1 **Cognitive ability is heritable and predicts the success of an**

2 **alternative mating tactic**

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9

9   **Summary**

10   The ability to attract mates, acquire resources for reproduction, and successfully outcompete

11   rivals for fertilisations may make demands on cognitive traits - the mechanisms by which an

12   animal acquires, processes, stores, and acts upon information from its environment.

13   Consequently, cognitive traits potentially undergo sexual selection in some mating systems.

14   We investigated the role of cognitive traits on the reproductive performance of male rose

15   bitterling (*Rhodeus ocellatus*), a freshwater fish with a complex mating system and alternative

16   mating tactics. We quantified the learning accuracy of males and females in a spatial learning

17   task and scored them for learning accuracy. Males were subsequently allowed to play the

18   roles of a guarder and a sneaker in competitive mating trials, with reproductive success

19   measured using paternity analysis. We detected a significant interaction between male mating

20   role and learning accuracy on reproductive success, with the best performing males in maze

21   trials showing greater reproductive success in a sneaker role than as a guarder. Using a cross-

22   classified breeding design, learning accuracy was demonstrated to be heritable, with

23   significant additive maternal and paternal effects. Our results imply that male cognitive traits

24   may undergo intra-sexual selection.

25   **Keywords:** alternative mating tactics, cognition, learning, mating system, sexual selection

26   **Running title**: Cognition and male reproductive success

1. Introduction

Cognitive traits, the neuronal processes concerned with acquiring, processing, retaining, and using information [1-3], equip an individual with the capacity to solve novel tasks. Cognitive traits may be favoured under natural selection if they influence individual fitness, for example through enhanced foraging ability or food caching [1]. Cognitive ability may also potentially undergo sexual selection [4,5]. Sexual selection is selection that acts on differences in fitness among individuals due to the number and identity of their mates, and is a powerful form of selection that shapes mating system evolution [6]. Hitherto, sexual selection has not been considered a major driving force in the evolution of cognition [1]. However, cognitive traits have the potential to influence mate finding, mate choice or success in fertilization, depending on the selective forces generated by a particular mating system. Variance in male reproductive success is typically greater than in females and often results in the evolution of elaborate phenotypic traits in males. In the case that cognitive traits undergo sexual selection, a prediction is that selection for cognitive ability will be stronger in males than females, in the same way that other traits, such as nuptial coloration, weapons or alternative mating behaviours, are typically more strongly expressed in males than in females. For selection to act on cognitive traits, there must also be heritable variation with consequences for fitness [3,4,7].

Some recent studies have implicated a role for male cognitive abilities in the capacity to attract mates, successfully father offspring and efficiently perform parental care, particularly in birds [8-12]. In *Drosophila melanogaster* it was shown that male cognitive traits were eroded under relaxed sexual selection, demonstrating a potentially significant role of cognition in the mating system of this species [13]. The results of this study imply a heritable basis to cognitive traits, and that their expression can carry a fitness cost in some circumstances. However, little emphasis has so far been placed on several key aspects of

52   cognition, namely understanding how cognitive traits evolve, how they are associated with

53   reproductive success, or what trade-offs constrain their evolution.

54   Here we investigated the role of cognitive ability in the mating system of rose

55   bitterling (*Rhodeus ocellatus*), freshwater fish that spawn their eggs in the gills of living

56   mussels. Dominant males aggressively defend territories to monopolize mussels and perform

57   elaborate courtship towards females [14,15]. Females place their eggs into the gills of a

58   mussel through its exhalant siphon. Males fertilize the eggs by ejaculating over the inhalant

59   siphon of the mussel, with water filtered by the mussel carrying the sperm to the eggs. Pre-

60   oviposition ejaculations, whereby males release sperm over a mussel before a female spawns,

61   are common. Bitterling spermatozoa remain viable within a mussel gill for an unusually

62   prolonged period and are capable of fertilizing eggs at least 14 minutes after ejaculation

63   [16,17]. The risk of sperm competition in bitterling is high [18]. Males that control access to

64   mussels enjoy high reproductive success and male dominance is determined by body size

65   [19,20], with smaller, subordinate males adopting alternative mating tactics. The dominant

66   'guarder' and subordinate 'sneaker' mating tactics are not fixed and male mating behaviour is

67   opportunistic, with males capable of playing either role depending on environmental context

68   [21,22], which is the typical situation in teleost fishes [15]. The sneaker tactic commonly

69   involves pre-oviposition ejaculations [14,15,17], with male reproductive success determined

70   by how males distribute their ejaculates among mussels in relation to the distribution of

71   spawning females, the spatial distribution of mussels, and the distribution of rival ejaculates

72   [17], while minimizing the risk of sperm depletion [18].

73   The bitterling mating system, therefore, would appear to favour males that can match

74   their mating role to the local environment, and that have the spatial cognitive ability to

75   distribute their ejaculates optimally among mussels to maximize their reproductive success

76   [17]. In particular, sexual selection for spatial cognition would be predicted to operate more

77   strongly on males in a sneaker role, since guarder males typically enjoy higher fertilization

4

78   success than sneakers, with sneaker success linked more directly to an ability to anticipate

79   spawning events with pre-oviposition ejaculations. Here we examine the potential role of

80   cognitive traits in mating system evolution by testing the predictions that: 1. performance in a

81   spatial learning task predicts male reproductive success; 2. spatial cognitive ability favours

82   males performing alternative mating tactics; 3. performance in a spatial learning task is

83   heritable.

84

85   2. Materials and methods

86   (a) Study system

87   Experimental *R. ocellatus* used in trials were the second generation descended from 200 wild-

88   caught fish from the River Yangtze basin, China. During the experiment they were 18 months

89   old. Prior to experiments, fish were housed in stock aquaria measuring 600 (length) x 300

90   (width) x 400 (depth) mm. For learning trials, groups of 16 randomly selected males and

91   females were taken from stock aquaria. The same 16 males were subsequently used in

92   competitive mating trials to assess the reproductive success of males of known performance

93   in spatial learning ability. To quantify the heritability of spatial cognition, males and females

94   from the same stock as the learning and mating trials were used, though not the same

95   individuals. Stock and holding aquaria, as well as maze and mating trial aquaria, were all

96   housed in an environmentally controlled room with a 16:8 h light: dark cycle at 23 °C. Fish

97   were fed a mixture of commercial dried fish flake food and bloodworm (*Chironomus* spp.)

98   twice daily.

99

100   (b) Learning accuracy

101   Learning accuracy was quantified using maze apparatus for fish [19,20], comprising a square

102   plastic box with opaque walls measuring 500 (length) x 500 (width) x 300 (depth) mm. A

103   central chamber (300 x 300 mm) with opaque walls was connected to four outer chambers by

104   separate 50 mm wide openings (figure S1*a*). The walls of each outer compartment were a

105   different colour, either red, blue, green or black, to act as clear landmarks for navigation. The

106   choice of experimental colours was based on cyprinid colour vision, which is tetrachromatic

107   [21]. Each outer chamber had a 40 mm diameter Petri dish placed at its farthest corner. A

108   webcam, connected to a laptop computer, was suspended directly above the maze so that a

109   fish in the maze could be observed remotely without disturbance.

110   Test fish were individually housed in holding aquaria measuring 300 (length) x 200

111   (width) x 220 (depth) mm. Each fish was randomly assigned to either the red, blue, green or

112   black chamber as a reward chamber. To measure learning accuracy, each fish was given a

113   single familiarity trial before testing. A food reward of 5-8 live whiteworms (*Enchytraeus*

114   spp.) was placed in the Petri dish in the test chamber to which the test fish was assigned. To

115   control for the effect of olfactory cues, water in which whiteworms were stored, and infused

116   with their odour, was pipetted into all test chambers immediately prior to each test. The test

117   fish were gently transferred to a clear plastic release cylinder in the central compartment of

118   the maze and allowed at least 2 min. to settle. The cylinder was then raised remotely, freeing

119   the fish and enabling it to explore the maze. All fish located and ate the food reward within

120   two hours of release.

121   On the day following their familiarity trial, the fish was returned to the release

122   cylinder for the start of the trial proper. A record was kept of the frequency with which the

123   fish made an error and entered a chamber without a food reward, scored as occasions when

124   the fish passed at least halfway through the chamber entrance. If after 10 min. the fish had not

125   located the food reward, it was gently guided into the rewarded chamber with a hand net and

126   allowed to feed. After feeding, fish were transferred back to their holding aquaria. Fish were

127   not fed prior to testing and so were motivated to locate the food reward. Every fish was tested

128   once each day for 7 days, with the total number of errors over this period summed as a

129   learning accuracy score. After completion of 7 trials, fish were measured for Standard Length

130   (SL; tip of the snout to the base of the tail fin).

131

132   ## (c) Emergence trials

133   To control for the potentially confounding effects of variation in 'shyness-boldness' *sensu*

134   [23], a behavioural assay was performed. The assay measured time to emerge from a refuge

135   (for simplicity, hereafter referred to as 'emergence time'), estimated as the time taken for an

136   individual to emerge from shelter in a novel environment [23].

137   Tests followed an established protocol [24] and were conducted in glass aquaria

138   measuring 300 (length) x 200 (width) x 200 (depth) mm. Test aquaria had a gravel substrate

139   and water to a depth of 150 mm. The aquarium was bisected with a sliding opaque partition

140   placed 100 mm from one end. Artificial plants that reached the water surface were placed

141   behind the screen to provide a refuge area of dense submerged vegetation. The remaining 200

142   mm section of the aquarium was bare of cover (figure S1*b*). Fish were gently released into the

143   vegetated end of the test aquarium. After a 5 min. acclimation period, the partition was raised

144   allowing the fish to explore the whole aquarium. An observer recorded the time taken for the

145   fish to emerge a full body length from the vegetated end into the open part of the aquarium. A

146   fish that failed to emerge within 10 min. was assigned a score of 600s. After completion of a

147   trial, the test fish was returned to its holding aquarium. On the following day, the fish was

148   retested using the same protocol. Repeatability was high ($r = 0.61$). The mean of the two

149   emergence scores was used as an index of shyness-boldness for that individual. After

150   completion of trials, males were retained singly in their holding aquaria for testing in

151   competitive mating trials. Females were returned to stock aquaria and were not used further.

152

153   ## (d) Competitive mating trials

154   The reproductive success of the 16 males used in learning and emergence trials was measured

155   in mating trials by permitting them to compete with rivals for fertilizations, acting in the role

156   of both a guarder and a sneaker. Rivals were from the same stock of fish, but played no other

157   role in the study. Trials were performed in an aquarium measuring 1250 (width) x 300

158   (length) x 300 (depth) mm. Two size-matched *Unio pictorum* mussels were placed in sand-

159   filled cups and situated at each end of the aquarium. The aquarium had a sand substrate and

160   was furnished with 20 artificial plants, distributed haphazardly, to add environmental

161   heterogeneity.

162   The focal male was gently released into the aquarium and randomly assigned either a

163   guarder or a sneaker role. In the case the male was to play the role of a guarder, a rival male

164   was released into the test aquarium that was 20% (by SL) smaller than the focal male. If the

165   male was to play the role of a sneaker, the rival was 20% larger. Male mating role in *R.*

166   *ocellatus* is determined by relative body size [25]. The two males were left for 24 h to

167   establish dominance roles. In every case the larger male played the role of guarder and the

168   smaller individual acted as a sneaker during matings. After 24 h, a female in reproductive

169   condition (with an extended ovipositor) was selected from a stock tank and gently released

170   into the aquarium. After 1 h (which is sufficient time for repeated spawning acts) the female

171   was captured, measured and a small portion of the tail fin was removed and fixed in 95%

172   ethanol. A second female was then released and the process repeated.

173   After completion of a trial, a finclip was taken from the focal and rival male and fixed

174   in ethanol for paternity analysis. The focal male was returned to his holding aquaria. The

175   Total Length of the mussels was measured and their gills checked for eggs. If eggs were

176   present, the mussel was dissected and the eggs allowed to develop in a water-filled 70 mm

177   diameter Petri dish in an incubator at 23 °C for five days. After five days, the embryos were

178   fixed in ethanol for parentage analysis. Rival males and females were released in stock tanks

179   and were not used again in trials.

180   Focal males were again tested a minimum of two days after the first trial (mean ± s.d.

181   of 2.8 ± 1.0 days), but in the opposite role to the one they played in their first trial. Thus, focal

182   males that had played a guarder role subsequently played the role of a sneaker, and *vice versa*,

183   with the order in which they played these roles randomised and using a new rival male and

184   pair of females. Finclips were collected from the rival male, both females, and fertilized eggs

185   were incubated and subsequently fixed in ethanol. It was not necessary to finclip focal males

186   again. At completion of trials, all 16 focal males had engaged in competitive matings with a

187   rival in both a guarder and sneaker role. The study generated a total of 439 fertilized embryos.

188   Of these, a total of 416 embryos (95%) survived five days to fixation in ethanol, with a mean

189   (± SE) of 13.0 (± 1.2) embryos per trial.

190

191   **(e) Parentage analysis**

192   For parentage analysis, DNA was extracted from ethanol preserved tissue using established

193   methods [26]. A set of 8 microsatellite loci [26,27] was chosen on the basis of their variability

194   and informative value and combined in two multiplex PCR reactions, with a mean of 13

195   (range: 6-23) alleles per locus. The length of the DNA fragments was analysed using

196   GeneMapper® software. DNA was successfully extracted and analysed for a total of 408

197   embryos. Of these, paternity was assigned with 95% confidence for 364 embryos in Cervus

198   3.0 (error rate set to 0.01) [28]. In one replicate, only three eggs were recovered and data for

199   this replicate were excluded from the subsequent analysis. All other embryos were included in

200   analyses, with a mean of 12 (range: 5-25) embryos per replicate. For one male, a fin clip from

201   a rival male was not properly fixed when the focal male played a guarder role. In this case

202   paternity could only be estimated with 95% certainty for the male in a sneaker role but not as

203   a guarder. Because of the paired nature of our subsequent analysis, this replicate was excluded

204   from the dataset.

205

(f) Heritability: *in vitro* fertilisations

The heritability of learning accuracy was measured using a North Carolina Type II breeding design using *in vitro* fertilizations (IVF) to generate a series of replicated half-sib families [29]. Eight blocks, each with a set of 2 × 2, male × female, factorial crosses were conducted using fish from the same stock. Within each block, both males were crossed with both females, with a replicate of each cross. This design generated 2 replicates of 4 families of maternal and paternal half-siblings, in each of eight blocks, with a total of 64 replicated families in the final combined design. A comparable design was used successfully in previous heritability studies with *R. ocellatus* [30], and permits the relative contribution of additive and nonadditive genetic effects for a trait of interest to be measured, and to identify maternal and paternal contributions to additive genetic variance.

To generate crosses, experimental females were isolated until they ovulated a batch of eggs; obvious from the female's extended ovipositor. The eggs were gently stripped from the female and divided into approximately two equal groups in separate 70 mm diameter Petri dishes containing freshwater (mean = 8.0 ± 3.1 s.d. eggs per group). Sperm was stripped from the two experimental males by gently pressing their abdomens and mixed in 9 ml of teleost saline [31]. A 1 ml subsample of this sperm solution was diluted with a further 9 ml of saline. Sperm suspensions were pipetted over the eggs and the covered Petri dishes were left on the laboratory bench for 30 min. The fertilized eggs were washed in freshwater and the long axis of every egg measured under a binocular microscope (Nikon Eclipse E200) with an eyepiece micrometer to the nearest 0.1 mm. They were subsequently incubated at 23 °C until the yolk sac was absorbed and the fish began exogenous feeding, a period of approximately 30 days. A daily record was kept of embryo survival. After the onset of exogenous feeding, fish were transferred in family groups to aquaria measuring 300 (length) x 200 (width) x 210 (depth) mm and fed twice daily on formulated zebrafish granules, supplemented with live *Artemia*.

(g) Heritability: offspring learning accuracy

Offspring learning accuracy was assayed after approximately 12 weeks (mean = 86.3 ± 2.9 s.d. days) with a simplified version of the learning trials used for adults. A simplified design was used to facilitate screening of a large number of fish. Mean (± s.e.) offspring SL at this age was 21.8 (± 1.9) mm. A single fish was tested from each family generated from factorial crosses, with 64 fish tested in total. Fish were sexually immature and were selected randomly from each family.

Learning accuracy was measured in a series of dichotomous choice chambers. These comprised a glass aquarium measuring 300 (length) x 200 (width) x 220 (depth) mm containing 7 L of fresh water. Halfway along the aquarium there was a sliding partition that retained the test fish in the rear portion of the aquarium (figure S1*c*). The front of the aquarium was partitioned into two 70 x 100 mm choice chambers, with 40 mm openings at each side. A single 30 mm diameter Petri dish was situated immediately inside each choice chamber, such that they could not be seen from outside the chamber. A red and blue plastic marker, measuring 5 x 5 mm, was attached to the front of the aquarium, so that it was visible to the test fish from the rear of the aquarium as landmarks for navigation. The side of the aquarium on which the blue or red markers were attached was randomised. Test fish were randomly assigned to either the red or blue chamber as a reward chamber.

To minimise isolation stress while confined at the rear of the aquarium, fish were able to see neighbours in adjacent aquaria. However, the front half of aquaria were screened from neighbours with an opaque barrier. Thus, when making the decision to enter the test chambers at the front of the test aquarium, the fish were visually isolated from their neighbours. This conformation ensured fish were not visually isolated between trials, but could not be influenced by the behaviour of neighbours during trials.

To measure learning accuracy, fish were initially given a familiarity trial. Test fish were introduced to the test chamber on the day prior to the start of trials, but not fed. The

258   following day, a food reward of 5-8 live whiteworms was placed in the Petri dish in the test

259   chamber to which the test fish was assigned (red or blue). To control for the effect of potential

260   olfactory cues, water in which whiteworms were stored, and infused with odour, was pipetted

261   into both chambers. The central partition was raised and the fish allowed to explore both

262   chambers and feed on the whiteworms. All fish located and ate the food reward within two

263   hours of release.

264   On the day following the familiarity trial, the fish were confined behind the central

265   partition. Whiteworms were replaced in the Petri dish and the partition removed. A record

266   was kept of the frequency with which the fish made an error and entered a chamber without a

267   food reward, scored as occasions when the fish passed at least halfway through the chamber

268   entrance. If after 10 min. the fish had not located the food reward, it was gently guided into

269   the rewarded chamber with a hand net and allowed to feed. After feeding, fish were confined

270   at the back of the aquarium, behind the partition. Fish were not fed prior to testing and so

271   were motivated to locate the food reward. Every fish was tested once each day for 6 days,

272   with the total number of errors over this period summed as a learning accuracy score.

273

274   (h) Statistical analysis

275   Before applying statistical models a data exploration was undertaken following the protocol

276   described in [32]. The data were examined for outliers in the response and explanatory

277   variables, homogeneity and zero inflation in the response variable, collinearity between

278   explanatory variables and the nature of relationships between the response and explanatory

279   variables. Data analyses were performed using R [33].

280   Sex difference in learning accuracy was modelled using a generalised linear model

281   (GLM) with log-link function to preclude negative fitted values. Assuming estimates of

282   learning accuracy ($accuracy_i$) were Poisson distributed with mean μ_i, the model contained a

283   linear effect for fish length (*sl*), emergence time (*emg*) and sex (*fSex*) as main terms and took

284   the form:

285

286        $accuracy_i \sim Poisson(\mu_i)$

287        $E(accuracy_i) = \mu_i$ and $var(accuracy_i) = \mu_i$

288        $\eta_i = \beta_1 + \beta_2 \times sl_i + \beta_3 \times emg_i + \beta_4 \times fSex_i$

289        $log(\mu_i) = \eta_i$

290

291   Male reproductive success from competitive mating trials was modelled using a

292   binomial generalised linear mixed model (GLMM) with an observation level random

293   intercept. Male length (SL) was collinear with emergence time, and rival SL was collinear

294   with male mating role. Consequently emergence time and rival SL were dropped from the

295   analysis [32]. The model took the form:

296

297        $success_{ij} \sim Binomial(\pi_{ij}, N_{ij})$

298        $E(success_{ij}) \sim N_{ij} \times \pi_{ij}$

299        $var(success_{ij}) \sim N_{ij} \times \pi_{ij} \times (1 - \pi_{ij})$

300        $\eta_{ij} = \beta_1 + \beta_2 \times sl_{ij} + \beta_3 \times mussel_{ij} + \beta_4 \times fRole_{ij} : \beta_5 \times accuracy_{ij} + m_i + \varepsilon_{ij}$

301        $logit(\pi_{ij}) = \eta_{ij}$

302        $m_i \sim N(0, \sigma^2_{male})$

303        $\varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$

304

305   *Success$_{ij}$* is the reproductive success in the *j*th competitive mating trial for male *i* out

306   of the N_{ij} trials conducted. π_{ij} is the probability of successful fertilisation of eggs by male *i* in

307   the *j*th competitive mating trial. The model contained a linear effect for male SL (*sl*) and

308   mussel length (*mussel*) as main terms and an interaction between male mating role (*fRole*) and

309   learning accuracy (*accuracy*). The random intercept m_i was included to introduce a correlation

310   structure between observations for the same male. ε_{ij} is an observation level random effect to

311   accommodate overdispersion in the data [32,34]. Continuous covariates were standardized to

312   enhance numerical optimisation of the model [32]. In the model, the interaction between male

313   role and learning accuracy measured the contribution of learning accuracy to reproductive

314   success in the roles of guarder and sneaker. As an additional measure of this effect, the

315   difference in reproductive success of males playing a sneaker compared with guarder role was

316   correlated with learning accuracy.

317   The colour of the test chamber in learning trials, and the order in which males played

318   either a sneaker or guarder role in competitive mating trials made no significant contribution

319   to models and were dropped from analyses.

320   In the analysis of the heritability of learning accuracy, two-way ANCOVA was used

321   for each 2×2 factorial block to compare effects of sire, dam, and their interaction on learning

322   accuracy at 12 weeks. Under this design the *k*th offspring phenotype from cross $i \times j$ takes the

323   form:

324

325          $z_{ijk} = \mu + s_i + d_j + I_{ij} + e_{ijk}$

326

327   μ is the mean population phenotype and s_i and d_j are the additive effects on phenotype from

328   the *i*th male (sire effect) and *j*th female (dam effect) respectively. I_{ij} is the non-additive sire $\times$

329   dam interaction and e_{ijk} is the deviation of observed phenotype of the *k*th offspring of male *i*

330   and female *j* from model predictions, and comprises phenotypic variance resulting from

331   segregation, dominance and environment [29]. The model assumes within family variance is

332   uncorrelated with among family variance, with total phenotypic variance the sum of sire, dam,

333   interaction and error variance:

334

335 $$\sigma^2_z = \sigma^2_s + \sigma^2_d + \sigma^2_I + \sigma^2_e$$

336

337 Sums of squares were combined to calculate mean squares and degrees of freedom for all

338 families combined [29]. Because the amount of egg yolk can significantly affect offspring

339 fitness [15,35], egg size was included as a covariate in the analysis as a maternal effect.

340 Narrow-sense heritability (h^2) was estimated as V_A/V_P, where V_A is additive genetic variance

341 and V_P total phenotypic variance [29].

342

343 2. Results

344 (a) Sex differences in learning accuracy

345 Males showed significantly better learning accuracy in maze trials than females (table 1).

346 Mean (± s.e.) learning accuracy score for males was 8.6 (± 0.74) errors and for females 11.0

347 (± 0.84). There was no significant effect of fish SL or emergence time (table 1) on learning

348 accuracy, and no sex difference in body size ($t_{30} = 1.47$, $p = 0.153$) or emergence time ($t_{30} =$

349 1.30, $p = 0.202$).

350

351 (b) Male reproductive success and learning accuracy

352 There was a significant interaction between male mating role and learning accuracy in

353 predicting reproductive success in competitive mating trials (table 2). When males played a

354 sneaker role, learning accuracy predicted mating success, but not as a guarder (figure 1).

355 There was a significant correlation between the difference in reproductive success in the

356 sneaker role compared with territorial (sneaker - territorial) with learning accuracy ($t_{13} = 2.68$,

357 $p = 0.019$). Overall, male reproductive success was significantly higher in a dominant guarder

358 role than as a subordinate sneaker (table 2), though some males performed better in the role of

359 sneaker than as a guarder (figure 2). Mean mussel size weakly predicted male reproductive

360 success (table 2). There was no significant contribution of male SL to reproductive success
361 (table 2).

362

363 (c) Heritability of learning accuracy

364 Learning accuracy showed significant additive male and female effects (table 3). There was
365 no male × female interaction on learning accuracy. Narrow-sense heritability of learning
366 accuracy was estimated as $h^2 = 0.27$.

367

368 4. Discussion

369 Darwin was the first to recognize that cognitive traits potentially undergo sexual selection [6].
370 Our results demonstrated a link between male performance in a spatial task and reproductive
371 success, which depended on mating context. Accuracy of learning predicted the reproductive
372 success of males adopting sneaky mating tactics, but not the success of males playing a
373 dominant, guarder mating role. We also measured significant paternally and maternally
374 inherited additive genetic variance for learning accuracy, raising the possibility that spatial
375 cognition may undergo sexual selection in rose bitterling.

376   Our findings implicate a possible causal link between male performance in a spatial
377 learning task in the capacity to fertilize the eggs of females in a competitive environment, and
378 especially in the role of a sneaker. The mechanism by which spatial cognition might
379 contribute to male reproductive success was not directly measured in this study. However,
380 male reproductive success in bitterling is closely linked to the way the male distributes
381 ejaculates in space and time, particularly for sneakers [16,17,26,36,37]. In nature and in the
382 lab, male bitterling systematically patrol mussels in their own territory, as well as those of
383 their neighbours, examine the exhalant siphons of mussels and frequently ejaculate over them
384 (termed pre-oviposition ejaculation) [14,15]. Non-territorial males also engage in the same

385 behaviour, which takes place even in the absence of females, though the presence of a female

386 who is ready to mate significantly increases the rate of male inspection and ejaculation

387 [17,37]. Males appear to obtain information about the presence of their own, and possibly

388 rival spermatozoa by examining mussel siphons, which may provide them with cues about

389 how to distribute their sperm among mussels [17,37,38]. To maximize their reproductive

390 success, males must anticipate female oviposition decisions, as well as the ejaculatory

391 behaviour of rivals, often among numerous mussels distributed over a wide area, and place

392 their sperm into particular mussels at appropriate time intervals to minimize their risk of

393 sperm depletion [17,18,38,39]. Males also modulate ejaculation size [39-41] in response to

394 the intensity of sperm competition. Thus in the bitterling mating system, optimizing the size,

395 distribution and timing of ejaculates may impose cognitive demands on males, particularly

396 those playing sneaker mating tactics, which selects for enhanced spatial cognitive ability.

397   For selection to operate on cognitive traits a requirement is that they must show

398 heritable variance. The heritability of cognitive ability has rarely been estimated, though

399 where it has, it appears to be significant [4,5]. In humans, more than half of individual

400 differences in intelligence are attributed to additive genetic variation [42,43]. For other taxa,

401 systematic analyses of cognition are lacking [3-5,7]. Our estimates of heritability of

402 performance in a spatial learning task in rose bitterling indicated that approximately one

403 quarter of variance in learning accuracy was heritable. The heritability of learning accuracy

404 was wholly additive, and both maternally and paternally inherited. Significant additive

405 variance for learning accuracy implies that the trait would respond positively to directional

406 sexual selection. It also implies that selection on spatial learning in the study population has

407 not been consistently strong, since variance in a trait is typically depleted under strong

408 positive selection [29]. However, while spatial cognitive ability may enhance fitness in some

409 circumstances, the evolution of cognitive traits face constraints and need not always

410 experience positive selection. Thus there are potential trade-offs between the fitness benefits

411 of enhanced cognitive performance, and costs associated with cognitive traits [44]. In a wild

412 population of great tits (*Parus major*), parents that were able to solve a cognitive task

413 produced larger clutches than those that failed to solve the task [9]. Task solvers spent less

414 time foraging and foraged over a smaller area than non-solvers, implying that they were more

415 efficient foragers than non-solvers. However, solvers were more sensitive to disturbance and

416 were more likely to desert offspring. The result was that, on average, solvers and non-solvers

417 fledged a similar number of offspring. In a lab population of the guppy (*Poecilia reticulata*),

418 artificial selection on brain size enhanced cognitive ability in females, but not males, despite

419 brain size responding to artificial selection in both sexes. Larger-brained individuals paid a

420 fitness cost in term of producing fewer offspring, potentially as a trade off between

421 energetically expensive brain tissue and investment into other organs [45]. In the present

422 study, cognitive traits were favoured when males played a sneaker role, but not in a guarder

423 role, implying there may be a trade-off in the traits that make a successful sneaker and

424 guarder. The guarder role in bitterling typically generates higher reproductive success than the

425 sneaker role, though this varies with fish density [16,26].

426   A prediction from our results is that those males suited to a guarder role will have

427 greater reproductive success at low densities, where the reproductive success of guarder is

428 known to be greatest [16,26]. In contrast, males with superior cognitive ability would be

429 predicted to perform better at high male densities, where sperm competition and male ability

430 to optimally distribute their ejaculates plays a more critical role in male reproductive success

431 [16,26,36,46,47]. A predicted outcome is that selection on male cognitive traits will vary

432 among populations, and within populations among breeding seasons, thereby maintaining

433 variance for cognitive traits. Bitterling populations occur at highly variable densities [48] and

434 males exhibit wide variation in behavioural and morphological traits [36], offering

435 exceptional material for examining selection on cognitive traits in nature. Further

436 investigation of the role of cognitive traits in species that express alternative mating tactics

437 will demonstrate the generality of our conclusions for other mating systems. It would also be

438 informative to examine domain specificity in rose bitterling and establish whether learning

439 accuracy can predict enhanced fitness in other contexts.

440   In conclusion, this study demonstrates a potential role for spatial cognitive traits in the

441 mating system of a fish. Male performance in a spatial learning task showed additive genetic

442 variance and may undergo intra-sexual selection, particularly under environmental conditions

443 that favour the expression of alternative mating tactics. This is the first non-human study to

444 show genetic variance for spatial cognitive ability with a direct link to reproductive success.

459

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459

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567

567 **Table 1.** Summary of the generalised linear model for Poisson distributed data to

568 examine sex differences in learning accuracy in rose bitterling (*Rhodeus ocellatus*).

569 N_{obs} = 32.

570

Model parameter	Estimate	s.e.	z	P
Intercept	1.510	0.591	2.55	0.011
sl	0.014	0.010	1.36	0.171
emg	-0.001	0.001	-0.32	0.750
sex$_{(female)}$	0.289	0.127	2.27	0.023

571

572 **Table 2.** Summary of the generalised linear mixed-effects model for binomial

573 distributed data to examine mating role differences in the reproductive success of

574 male rose bitterling (*Rhodeus ocellatus*). Individual males were fitted as random

575 intercepts, with standard deviation of 0.75. An observation level random intercept

576 was included in the model with standard deviation of 0.43. N_{obs} = 30.

577

Model parameter	Estimate	s.e.	z	P
Intercept	-0.223	0.297	-0.77	0.441
role$_{(guarder)}$	1.054	0.327	3.23	0.001
accuracy	-0.739	0.372	-1.97	0.047
sl	0.560	0.307	1.82	0.068
mussel	0.434	0.200	2.17	0.030
role$_{(guarder)}$ x *accuracy*	0.754	0.335	2.25	0.024

578

579

579 **Table 3.** ANCOVA for rose bitterling (*Rhodeus ocellatus*) offspring learning accuracy for *in*

580 *vitro* fertilizations.

581

Source	df	SS	MS	F	p	variance	%
Egg size	8	5417	677.1	1.88	0.118	39.6	8
Female (F)	8	6741	842.6	3.74	0.040	30.2	6
Male (M)	8	10964	1370.4	6.09	0.010	63.1	13
F x M	8	1801	225.2	0.63	0.747	0	0
Error	21	7561	360.1			360.1	73

582

583 **Figure Legends**

584 **Fig. 1.** Fitted values for male reproductive success against standardized learning accuracy

585 scores for males playing a guarder and sneaker role in competitive mating trials modelled

586 using a binomial GLMM. Grey bands indicate 95% confidence intervals around the fitted line.

587 Black circles are observed values for male reproductive success. Note that a low

588 standardized learning score indicated completion of the maze task with few errors.

589 **Fig. 2.** Observed reproductive success of males playing a guarder and sneaker role in

590 competitive mating trials. Linked black circles are the same individual, open circles represent
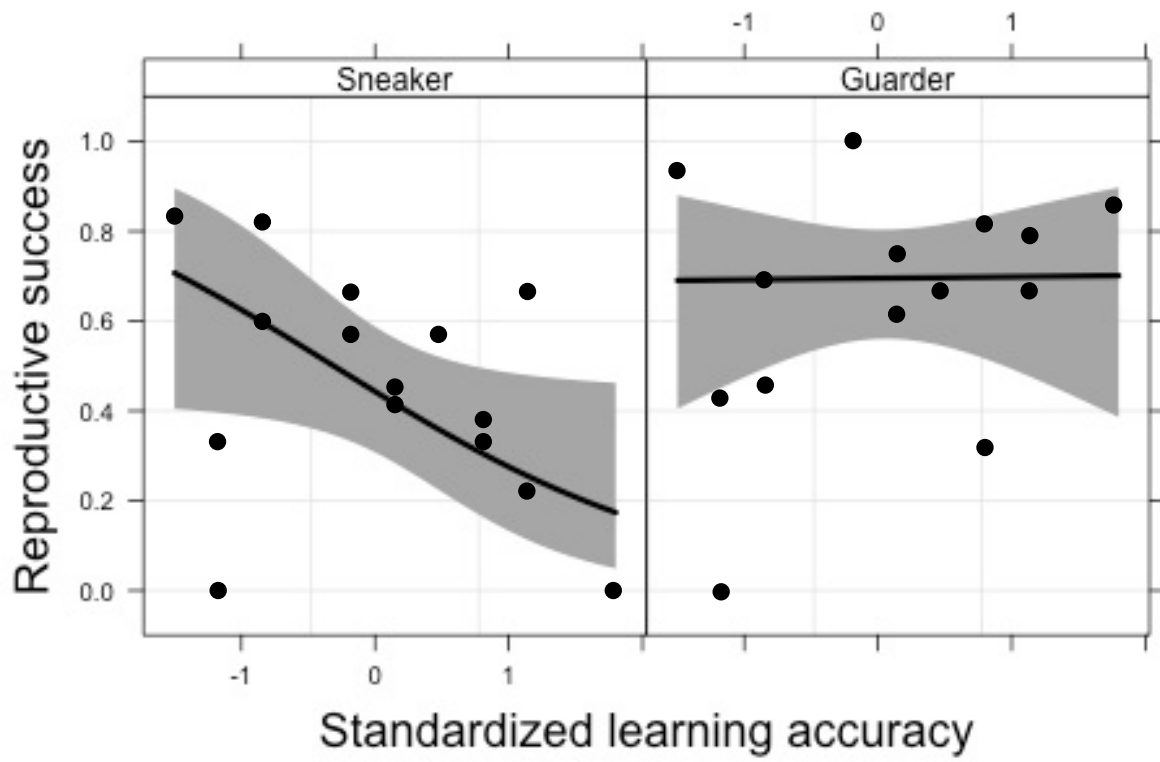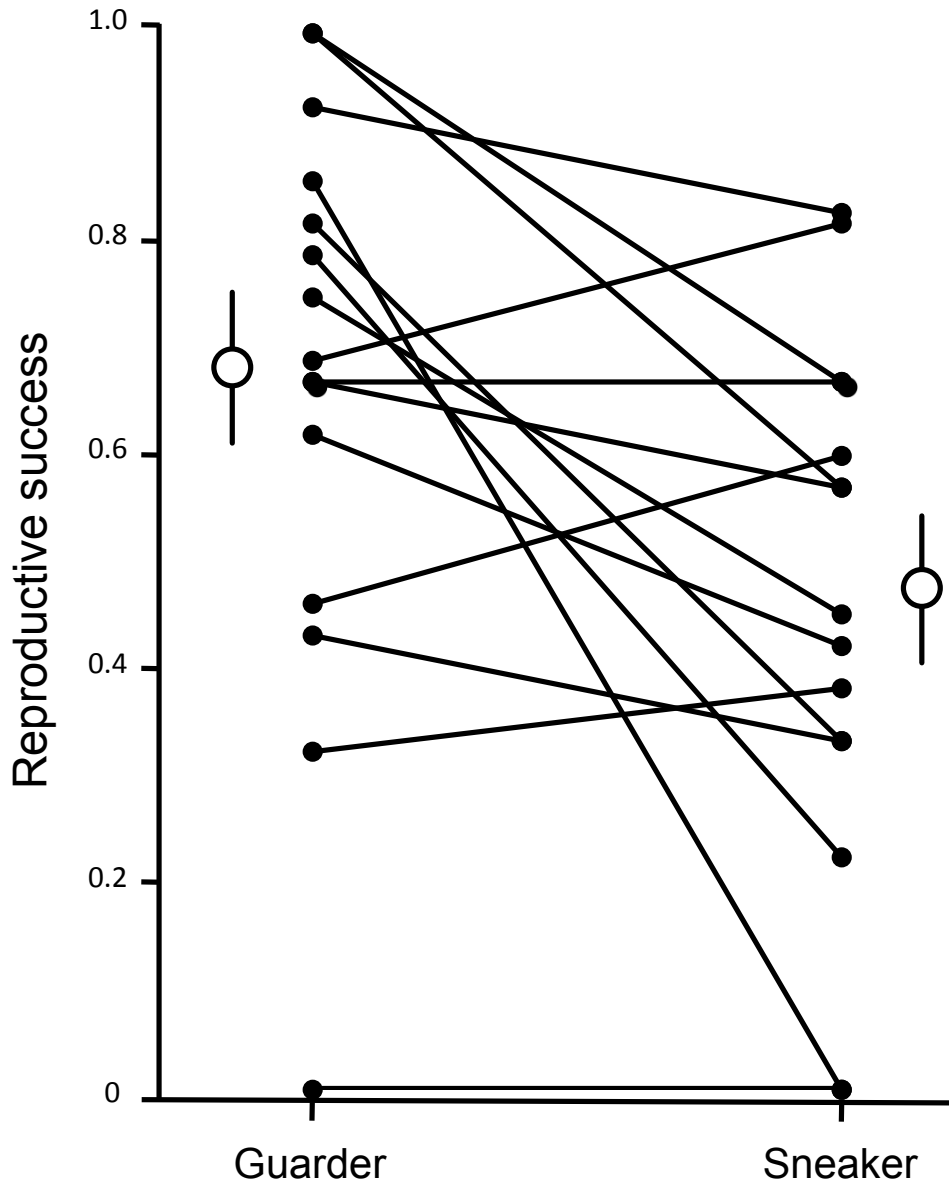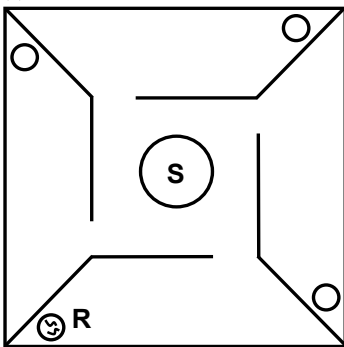
591 mating role means (± s.e.).

Figure 1

Figure 2

Cognitive ability is heritable and predicts the success of an alternative mating tactic

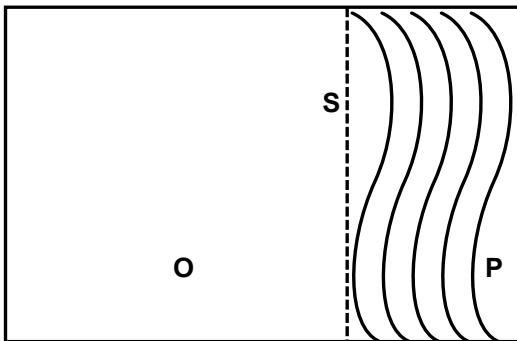C. Smith *et al.* Online Appendix

1. Supplementary figures

Figure S1. Diagrams showing plan view of test apparatus for: (*a*) adult maze trials, showing release cylinder (S) in central compartment and Petri dish containing food (R) in reward compartment; (*b*) test tank for measuring emergence speed, showing refuge area containing artificial plants (P), sliding opaque partition (S), and open area (O); (*c*) juvenile maze trials, showing removable barrier (S) and Petri dish containing food (R) in reward compartment.

(*a*)

(*b*)

(*c*)