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Heritability estimation via molecular pedigree reconstruction in a wild fish population reveals substantial evolutionary potential for age at maturity, but not size within age-classes.

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21   ABSTRACT

22   While evolutionary responses require heritable variation, estimates of heritability (h^2) from

23   wild fish populations remain rare. A 20-year molecular pedigree for a wild Scottish

24   population of Atlantic salmon (*Salmo salar*) was used to investigate genetic contributions to

25   (co)variation in two important, correlated, phenotypic traits: "sea-age" (number of winters

26   spent at sea prior to spawning) and size-at-maturity (body length just prior to spawning). Sea-

27   age was strongly heritable ($h^2 = 0.51$) and size exhibited moderate heritability ($h^2 = 0.27$). A

28   very strong genetic correlation ($r_G = 0.96$) between these traits implied the same functional

29   loci must underpin variation in each. Indeed, body size within sea-ages had much lower

30   heritability that did not differ significantly from zero. Thus, within wild *S. salar* populations,

31   temporal changes in sea-age composition could reflect evolutionary responses, whereas rapid

32   changes of body-size within sea-ages are more likely due to phenotypic plasticity. These

33   inheritance patterns will influence the scope of evolutionary responses to factors such as

34   harvest or climate change and, hence, have management implications for salmonid

35   populations comprising a mix of sea ages.

36

37   Keywords. salmonids; population dynamics, quantitative genetics, Bayesian, evolvability,

38   adaptation, management, conservation genetics

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INTRODUCTION

Life history traits such as age and size at first reproduction are typically expected to be under strong selection, given their close links with Darwinian fitness (Stearns 1992, Roff 2002). All else being equal, genotypes that reproduce as early as possible in life should have higher relative fitness than those that first reproduce at older ages (Brommer et al. 2002). In species with indeterminate growth such as most fishes, however, there may be strong breeding advantages (especially for females) to delaying first reproduction to older ages, and hence larger sizes (Heino and Kaitala 1999). The optimum age and size at first reproduction will then depend on factors such as (seasonal) environmental opportunities for growth, age-specific extrinsic mortality schedules, and the extent to which reproductive success depends on body size (Stearns 1992, Charlesworth 1994, Roff 2002). Human-induced changes to any of these parameters can alter the selective pressures on populations; for example, harvests of wild animals can drive "unnatural selection" on phenotypic traits such as age at maturity and body size (Allendorf and Hard 2009, Heino et al. 2015, Harvey et al, 2017), while climate change can impact selective dynamics in a range of complex ways (Munday et al. 2013, Sydeman et al. 2015). Inferring the potential for evolutionary responses, however, requires information on the extent to which observed phenotypic (co)variation is underpinned by genetic (co)variation (Kuparinen and Hutchings, 2017). Detailed, long-term, studies of naturally regulated populations, in which the life histories, reproductive success and pedigrees of individual animals are measured or inferred can provide this information (Naish and Hard 2008, Clutton-Brock and Sheldon 2010).

In Atlantic salmon (*Salmo salar* L.) and other salmonid fishes, the sea-age of adults returning to spawn for the first time is an important trait from both ecological and economic perspectives, as older fish tend to be larger and therefore more valuable from a commercial and recreational fishing perspective, while also having higher fecundity and therefore

contributing more towards total annual egg numbers. Sea-age variation may also contribute

towards ecological and evolutionary portfolio effects via bet-hedging mechanisms (Schindler

et al., 2015), where risk is spread across multiple sea-age cohorts. Some individuals, and

populations of Atlantic salmon, return after just one winter at sea (1SW), whereas others

known as multi-sea winter (MSW) fish return after two or more winters at sea (Klemetsen et

al, 2003). The marine feeding destination, sea-age, adult-return size and adult run-timing are

all inter-related (Malcolm et al, 2010). The average size of returning adults generally

increases with time spent at sea, both across and within sea-age classes; for example, MSW

fish tend to be on average much larger than 1SW, and later-running fish of a given sea-age

class are typically larger than early-running fish (Hutchings & Jones 1998, Bacon et al. 2009,

2010, Barson et al. 2015). A substantial genetic component underlying sea-age variation has

been shown or implied by several studies of Atlantic salmon (Nævdal 1983, Gjerde 1984,

Johnston et al. 2014, Ayllon et al. 2015, Barson et al. 2015) and related species such as

Chinook salmon (*Oncorhynchus tshawytscha*) (Hankin et al. 1993, Quinn et al. 2001) and

steelhead/rainbow trout (*Oncorhynchus mykiss*) (Tipping 1991, Kause et al. 2003).

Body-length at maturity has also been shown to be heritable in salmonids generally,

with Carlson & Seamons (2008) reporting a median heritability (the fraction of phenotypic

variation explained by genetic variation) estimate of 0.21 across six salmonid studies. Only

one of these studies (Dickerson et al. 2005), however, involved a situation where the fish (in

that case, pink salmon, *Oncorhynchus gorbuscha*) spawned and reared naturally in a wild

river environment; the rest all involved either hatchery/farm-origin broodstock and/or

artificial rearing conditions (Carlson and Seamons 2008). Since then a limited number of

additional salmonid studies have appeared that estimate heritability of length-at-age in

freshwater in fully natural environments (Serbezov et al. 2010, Morrissey and Ferguson 2011,

93   Reed et al. 2015), but these studies did not focus on heritability of size-at-maturity

94   specifically.

95   Availing from the (still rare) existence of a multi-generation data set from a wild

96   Atlantic salmon population, the specific aims of this study were to: (1) construct a pedigree

97   based on molecular data, (2) estimate the heritability of both adult body-length and sea-age-

98   at-maturity; (3) test for any genetic correlation between these traits; and (4) test for temporal

99   trends in estimated breeding values for both traits, which would indicate microevolutionary

100   responses over the study period. Significant phenotypic trends in body size and sea-age are

101   apparent in this population during our 1991-2011 study period (Glover, pers comm.) and

102   other populations in the region more generally (Bacon et al. 2009), but it remains unknown

103   whether there is any genetic component to these trends. Answering the latter question could

104   suggest fruitful new avenues for research and interpretation of monitoring data.

105

106   METHODS

107   *Study site*

108   The Girnock Burn (Fig.1) is an intensively studied upland catchment of c. 30 km^2 on the

109   Aberdeenshire Dee (confluence 57 ° 0.6' N, 3° 5.3' W), in north-eastern Scotland, which has

110   been operated by Marine Scotland Science Freshwater Fisheries Laboratory since 1966. Full

111   details of the site, monitoring data and a list of associated publications are available at URL1.

112   In brief, the catchment contains some 8 km of nursery stream for Atlantic salmon ranging in

113   altitude from 230 to 900m. Adult salmon entering the Girnock are caught in an ascending trap

114   near the confluence with the mainstem of the Aberdeenshire Dee, given an individual

115   identification (floy) tag, sexed based on phenotypic characteristics, measured (adult fork

116   body-length, hereafter body-length) and aged by scale-reading, following international

117   protocols.

118   Apart from unusual scale- or tissue-sample loss, the DNA samples obtained from adults can

119   be taken as a complete set of breeders (only one adult is known to have got above the traps

120   without being caught in 50 years, during emergency trap-maintenance during an autumn

121   spate). Since the early 1990s tissue samples (adipose-fin clips) of adults were also taken, and

122   stored in molecular grade (99%) ethanol.

123   The Girnock provided an example of a fully wild Atlantic salmon population from the

124   start of the historic study (1966) until 1999, with adult fish being allowed to spawn naturally

125   above the traps. During the late 1990s, numbers of adult females fell very low (9, 11 & 22

126   over three years), and from 2000 to 2010 increasing proportions of adults were temporarily

127   held, stripped and their eggs stocked into the river section above the trap (see Bacon et al.

128   2015; URL2; Glover, *pers. comm.).* The fish were artificially mated with a crossing protocol

129   designed to increase mating variation and promote the maintenance of broad genetic variation

130   (see Bacon et al. 2015 for full details). From 2011 the system was returned to fully natural

131   spawning (but no offspring from that period returned during this study). Most juveniles from

132   the Girnock undergo parr-to-smolt transformation and seaward migration at age 2+ (third

133   year of life) or 3+ (fourth year of life). A minority of adults return after 1SW, the majority

134   after 2SW, typical of upland Scottish salmon sites (Gurney et al. 2015; URL3). 3SW adults

135   are now uncommon, and repeat spawners are regionally relatively rare (less than 2%, as is

136   typical for eastern Scotland, Bacon et al. 2012). The life span of Atlantic salmon from the

137   Girnock is therefore typically 5 to 6 years, but can range from 4 to 8 years. 1SW adults

138   typically suffer only about half the marine mortality of 2SW fish, but, being around half the

139   size (biomass), female 1SW salmon produce about half as many eggs as 2SW females

140   (Gurney et al. 2012, 2015).

141

DNA samples and microsatellite genotyping

DNA samples were derived from three sources during our study period 1991-2011. In the

mid-1990s, aliquots of genomic DNA extracted during earlier studies were available,

prepared according to protocols given in Taggart et al. (2001). Most of the other samples

from the late 1990s onwards were obtained from adipose fin-clips taken from adults, stored in

molecular grade (99%) ethanol, and with genomic DNA extracted using the Promega,

Wizard® SV 96 Genomic DNA Purification System (www.promega.com) (see Keenan et al.

2013a). The remaining samples, mainly from the early 1990s, plus some fish that were not

adipose-clipped in later years, were obtained from scale samples, archived in paper packets,

air-dried and with the genomic DNA extracted as above. Genomic DNA for all samples was

checked for quality and concentration through visual comparison with a *Hind*III digested ʎ

DNA size standard on Ethidium Bromide stained 0.8% 0.5X TBE agarose gels.

All samples were examined for a panel consisting of 17 putatively neutral

microsatellite marker loci organised in two multiplex PCR. These microsatellites were

selected based on information content (polymorphism), consistent co-amplification reliability

and non-overlapping size compatibility. Multiplex- 1 comprised ten markers and Multiplex 2

consisted of the seven remaining microsatellites (see Appendix A for details); , in addition to

a sex specific marker based on the sdY locus, which is a conserved region for sex

determination in salmonids (Yano et al. 2012, 2013). This later marker was used to double-

check concordance between the biometric records and the genotype results (to guard against

recording errors, including samples potentially omitted from sequences).

PCRs were carried out in 96 well microtitre plates in 3.5μl volumes consisting of 1μl

DNA (~2-5 ng/μl), 1.75μl of 2x PPP Top-Bio mastermix (Top-Bio) and 0.75 μl of a cocktail

165   of ABI fluorescent labelled forward and/or unlabelled reverse 'pig' tailed PCR primers

166   (Appendix A.1 for details on labelled and unlabelled primers, and specific primer

167   concentrations per marker loci). Samples were overlain with 10 µl of mineral oil to prevent

168   evaporation. Thermocycling conditions for both multiplex panels were as follows: an initial

169   denaturation step of 15 min at 95°C, followed by 28 cycles of 95°C for 30 sec, 57°C for 90

170   sec and 72°C for 60 sec. This was followed by a final extension step of 30 min at 60°C. All

171   reactions were carried out using Techne TC-Plus thermal cyclers, with a heated lid at 105°C.

172   Amplified fragments were diluted one-tenth with double-distilled H_2O, and 1µl of this

173   dilution was added to 9 µl of HiDi formamide (Thermo Fisher Scientific) mixed with Gene

174   Scan 600-LIZ size ladder (Thermo Fisher Scientific).

175   Diluted PCR products were analysed on a 96 capillary ABI 3730XL DNA analyser

176   (Thermo Fisher Scientific), and the fragment size analysis (i.e. allelic calls) for genotypes

177   carried out using GENEMAPPERv4.1 (Thermo Fisher Scientific). Genotypes for each

178   microsatellite locus/specimen were individually checked and manually confirmed prior to

179   their addition to an electronic database. Over 50% of the genotyping was independently

180   repeated to ensure consistent scoring (i.e. to minimise scoring errors). Locus-specific

181   statistics including allele numbers, heterozygosity, allelic richness and deviations from

182   Hardy-Weinberg expectations were estimated both on annual caught samples (i.e. candidate

183   parents) and the pooled data set using the R package *diveRsity* (Keenan et al. 2013b). The

184   power of the markers to correctly assign individuals to unique families was carried out using

185   Family Assignment Program (FAP; Taggart 2007).

186

187   *Pedigree reconstruction*

188 Parental cohorts were defined as adults returning to spawn at the Girnock in each autumn. For

189 each such spawning cohort, a sub-set of later returning adults that comprised the full span of

190 their sea-going progeny, by all possible river-age (1 to 4) and sea-age (1 to 3) range

191 combinations, was assembled as putative offspring. For instance, returning adults caught in

192 the trap between 1993 and 1998 were analysed as putative offspring for the 1991 spawning

193 cohort. Pedigree reconstruction was carried out using FAP (Taggart 2007), which estimates

194 exclusion-based family assignment probabilities within family mixtures where all parental

195 genotypes are known. In summary, FAP was used to search all potential offspring for the

196 given spawning cohort and to exclude those that could not derive from a given putative pair

197 of male and female adult parents (based on field-observed sexes). The output was a list of

198 'family-trio' identities (potential female parent, male parent, offspring), together with

199 information on the number of loci matching exactly, the number of evidently mismatching

200 loci, and the list of such mismatching offspring loci. This process was repeated, separately,

201 for each adult cohort from 1991 to 2006 (i.e. for all spawning cohorts for which full-sets of

202 putative returning adult offspring were available in the sample database).

203   The resulting cohort-specific lists of putative family trios were manually examined

204 and any mismatching loci for the putative offspring and its parents were double checked back

205 to the original ABI raw chromatogram data. Most allele mismatches involved single marker

206 loci and resulted from one individual (one parent or the offspring) being incorrectly assigned

207 an adjacent-sized allele, rather than the likely-true parental one. These could be easily

208 rectified following visual inspection. Following this second stage of quality control, the

209 validated family trios were used as the basis for the pedigree. Only complete parent-offspring

210 records were used in this study (a sampled offspring and both its anadromous father and

211 mother), excluding any offspring with only one known adult female parent, presumably sired

212 by an un-sampled precocious parr male. FAP was also used to estimate the 'parental

213   exclusion power' of the data (i.e. power of the markers to correctly assign individuals to a

214   given set of known families).

215

216   *Power analyses for heritability estimation*

217   In order to quantify the statistical power in this dataset to detect true non-zero h^2, a power

218   analysis (see Appendix B.3 for full details) was conducted in which a range of h^2 values were

219   simulated (h^2 = 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, 0.50, 0.75) using the *rbv* function in

220   *MCMCglmm* (Hadfield 2010). One thousand replicate simulations were run for each

221   simulated h^2 value, and each time the estimated h^2 and its associated P-value based on

222   likelihood ratio tests were returned and saved. Power was then calculated as the proportion of

223   P-values that were <0.05.

224

225   *Estimation of quantitative genetic parameters using animal models*

226   Bayesian animal models were used to estimate quantitative genetic parameters of interest

227   using the R package *MCMCglmm* (Hadfield 2010). Full details on the estimation procedures

228   for the quantitative genetic parameters, as well as more details on the pedigree structure, are

229   given in Appendix B.1

230   Univariate animal models for the data were first devised (one trait as a single response

231   variable) to estimate the narrow-sense heritability *(h^2)* of each of the phenotypic traits of

232   interest: adult body-length and sea-age-at-maturity. These univariate models partitioned the

233   total phenotypic variance V_P in each trait into contributions from additive genetic effects

234   (V_A), maternal effects (V_M), year effects (V_Y) and residual effects (V_R), the latter assumed to

235   arise from environmental effects and non-additive genetic effects. Thus $V_P = V_A + V_M + V_Y +$

236   V_R, and $h^2 = V_A/V_P$. A fixed effect of sex (two level factor: adult males and adult females)

237   was included in the univariate models for both traits, to control for the fact that males may

238   mature at a younger age, on average, and hence also are smaller as adults, than females. Year-

239   to-sea (the year the focal individual migrated to sea as a smolt, known from scale readings)

240   was used to define temporal cohorts (i.e. V_Y, see Appendix B.2).

241   Adult body size (measured in centimetres and treated as a Gaussian variable) was first

242   natural logarithm (hereafter simply log) transformed before fitting the animal models, as

243   preliminary exploratory analyses indicated that this transformation minimised autocorrelation

244   between MCMC samples and yielded less skewed posterior distributions of the parameters.

245   In Atlantic salmon, among-individual variation in adult body size is strongly affected

246   by sea-age, as fish that remain at sea an extra year have more time for marine growth. The

247   mean size of 1SW salmon in our 1990-2012 sample, for example, was 55.8 ± 2.1 (S.E.) cm,

248   whereas the mean size of MSW salmon was 68.4 ± 2.1 cm. If sea age itself is heritable (i.e. if

249   the propensity to stay >1 year at sea has a genetic basis), then the h^2 of adult body size as

250   estimated from a univariate animal model will conceptually encompass the effects of genes

251   influencing sea age (which in turn drive size variation among sea-age classes), as well as,

252   genes influencing body size variation within sea-ages. To capture this heritable effect, a new

253   variable "sea-age-corrected body-length", (denoted $\hat{S}_{i,a}$), was constructed as follows:

254
$$\hat{S}_{i,a} = S_{i,a} - \bar{S}_a,$$

255   where $S_{i,a}$ corresponds to the log body-length of individual i of sea-age category a (1SW or

256   MSW) and $\bar{S}_a$ is the mean log-body-length of all individuals of sea-age a. A univariate

257   animal model of $\hat{S}_{i,a}$ was then run (UV model 2) with the same MCMCglmm settings and

258   model structure as UV model 1 to estimate h^2 of body-length conditional on sea-age.

259   The trait sea-age was treated as a binary variable, with a value of 0 corresponding to

260   maturation after one sea-winter and a value of 1 to maturation after two or more sea-winters

261   (178 of the records in the dataset were 1SW adults; among the MSW adults, only 5 out of 323

262   spent three winters at sea, and only one fish spent four winters at sea). A probit link function

263   (family = "threshold" in *MCMCglmm*) was used, where the resulting residual variance refers

264   to the variance of the link function, here fixed at 1 in order to render V_A estimable. The h^2

265   reported from this univariate model of sea-age (UV model 3) thus refers to h^2 on the liability

266   scale, rather than the observed binary scale (see Appendix B.2).

267   In the next step, bivariate animal models (both traits included as response variables)

268   were used to partition the overall phenotypic (co)variance between body-length and sea-age-

269   at-maturity into additive genetic versus environmental (residual) components. Maternal

270   effects and year effects were not included in these models (see Appendix B.1). In the first

271   bivariate animal model (BV model 1), trait 1 was log adult body-length and trait 2 was binary

272   sea-age. Unstructured covariance matrices for the random effects were specified, in order to

273   provide estimates of COV_A and the residual covariance, COV_R. The additive genetic

274   correlation (r_G) between body-length and sea-age was calculated by dividing COV_A by the

275   product of the square root of V_A for each trait. Similarly, the environmental (residual)

276   correlation (r_E) was calculated by dividing the residual covariance by the product of the

277   square root of V_R for each trait. Statistical support for r_G and r_E was assessed by checking

278   whether the 95% highest posterior density (HPD) interval for each included zero.

279   A second bivariate animal model (BV model 2) was run where this time the two

280   response variables were sea-age-corrected body length (on the log scale, i.e. $\hat{S}_{i,a}$) and sea-age.

281   This effectively corrected adult body size for variation due to sea-age, and the resulting r_G

282   and r_E estimates from BV model 2 correspond to the genetic and environmental correlations

283   (respectively) between "body size-within-sea-age" and sea age.

284   All quantitative genetic parameter estimates are reported as posterior means ± HPD intervals.

285

286

287   *Testing for microevolutionary trends*

288   In a quantitative genetic sense, microevolutionary responses are apparent when estimated

289   breeding values (estimates of the additive genetic "merit" of an individual for a given trait)

290   exhibit a temporal trend at the population level. We used the procedure recommended by

291   Hadfield et al. (2010 b) to test for evolutionary change in body-length corrected for sea-age,

292   and in sea-age itself (see Appendix B.4 for full details).

293

294   RESULTS

295   *Population composition and phenotypic trends*

296   During our 1991-2011 study, a total of 1,733 anadromous adults came to the Girnock to

297   spawn [annual average 75.3 (range 29 to 144, total adults)], of which the great majority

298   (97%, range 88% to 100%) in every year, were of known sex, sea-age and river-age. The

299   annual proportion of returning adults that had prior-clipped adipose fins, indicating that they

300   were both reared above the Girnock trap and trapped as juvenile emigrants, averaged 47%

301   (range 31% to 76%). The remaining 53% of unclipped returning adults are assumed to have

302   either: 1) passed over the smolt trap and not been marked; or, more likely, (2) to have been

303   reared below the Girnock trap. If reared below the Girnock trap, they were probably reared

304   outside of the Girnock catchment (only 18% of fully-grown parr are estimated to be reared

305   within the Girnock catchment but also below the Girnock trap, a region comprising just 8%

306   of the Girnock's wetted area (Glover pers comm.)). An annual average of 47% of known-sex

307   adults were females (range 30% to 60%; male% ≈ 100% - female%). During the sub-period

308   of ova-manipulation (2000 to 2010) an average of 80% of females (range 48% to 100%) were

309   artificially spawned. Fluctuations and/or trends in population numbers and composition

310   occurred (see Discussion).

311

312   *Molecular genetics and pedigree reconstruction*

313   The rate of genotyping success was high, with 92% of all individual samples amplifying for

314   at least 14 or more marker loci (i.e. 82% of screened markers per sample). Full genotype

315   information (17 loci) was obtained for 83% of all samples screened. Loci summary statistics

316   are detailed in Appendix A.2. Across the 17 loci, excluding the sex marker, there were, on

317   average across the 20 cohorts, 11.5 alleles per locus per year (± 7.9 S.D., range 2 to 47).

318   Within annual cohorts, observed heterozygosity values averaged 0.748 across loci per year (±

319   0.178 S.D., range 0.150 to 1.000). Allelic richness within loci was similar over all the study

320   years. There was no evidence for departures from Hardy-Weinberg expectations (HWE) or

321   linkage disequilibrium from any cohort.

322   Power analysis indicates that the marker panel (17 loci) used provides over 99% accuracy to

323   identify putative offspring to family. There is only a very minor decrease in power when 14+

324   markers are considered with some variation related to the particular marker loci also being

325   noted (i.e. high versus moderately polymorphic marker).

326   From some 1,600 records of adult genotypes available for paternity assignment during 1991-

327   2011, 273 full parent-offspring trios (mother, father, offspring) were discerned and retained

328   after quality-controlling. 1991 to 1995 and 2007 to 2011 have records of incomplete families

329   (not all eventual 'older' offspring will have returned and been sampled). Inclusion of such

330   records could bias interpretation of the data. The retained complete parentage-records (both

331   parents and all offspring of varying river- and sea-ages) mainly spanned the years

332   1995~2006; these years happened also to span a period of low returning adult numbers.

333   Consequently, the estimated annual values of sea-age and body-length are particularly

334   variable.

335   In order to retain only those parent-offspring trio records that were informative for the

336   quantitative genetic analyses, trios were excluded in which any of the involved individuals

337   were repeat-spawners (i.e. had spawned in previous years); the sex of either parent was

338   unknown; body-length was not measured; or neither sea-age nor river-age were readable from

339   scales (see below). This rigorous filtering reduced the number of useable trios from an initial

340   336 to 276 (82% retained).

341   After such filtering, the years of spawning for both putative parents matched in every case.

342   For one of the 276 trios, a missing value for the offspring's sea-age was estimated based on

343   its year of spawning, its known river-age, and the pedigree-determined year of spawning of

344   its parents. Similarly, for 14 of the 276 trio records, the offspring's river-age was estimated

345   from its year of spawning, its known sea-age, and the pedigree-determined year of spawning

346   of its parents. The offspring's total scale-read age closely matched its pedigree-determined

347   total-age in most cases: in 78% of cases (216/276 trios) the match was exact; in 19% the age-

348   comparisons differed by only a single year (53/276); the remaining seven age-discrepancies

349   comprised four records where age-comparisons differed by 2-years; one at 3-years; and two

350   at 5-years. The latter three records involving offspring-ageing discrepancies of >2 years were

351   removed, resulting in 273 rigorously useable parental-trio records.

352   Further details on the pedigree are given in Table 1 and Appendix B.1.

353

354   *Animal models*

355 Convergence was good for all animal models (results not shown). The univariate model of

356 body-length (UV model 1) showed that additive genetic effects (V_A) accounted for

357 approximately one-quarter of the total phenotypic variance (Table 2, h^2 of log adult body-

358 length = 0.27). Of the remaining variance (i.e. not including V_A), maternal effects (V_M)

359 accounted for approximately 13% (9% of the total phenotypic variance V_P), year-to-sea

360 effects (V_Y) for 15% (11% of V_P) and residual environmental effects (V_R) for 72% (53% of

361 V_P). Males were on average smaller (61.62 ± 0.58 cm) than females (66.10 ± 0.33cm). When

362 body-length was corrected for sea-age (UV model 2), V_A then accounted for a considerably

363 smaller fraction of V_P (Table 2, h^2 of body length within sea-ages = 0.14). V_M accounted for

364 approximately 14% of V_P, V_Y for 9%, and V_R accounted for the remaining 64%. The

365 univariate animal model of sea-age (UV model 3) showed that V_A accounted for a little over

366 half of V_P (Table 2, h^2 of sea-age = 0.51). Of the remaining variance (i.e. not including V_A),,

367 V_M accounted for approximately 10% (5% of V_P), V_Y for 44% (20% of V_P) and V_R for 46%

368 (21% of V_P).

369 The results of the bivariate animal model of adult body length and sea-age (BV model

370 1) mirrored those of the univariate animal models in terms of the estimated heritabilities for

371 the two traits (h^2 of adult body length = 0.26 and h^2 of sea-age = 0.48 in BV model 1; Table

372 3). The estimated genetic correlation between these traits was very high in the bivariate

373 model where body length was not adjusted for sea age (r_G = 0.96; Table 3). The results of the

374 bivariate animal model of sea-age-corrected body length and sea-age (BV model 2) also

375 largely mirrored those of the univariate models in term of the estimated heritabilities for the

376 two traits, although the h^2 of sea-age-corrected body length was somewhat lower (h^2 of sea-

377 age-corrected body length = 0.06 and h^2 of sea-age = 0.53 in BV model 2; Table 3). The

378 estimated genetic correlation between these traits was positive (r_G = 0.33) but the HPD

379   intervals overlapped zero (Table 3), indicating that the r_G was not significantly different from

380   zero.

381   A power analysis showed that the Girnock data had sufficient power to detect $h^2 >$

382   0.22 with >80% confidence with no inherent bias in the data-set (Fig.2).

383

384   *Testing for microevolutionary trends*

385   The temporal trend in estimated breeding values for the trait "sea-age corrected body-length"

386   was estimated at -0.0001 log(cm)/year (95% quantiles: -0.0008 to 0.0004). This trend in

387   estimated breeding values was not statistically significantly negative, as only 67.4% of the

388   posterior distribution of estimated temporal slopes was less than zero. The probability that

389   this very small trend in estimated breeding values was more negative than one would expect

390   based on random genetic drift was 63.3%, again indicating a lack of any evidence for a

391   microevolutionary response to selection. .

392   The temporal trend in estimated breeding values for the trait sea-age was estimated at

393   -0.017 liability units per year (95% quantiles: -0.053 to 0.009). This trend in estimated

394   breeding values was not statistically significantly negative, as only 81.8% of the posterior

395   distribution of estimated temporal slopes was less than zero. The probability that this trend

396   was more negative than one would expect based on random genetic drift was 74.3%, again

397   indicating a lack of solid evidence for a microevolutionary response to selection.

398

399   DISCUSSION

400   Our data from a well-studied, single, wild population of Atlantic salmon show that sea-age

401   and body-length are both quite strongly heritable and, importantly, that the two traits are

402   strongly genetically correlated. However, when sea-age is alternatively correlated to body-

length *within* each sea-age class (i.e. sea-age corrected body-length), the genetic correlation is then not significantly different from zero, and nor is the h^2 for sea-age-corrected body-length, implying that individual variation in size within sea-ages does not have a heritable basis. Most studies estimate quantitative genetic parameters in laboratory, or in the case of fish, aquaculture settings, which may not reveal much about evolutionary potential under wild natural conditions (Charmantier and Garant 2005). Our results thus add important general information regarding the potential for wild fish populations to respond to natural selection (or "unnatural selection", c.f. Allendorf & Hard 2009) and complement recent findings showing strong genetic components to age and size at maturity in Atlantic salmon (e.g. Ayllon et al. 2015, Barson et al. 2015, Lepais et al. 2017).

Inheritance patterns

Substantial amounts of additive genetic variance in sea-age were not entirely unexpected, given that recent studies of Atlantic salmon discovered several genetic loci underpinning variation in sea-age, including a single gene (*VGLL3*) that explains almost 40% of the total phenotypic variation (Ayllon et al. 2015, Barson et al. 2015). These studies used a very different approach – genome-wide associations combining data from a large number of populations across a broad geographic region – and homed-in on specific quantitative trait loci (QTL), whereas we followed a classical quantitative genetics approach that treats the underlying genetic architecture as a "black box" and focussed on a single population. The concordant inferences between studies conducted at very different scales using different methodologies – namely, that sea-age variation in wild Atlantic salmon populations has a strong genetic basis –points towards a general finding for the species. Studies of other fish species have also documented heritable differences in age-at-maturity within and among populations (e.g. mosquitofish *Gambusia affini* introduced to Hawaii, (Stearns 1983); guppies *Poecilia reticulata* in Trinidad, (Reznick et al. 1990); Chinook salmon *Oncorhynchus*

428 *tshawytscha* introduced to New Zealand, (Quinn et al. 2001). This raises interesting questions

429 regarding the evolutionary forces that maintain genetic variation in this key life-history trait,

430 which may include sex-dependent dominance (Barson et al. 2015), frequency-dependent

431 selection and spatiotemporal variation in environmental selection pressures (Gurney et al.

432 2012). In this study we ignored potential sex-specific inheritance patterns, which is justified

433 if inter-sex genetic correlations for our traits of interest are positive and high, but this

434 assumption should be tested in future studies, given sufficient statistical power. It is also

435 possible that the period of captive propagation might have had an influence on the results.

436 The crossing protocol, which involved mating multiple males to females and vice versa, may

437 have increased the number of links in the pedigree beyond that which might have occurred in

438 the absence of captive propagation, in which case it could have afforded increased power and

439 accuracy to our animal models. On the other hand, this represented a deviation from natural

440 spawning behaviours and mate choice, which may have affected the expression of genetic

441 variation (Pigliucci 2006), but we have no way of knowing *a priori* the direction and

442 magnitude of such influences.

443 Despite these strong genetic effects, environmental influences clearly also play a role;

444 in our study, for example, approximately half of the variation in sea-age was attributable to

445 environmental variation, although some of this may have been due to non-additive genetic

446 effects (dominance and epistasis) which we could not separate out with our pedigree structure

447 (indeed, Barson et al. 2015 showed that dominance effects occur at the *VGLL3* locus). To put

448 this in practical terms, we observed over our study period that of the 114 offspring in total

449 produced by matings between two MSW parents, 84 (74%) of the resulting offspring

450 themselves returned as MSW; thus a high heritability "biases" this probability towards "like

451 producing like". Friedland & Hass (1996) found that the fraction of 1SW adult returns from a

452 hatchery-dependent stock of Atlantic salmon was positively associated with late summer

453   marine growth, implying that age-at-maturity responds plastically to marine environmental

454   conditions. The literature suggests that freshwater conditions may also affect the sea-age of

455   anadromous Atlantic salmon, as implied by positive correlations between smolt size and the

456   proportion of fish returning after 1SW (O'Connell and Ash 1993, Salminen 1997). Similarly,

457   inverse relationships between freshwater age and ocean age have been found in wild

458   steelhead trout *Oncorhynchus mykis* (Ward and Slaney 1988). However, if sub-populations

459   are spatially structured, as in the North Esk in Scotland (Gurney et al., 2015; Bacon et al.

460   2012), with higher fractions of 1SW fish in lower parts of the catchment, where smolts also

461   tend to get larger quicker (e.g. due to better growth at higher temperatures), then such

462   correlations would arise from the spatial structuring alone, even if freshwater conditions have

463   no causal effect on sea-age variation. Thus it remains unclear to what extent such patterns

464   reflect plastic responses, spatial structuring, or genetic correlations among traits expressed at

465   different life stages.

466   Genetic covariance among suites of growth-related and life-history traits have been

467   demonstrated in rainbow/steelhead trout (*Oncorhynchus mykiss*) (Hecht et al. 2015) and

468   brook charr (*Salvelinus fontinalis*) (Thériault et al. 2007), while some QTL for early male

469   maturation status in Atlantic salmon also collocated with those affecting spring weights of

470   juveniles (Lepais et al. 2017). We lacked individual-level data on freshwater or marine

471   growth rates, and hence could not test explicitly for plastic effects of specific traits/cues on

472   sea-age, nor could we test for genotype-by-environment interactions, which may be important

473   here. Lepais et al. (2017) recently demonstrated that early male maturation in Atlantic salmon

474   emerges as an interaction between individual growth rate (an environmentally-sensitive status

475   trait) and a genetically variable maturation threshold (underpinned by at least three detectable

476   QTL), using a powerful new approach called the 'latent environmental threshold model"

477   (LETM). It would be very interesting to apply the LETM approach to future studies of sea-

478 age variation in Atlantic salmon or other anadromous fish; obtaining individual-level data on

479 relevant status traits, such as post-smolt growth, that may act as cues for sea-age decisions

480 will be challenging in many situations, but circuli spacing on scales could be used, for

481 example (e.g. Friedland & Hass 1996). Furthermore, it would be very interesting to dissect

482 the mechanisms by which genetic variation in sea-age comes about; for example, Scottish-

483 origin Atlantic salmon that eventually become MSW evidently use different oceanic feeding

484 areas (e.g. the waters off Greenland) than those than adopt the 1SW life history (which are

485 not recorded as going to Greenland, e.g. Malcolm et al. 2010), raising the intriguing

486 possibility that genetic variation in migration routes and/or destinations might partially

487 explain variation in sea-age, although the correlation between sea-age and migration patterns

488 may not reflect any causal connection between these traits.

489　　　　The significant positive genetic correlation we documented between sea-age and

490 overall body-length is also consistent with the results of Barson et al. (2015), who

491 documented pleiotropic effects of specific genomic regions on sea-age and size-at-maturity.

492 Such pleiotropic effects are inevitable to a degree, in that alleles predisposing individuals

493 towards later maturation at older sea-ages should also produce larger fish, given that Atlantic

494 salmon keep growing the longer they remain at sea. A more surprising result was the fact that

495 the genetic correlation between sea-age and body-length was so high ($r_G = 0.96$) as to imply

496 that these are effectively the same trait at a genetic level. That does not mean, however, that

497 body size is completely genetically-determined and indeed, in our case, it appears that

498 individual variation in marine growth – a key determinant of body size at breeding – was

499 predominantly environmental, rather than genetic, in origin. This is because sea-age-

500 corrected body-length exhibited a low h^2 that did not differ significantly from zero (although

501 we only had the statistical power to detect $h^2 >$ ca. 0.22, so a true h^2 of less than this cannot be

502 ruled out). While this trait is not a direct measure of marine growth, Atlantic salmon put on

503   most of their growth at sea (smolt sizes are very small, see URL4) and thus variation in

504   marine growth rates and/or variation in coastal return-times must explain a large fraction of

505   the variation in size of returning adults within each sea-age class. Smolt size could also

506   indirectly account for some of the variation in adult size if, for example, larger smolts grow

507   faster at sea (e.g. due to feeding advantages of initially larger size within shoals of post-

508   smolts, which would set up a positive feedback).

509   A lack of (strong) genetic influences on marine growth is somewhat unexpected,

510   given that genetic variation could operate via a range of mechanisms here; e.g. some

511   genotypes could be better foragers, or target different prey types, or have different inherited

512   marine migration pathways or destinations, that expose them to more or less growth

513   opportunity, or be more efficient at converting food into somatic growth, etc. Moreover,

514   return migration timing has been shown to have a heritable basis in Atlantic salmon (Stewart

515   et al. 2002; Cauwelier et al. In Press) and other salmonids (Smoker et al. 1998, Quinn et al.

516   2000, O'Malley and Banks 2008, Kovach et al. 2012). This alone could produce

517   corresponding genetic variation in body-length within sea-age classes, unless return periods

518   were very short or if late-returning genotypes have lower marine growth rates than early-

519   returning genotypes, such that final size differences among them are minimal. At present we

520   can only speculate on this, as we do not have reliable information on coastal return-dates to

521   estimate the h^2 of that trait and potential genetic correlations with body size, or indeed sea-

522   age. Our tentative conclusion at this point is that environmental drivers of body-length

523   variation within each sea-age class are simply much larger in magnitude than any genetic

524   influences.

525   Our tests for microevolution during the period 1990~2012 did not reveal any

526   genetically based trends towards reduced average sea-age, nor towards smaller body-size.

527   However, over the longer period 1966~2016, Atlantic salmon from two Deeside streams (the

528   Girnock and Baddoch) have shown a significant decline in MSW numbers whilst 1SW

529   numbers remained stable, implying a significant decrease in average sea-age ratios, slightly

530   reducing the average sea-age; they also showed concomitant downward trends in body size,

531   which were coherent for both 1SW and MSW fish, (Glover et al. 2018 and *pers comm*). It

532   may simply be that the phenotypic trends documented by Glover et al. are entirely driven by

533   phenotypic plasticity, i.e. environmental influences on average sea-age and body size, rather

534   than microevolutionary responses to any directional selection pressures that might have

535   occurred over this period. Mortality pressures from both marine fisheries and freshwater

536   angling were strong on Scottish Atlantic salmon during the period 1960 to 1990, which

537   conceivably could have led to fisheries-induced selection (FIS) on, and subsequent evolution

538   of, sea-age and/or body size. The data analysed in this study fall mainly into a period when

539   marine fishery pressures were much reduced and freshwater angling pressures decreasing. As

540   evolutionary recovery of phenotypic traits from FIS is expected to be much slower than

541   declines caused by it (Heino et al. 2015), our findings with respect to microevolution could

542   simply reflect a period when any real changes were quite small and too low for our sample

543   sizes to detect. Moreover, the current study was focussed on a shorter time period and

544   involved smaller sample sizes than the Glover et al. study, and thus the power to detect a real

545   but small-magnitude evolutionary response may have been limiting. A further complicating

546   factor is that broad-scale climatic changes also occurred over this period, which may have

547   contributed to the observed phenotypic trends in Girnock salmon (Glover et al. 2018), either

548   via phenotypic plasticity, microevolution, or both. Given the apparently very low heritability

549   of body size within sea-ages, phenotypic plasticity – possibly driven by climate change –

550   represents the most parsimonious explanation for the observed length decreases in 1SW and

551   MSW Girnock salmon. The fact that these trends are parallel between sea-ages, despite

552   differing marine feeding zones, may point towards coherent climate effects acting across a

553   large geographic scale, but factors other than climate may of course be at play. A clearer

554   understanding of the relative roles of microevolution and plasticity and their

555   environmental/anthropogenic drivers could be achieved by extending this pedigree study over

556   a longer period to match the longer-term phenotypic data..

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576   Author Contributions

577   The Girnock Atlantic Salmon Pedigree project was envisaged by PJB and organised by him

578   and PMcG, in collaboration with PP, JG and CRP. FFL contributed the tissue-samples and

579   biometric-data. DNA extraction and genotyping were undertaken at QUB by CB and PP. PP

580   and PJB merged the biometric and genotype data, PP undertook the parentage-assignments

581   and he and PJB thereby quality-controlled the original genotype scorings. PJB extracted and

582   quality-controlled the merged sub-set of data for this paper, including preliminary analyses.

583   TR fitted and interpreted the animal models. PP wrote the Methods and Results for parentage

584   analyses. TR and PJB wrote the Methods and Results for the quantitative genetic analyses

585   and an outline for the paper. All authors contributed to the final draft

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587

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Appendices:

Appendix A:

      A.1 Microsatellite marker information

      A.2 Summary sample statistics per locus / cohort

Appendix B:

      B.1 Details on the reconstructed pedigree

      B.2 Full details on the estimation procedures for the quantitative genetic parameters

      B.3 Power analyses for heritability estimation

      B.4 Testing for microevolutionary trends

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796 **Table 1** Summary of annual sample sizes of adult Atlantic salmon within the reconstructed

797 Girnock pedigree. Column 2 shows the total number of individual fish spawning in that year

798 (Column 1) that appeared either as parents or offspring within "parent-offspring trios" (where

799 a trio represents one offspring and both its genetically-assigned parents). For example, an

800 adult returning in 1996 could be recorded as being in a trio in that year (i) because it mated

801 with another fish in that year and produced at least one surviving offspring that itself was

802 DNA-sampled as a spawning adult (e.g. six years later in 2002); (ii) because it was itself

803 assigned two parents (which might have spawned and been DNA-sampled in 1991, for

804 example), or (iii) for both reasons (i.e. some fish appear in the pedigree as both offspring and

805 parents). Columns 3 and 4 break Column 2 down by sex. Column 5 specifies how many of

806 the fish enumerated in Column 2 were themselves assigned two parents, while Column 6

807 specifies how many were assigned no parents.

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Adult Return Year	Total	Females	Males	Number with both[†] parents known	Number with neither parents known
1991	**8**	5	3	0[††]	8
1992	**9**	4	5	0	9
1993	**6**	3	3	0	6
1994	**2**	1	1	0	2
1995	**13**	7	6	0	13
1996	**12**	8	4	7	5
1997	**6**	3	3	3	3
1998	**10**	6	4	6	4
1999	**15**	6	9	5	10
2000	**28**	11	17	8	20
2001	**10**	6	4	5	5
2002	**11**	3	8	4	7
2003	**13**	5	8	5	8
2004	**51**	28	23	9	42
2005	**40**	25	15	14	26
2006	**46**	28	18	9	37
2007	**23**	13	10	5	18
2008	**19**	7	12	14	5
2009	**37**	14	23	37	0
2010	**60**	25	35	60	0
2011	**65**	42	23	65	0
Grand totals:	**484**	**250**	**234**	**256**	**228**

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818 † Note that this pedigree study focussed only on "full trios", i.e. cases where both parents of a given offspring
819 were assigned. An unknown fraction of offspring each year are sired by precociously maturing males, which are
820 not DNA-sampled and therefore cannot be assigned as true fathers, but the numbers here refer only to full
821 parent-offspring trios.

822 ††Note that prior to 1996, the parents of sampled spawning adults were not discernible as these parents would
823 have themselves spawned prior to 1991, when DNA samples were not available.

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830 **Table 2** Results of the univariate animal models. The values for adult body-length are on the

831 natural logarithm scale, while the values for sea-age-at-maturity are on the underlying

832 liability scale from the probit model (note the residual variance was fixed at 1). The intercept

833 refers to the fixed effect of the reference sex, females. HPD = highest posterior density.

Model	Parameter	Mean	Lower HPD interval	Upper HPD interval
(*UV model 1*) Univariate for adult body-length	Intercept (females)	4.197	4.173	4.2223
	Sex: males	-0.072	-0.090	-0.049
	Additive genetic variance V_A	0.0039	0.0016	0.0062
	Maternal variance V_M	0.0014	0.0002	0.0029
	Year variance V_M	0.0016	0.0004	0.0033
	Residual variance V_R	0.0078	0.0058	0.0101
	Heritability h^2	0.265	0.125	0.414
(*UV model 2*) Univariate for sea-age-corrected body length	Intercept (females)	0.000	-0.0148	0.0136
	Sex: males	0.0179	-0.0048	0.0307
	Additive genetic variance V_A	0.0008	0.0003	0.0015
	Maternal variance V_M	0.0008	0.0003	0.0015
	Year variance V_M	0.0005	0.0001	0.0010
	Residual variance V_R	0.0037	0.0030	0.0045
	Heritability h^2	0.140	0.044	0.261
(*UV model 3*) Univariate for sea-age-at-maturity	Intercept (females)	2.207	1.299	3.363
	Sex: males	-2.511	-3.647	-1.539
	Additive genetic variance V_A	2.580	0.114	6.143
	Maternal variance V_M	0.212	0.000	0.809
	Year variance V_M	0.937	0.081	2.158
	Residual variance V_R	1.000	1.000	1.000
	Heritability h^2	0.511	0.267	0.734

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845 **Table 3** Results of the bivariate animal models of (1) adult body-length and sea-age (binary

846 trait), and (2) sea-age-corrected body length and sea-age. The values for adult body-length

847 and sea-age-corrected body length are on the natural logarithm scale, while the values for

848 sea-age are on the underlying liability scale from the probit model (note that V_R for sea-age

849 was fixed at 1). The fixed effects are not reported as they mirror the findings of the univariate

850 models.

Model	Parameter	Mean	Lower HPD interval	Upper HPD interval
(*BV model 1*) Bivariate for adult body-length and sea-age	V_A for body length	0.0042	0.0019	0.0065
	V_A for sea-age	1.0914	0.1436	2.2986
	Additive genetic covariance COV_A	0.0633	0.0171	0.1157
	V_R for body length	0.0116	0.0095	0.0138
	V_R for sea-age	1.0000	1.0000	1.0000
	Residual covariance COV_R	0.0635	0.0471	0.0791
	Heritability h^2 for body length	0.2637	0.1261	0.3878
	Heritability h^2 for sea-age	0.4823	0.2174	0.7284
	Genetic correlation r_G	0.9551	0.9096	0.9879
	Environmental correlation r_E	0.5893	0.4586	0.6834
(*BV model 2*) Bivariate for adult body-length within sea-ages, and sea-age	V_A for body length within sea-ages	0.0004	0.0002	0.0007
	V_A for sea-age	1.5715	0.1436	2.2986
	Additive genetic covariance COV_A	0.0079	-0.0077	0.0256
	V_R for body length within sea-ages	0.0069	0.0061	0.0078
	V_R for sea-age	1.0000	1.0000	1.0000
	Heritability h^2 for body length within sea-ages	0.0560	0.0295	0.0976
	Heritability h^2 for sea-age	0.5347	0.2316	0.8495
	Genetic correlation r_G	0.3334	-0.2604	0.7205

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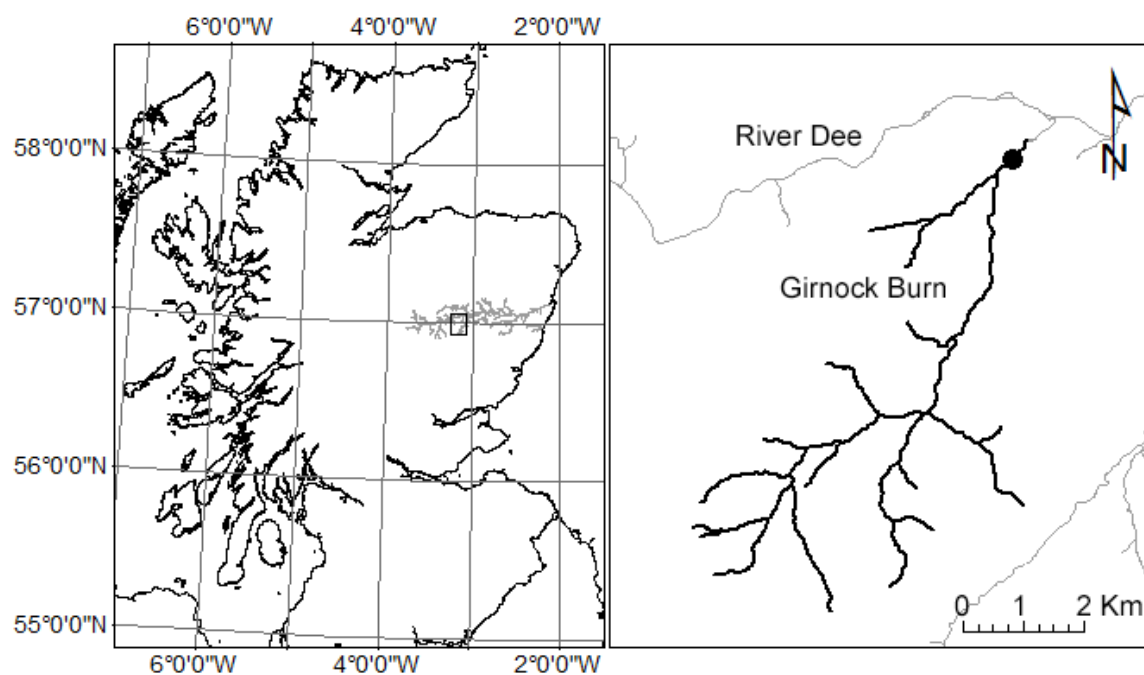
Fig.1 Map of the Girnock Burn study system, a tributary of the River Dee, Aberdeenshire,

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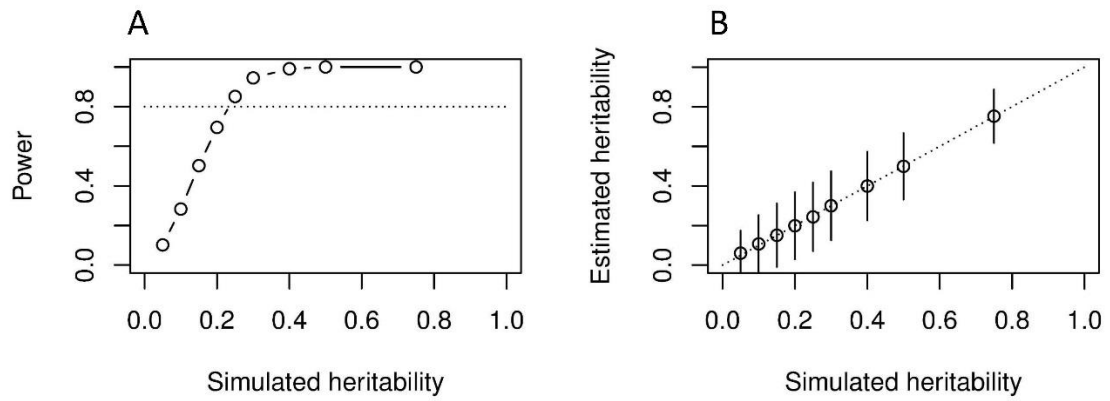
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878 **Fig.2** Power analysis for heritability estimation for an arbitrary trait given the observed

879 pedigree and phenotypic data structure, with statistical power (A) and estimated heritability

880 (mean plus 95% confidence intervals, B) plotted against simulated heritability. Dotted line in

881 A corresponds to a power of 80%; dotted line in B shows the 1:1 line.