\text{sire}:wR) + (F.\text{sire}:mR) \\ 167 \quad \quad \quad + (F.\text{dam}:mR) + (1|\text{dam}) \end{aligned} \quad (1)$$

168

169 where y is the offspring survival coded as a two-column vector; colons indicate fixed terms interaction
170 and (1|dam) corresponds to the notation of random effect of dam identity in the *lme4* syntax. We ran
171 the model in equation (1) for all four combinations of SNP data filtering (pruned/unpruned) and
172 genomic estimator ($F_{\text{UNI}}/F_{\text{HOM}}$) while keeping consistency between software used to estimate F_G and
173 IBD (*i.e.*, using $F_{\text{UNI}} / \text{IBD}_{\text{YanG}}$ and $F_{\text{HOM}} / \text{IBD}_{\text{VR}}$ together in the same models). Finally, as the use of a

174 logarithmic link function to GLMMs has been advocated to ensure unbiased estimates of inbreeding
175 load [55], we also ran all models using the “log” link option in *lme4* (see *Supplementary material*).

176

177 *Estimation of inbreeding load*

178 Inbreeding depression can be quantified using the inbreeding load (B) expressing the number of lethal
179 equivalents per gamete [64] and estimated as the negative slope of the regression of offspring inbreeding
180 coefficients on the trait values on the logarithmic scale [55, 65]. Inbreeding coefficients of offspring are
181 usually obtained from pedigree relationships which allow estimating the expected IBD relationships
182 within individuals [66]. Here, as pedigree relationships would not capture any fine-scale variation of
183 realized relatedness among parents (all parents are assumed to be unrelated), we estimated the
184 magnitude of inbreeding load as the slope of the regression of parental relatedness (mR) on offspring
185 survival from model (1) above. Finally, we calculated the mean and 95% confidence interval (CI) of B
186 from 1000 bootstrap iterations for each combination of SNP data and genomic inbreeding estimator.

187 To ensure consistency of our results across statistical approaches and to make our results comparable
188 to other studies, we also estimated B following the traditional approach using offspring inbreeding
189 coefficients instead of parental relatedness as the main model’s fixed effect (see *Supplementary*
190 *material*).

191

192 *Heritability estimates*

193 We estimated the proportion of genetic variation explaining the variance of offspring survival by
194 computing the heritability (h^2) of survival rate using an animal model [13, 67] of the form:

195

$$196 \quad y = X\beta + Z_A + Z_{DAM} + \varepsilon \quad (2)$$

197

198 where y is the vector of offspring survival coded as a binary response variable (0 and 1 for dead and
199 surviving individuals, respectively); X is the incidence matrix associated to the vector of fixed effect β ,
200 Z_A is the matrix of relatedness between individuals obtained from pedigree relationships; Z_{DAM} is the
201 random effect of dam identity and ε is the error term.

202 We further tested if offspring survival variation resulted from inbreeding depression by adding
203 individual F_G as a covariate in model (2). Indeed, inbreeding depression is expected to bias the
204 estimation of the genetic variance components from animal models [68] and we hypothesized that a
205 downward bias in h^2 resulting from the added fixed effect of F would give further support to the role of
206 inbreeding in survival rate variation in our data. Because genomic data to estimate offspring F_G was
207 only available for surviving individuals, we assigned to each offspring the mean F_G value calculated
208 from the survivors of each family (see also *Supplementary methods*). Animal models were fitted with
209 the *MCMCglmm* R package [69] using the “threshold” family option appropriate for binomial
210 regressions [70] and ran for 10,400,000 iterations with a burn-in period of 400,000 and sampling every
211 1000th iteration. We ran standard model diagnostics for *MCMCglmm* models [69, 70] by investigating
212 the trace plots of all variance components and computing their effective sample size using the
213 *effectiveSize* function implemented in the *coda* R package [71] and checking for autocorrelations
214 between iterations using the *autocorr* function from *coda*. We computed h^2 both on the latent and
215 observed scales using the *QGparams* function of the *QGglmm* R package [72].

216

217 **Results**

218 *Inbreeding coefficients and relatedness among the parents*

219 We found differences in inbreeding coefficients between sexes using both metrics, with males having
220 lower inbreeding coefficients than females (F_{UNI} : $t = -5.497$, $p < 0.001$; F_{HOM} : $t = -5.541$, $p < 0.001$).
221 Relatedness coefficients estimated with PLINK and GCTA were moderately positively correlated ($r =$
222 0.573 ; $p < 0.001$). Overall, relatedness between pairs of mates was low (Table S1), as expected from a
223 random sample of individuals mated at random. The pairwise IBD estimated with PLINK ranged from
224 0 to 0.237 and from -0.029 to 0.090 with GCTA. Negative values estimated with GCTA indicate that
225 these pairs of individuals were less related than the pairs in the sample on average [48].

226

227 *Inbreeding depression*

228 There was a negative effect of parental relatedness on offspring survival across all models (Fig. 1, Table
229 1), but model complexity (*i.e.*, inclusion of fixed terms and their interactions) varied depending on SNP

230 data filtering and the estimator (F_G and IBD) used (Table 1). Using pruned SNP data, the full model
231 using PLINK estimators (F_{HOM} & IBD_{VR}) indicated a negative effect of parental relatedness on offspring
232 survival (Table 1; $p = 0.022$) and that relatedness within half-sib families was positively associated with
233 offspring survival (Table 1; $p = 0.048$). In other words, triads of parents that were on average more
234 genetically related had fewer survivors among their progeny while females tended to have fitter
235 offspring when mated with more related males. We also found contrasting effects of parental F_{HOM} on
236 survival probability with more inbred females showing lower offspring survival (Table 1 F_{DAM} ; $p =$
237 0.022) but more inbred males showing higher offspring survival (Table 1, F_{SIRE} ; $p < 0.001$). We could
238 not test for any further interaction terms due to a high degree of multicollinearity among fixed terms
239 resulting in high VIF. Using unpruned SNP data, the model based on PLINK estimators yielded similar
240 results with parental relatedness having a strong negative effect on offspring survival (Table 1; $p <$
241 0.001). Paternal inbreeding coefficient in this model was also found to be positively related to offspring
242 survival (Table 1, F_{SIRE} ; $p < 0.001$). The relatedness within half-sib families had a negative effect on
243 offspring survival in this model (Table 1, wR ; $p < 0.001$).

244 Models based on the GCTA estimators (F_{UNI} & IBD_{Yang}) also indicated a negative effect of parental
245 relatedness on offspring survival (Fig. 1, Table 1). When using pruned SNP data, we found again
246 opposing effects of parental inbreeding coefficients on offspring survival (Table 1). This was also
247 reflected by the significant negative interaction between the F_{DAM} and F_{SIRE} terms in the model (Table
248 1, $p = 0.002$). Finally, the GLMM based on unpruned SNP data showed a negative effect of parental
249 relatedness on offspring survival (Table 1, $p = 0.008$) and a positive relationship between paternal
250 inbreeding coefficient and survival (Table 1, $p < 0.001$).

251

252 Overall, estimates of B varied depending on the type of filtering and genomic predictor used, and
253 showed relatively large confidence intervals around the mean (Table 2). The mean B across all models
254 was 15.896. Results based on the clutch-averaged inbreeding coefficients (see *Supplementary methods*)
255 were concordant with the above estimates with a mean $B = 16.946$ across models (Table S2). Finally,
256 results obtained using the log-link function gave highly similar results for all GLMMs (Table S3).

257

258 *Heritability of offspring survival*

259 We found moderate h^2 in offspring survival with $h^2 = 0.17$ [0.04;0.32] on the latent scale and $h^2 = 0.13$
260 [0.04; 0.25] on the observed scale. Adding offspring inbreeding coefficients as covariate in the animal
261 model reduced estimates of h^2 on both scales with $h^2 = 0.05$ [1.7e-09; 0.12] and $h^2 = 0.06$ [2.12e-09;
262 0.14] on the latent and observed scales, respectively.

263

264 **Discussion**

265 The most salient finding of this study was the fairly severe inbreeding load observed across all GLMMs,
266 seemingly much more severe than in other taxa for juvenile survival. This result shows that large
267 outbred populations can harbour substantial inbreeding load, and more importantly, that inbreeding
268 depression can ensue even when levels of parental relatedness is low. In the following, we discuss the
269 implications of these results for the study of inbreeding depression in the wild.

270

271 The magnitude of observed inbreeding depression exceeds that of a wide array of taxa previously
272 reported in the literature. Early estimates of lethal equivalents in several mammals and bird species
273 were on average one order of magnitude lower for survival traits ([13], see their Table 1 p. 279)
274 compared to our estimates. Recent surveys of the literature [55] are in line with this observation and in
275 the particular case of juvenile survival, inbreeding loads rarely exceed 1-3 homozygote lethals.
276 Although our estimates present relatively wide confidence intervals and vary among the genomic
277 estimators used, the order of magnitude and range of the inbreeding loads reported here is on par with
278 severe loads previously reported from some natural populations [73-76]. This has important
279 conservation implications [77] as the results suggest that large-sized populations may be at a high risk
280 of extinctions if rapid decline in population size or habitat fragmentation would increase the probability
281 of inbred mating.

282

283 The severity of inbreeding depression is known to depend on the environmental conditions under which
284 it is expressed, with stressful conditions amplifying its severity [78, 79]. Since our study was carried
285 out in a laboratory, we may have underestimated its real magnitude. Indeed, wild *P. pungitius* encounter

286 parasites [80], predators and other severe abiotic challenges which could potentially amplify the
287 negative consequences of inbreeding. Conversely, it is difficult to dismiss the possibility that the
288 unnatural laboratory environment could also lead to overestimation of the magnitude of inbreeding
289 depression, although this possibility seems unlikely. Regardless of the ‘true’ natural level of inbreeding
290 depression, the results show potential for it to exist in this outbred marine population.

291

292 An unexpected and intriguing result was the positive effect of paternal inbreeding on offspring survival:
293 offspring sired by more inbred males showed increased survival probability, regardless of the estimator
294 of inbreeding coefficient used. Given the negative relationship between parental relatedness and
295 offspring survival, it is possible that inbred males would benefit from mating with more distantly related
296 females. Similar results have been reported in the gopher tortoise (*Gopherus polyphemus*) in which
297 increased maternal inbreeding was associated with higher offspring fitness despite a strong negative
298 effect of parental relatedness [82]. In their study, Yuan *et al.* [82] propose that such mating strategy (kin
299 avoidance by inbred individuals) would alleviate the fitness cost of inbreeding, particularly in the sex
300 investing more resources in reproduction. In *P. pungitius*, males provide parental care through nest-
301 building, guarding and fanning of eggs and could thus benefit from this mating strategy. Unfortunately,
302 due to multicollinearity among variables, our current data did not allow us to model the interaction
303 between paternal inbreeding and parental relatedness and test this hypothesis further. However, we
304 found that survival rate was heritable, indicating that genetic differences between families explain some
305 amount of variation in offspring viability. Hence, certain combinations of parental inbreeding
306 coefficients and genetic relatedness yielding higher survival could be favoured by selection and provide
307 basis to the evolution of mate choice in this species (cf. [83, 84]). However, heritability of offspring
308 viability was lowered substantially when accounting for individual inbreeding coefficients, suggesting
309 that inbreeding affected survival to a larger extent than among-family genetic differences. Experimental
310 studies of mate choice controlling for the effect of parental inbreeding coefficient and parental
311 relatedness should be particularly interesting to address these questions.

312

313 While the results demonstrate that potential for inbreeding depression is present in the study population,
314 the results should not be extrapolated to say that it would be realized in wild *P. pungitius* populations.
315 This because all mating in this study were artificial, and therefore eluded all aspects of mate choice,
316 including kin recognition and active inbreeding avoidance. Such behaviours have been studied in the
317 related three-spined stickleback (*Gasterosteus aculeatus*) in which gravid females prefer to lay eggs in
318 nests built by unrelated males [85]. However, such studies of mate choice and inbreeding avoidance
319 often use very different levels of relatedness by contrasting completely unrelated to relatively closely
320 related individuals (*e.g.*, full-sib). In the present study, the range of relatedness among parental fish was
321 narrow, and well below full-sib relationship (the maximum value of $IBD_{VR} = 0.237$ corresponding to
322 mating with aunt/uncle or half-sib). Hence, it is unclear whether kin recognition in sticklebacks would
323 be fine-tuned to the levels of relatedness measured in our study and whether wild females could discern
324 between males with such low levels of relatedness. Furthermore, high levels of local kinship have been
325 reported in other marine fish [39, 86] and the relatively strong population structure found in marine
326 populations of *P. pungitius* [41, 87] could promote similar local kinship patterns and increase the
327 probability of inbred mating despite large population sizes.

328

329 In conclusion, as predicted by theory, the results show that there is an opportunity for severe inbreeding
330 depression to occur in large outbred marine populations. Future studies of inbreeding depression in the
331 wild should benefit from the inclusion of fine-scale measurement of parental relatedness in models of
332 inbreeding depression.

333

334 **Ethical statement**

335 This experiment was approved by the National Animal Experiment Board, Finland (permission
336 numbers: ESLH-STSTH223A and STH037A)

337

338 **Conflict of interest**

339 The authors declare no competing interests.

340

341 **Data accessibility statement**

342 Sequence data that support the findings of this study have been deposited in European Nucleotide
343 Archive (ENA) under the Accession nos PRJEB39736 (linkage map parents), PRJEB39760 (linkage
344 map offspring).

345

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354

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TABLES AND FIGURES

Table 1. Results from the GLMMs on the effect of parental relatedness on offspring survival. For each model, the slope of regression for each coefficient of the fixed terms and interactions is given along with the standard error (SE) and the p -value. mR : mean relatedness between pairs of parents; wR normalized within-half-sib relatedness; F_{DAM} : inbreeding coefficient of the dam; F_{SIRE} : inbreeding coefficient of the sire. Colons indicate interactions between terms. Statistically significant p -values ($p < 0.05$) in bold.

SNP data	IBD Estimator	F_G Estimator	Fixed effects and interactions	Slope	SE	p -value		
Pruned	IBD _{VR}	F_{HOM}	mR	-6.797	2.979	0.022		
			wR	3.465	1.759	0.048		
			F_{DAM}	-7.180	3.147	0.022		
			F_{SIRE}	9.828	1.479	< 0.001		
			IBD _{Yang}	F_{UNI}	mR	-19.380	6.5040	0.003
					wR	-7.940	5.752	0.167
					F_{DAM}	-15.446	4.961	0.002
	F_{SIRE}	14.955			2.177	< 0.001		
			$F_{DAM}:F_{SIRE}$	-195.637	64.489	0.002		
			wR: F_{DAM}	475.043	201.783	0.018		
	Unpruned	IBD _{VR}	F_{HOM}	mR	-12.563	1.640	< 0.001	
				wR	-9.978	2.571	< 0.001	
				F_{SIRE}	20.966	2.761	< 0.001	
		IBD _{Yang}	F_{UNI}	mR	-25.334	9.494	0.008	
wR				1.8537	5.909	0.754		
F_{SIRE}				18.095	3.584	< 0.001		

Table 2. Bootstrap estimates of the inbreeding load. The mean values for the inbreeding load (B) are reported along with their 95% Confidence Intervals (CI). The type of SNP data, IBD and F_G estimators are as defined in the main text.

SNP data	IBD Estimator	F_G Estimator	B	95% CI
Pruned	IBD _{Yang}	F_{UNI}	19.332	[7.430 - 31.274]
Unpruned	IBD _{Yang}	F_{UNI}	24.847	[9.243 - 42.119]
Pruned	IBD _{VR}	F_{HOM}	7.039	[2.974 - 12.386]
Unpruned	IBD _{VR}	F_{HOM}	12.367	[9.529 - 15.658]

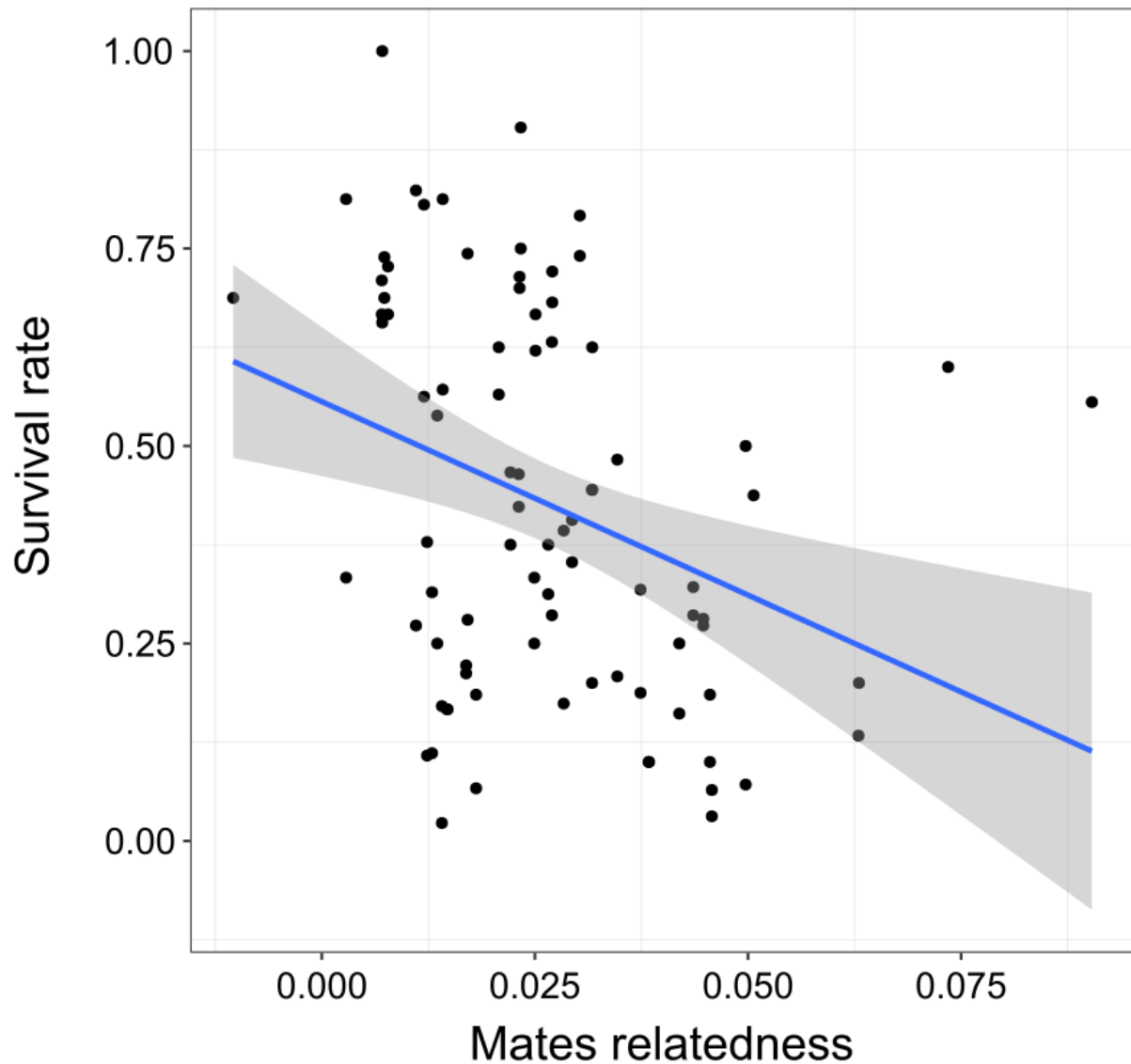


Figure 1. Inbreeding depression in juvenile survival rate of nine-spined sticklebacks. The survival rate for each family (black filled circles) is shown as a function of the relatedness coefficients between pairs of mates estimated with GCTA (see *Methods*). Blue line represents the fitted regression line from the ggplot *smooth* function and grey shadings are the 95% confidence intervals around the predicted values.