

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

## 27 1. Introduction

28 Cognitive traits, the neuronal processes concerned with acquiring, processing, retaining, and  
29 using information [1-3], equip an individual with the capacity to solve novel tasks. Cognitive  
30 traits may be favoured under natural selection if they influence individual fitness, for example  
31 through enhanced foraging ability or food caching [1]. Cognitive ability may also potentially  
32 undergo sexual selection [4,5]. Sexual selection is selection that acts on differences in fitness  
33 among individuals due to the number and identity of their mates, and is a powerful form of  
34 selection that shapes mating system evolution [6]. Hitherto, sexual selection has not been  
35 considered a major driving force in the evolution of cognition [1]. However, cognitive traits  
36 have the potential to influence mate finding, mate choice or success in fertilization, depending  
37 on the selective forces generated by a particular mating system. Variance in male reproductive  
38 success is typically greater than in females and often results in the evolution of elaborate  
39 phenotypic traits in males. In the case that cognitive traits undergo sexual selection, a  
40 prediction is that selection for cognitive ability will be stronger in males than females, in the  
41 same way that other traits, such as nuptial coloration, weapons or alternative mating  
42 behaviours, are typically more strongly expressed in males than in females. For selection to  
43 act on cognitive traits, there must also be heritable variation with consequences for fitness  
44 [3,4,7].

45         Some recent studies have implicated a role for male cognitive abilities in the capacity  
46 to attract mates, successfully father offspring and efficiently perform parental care,  
47 particularly in birds [8-12]. In *Drosophila melanogaster* it was shown that male cognitive  
48 traits were eroded under relaxed sexual selection, demonstrating a potentially significant role  
49 of cognition in the mating system of this species [13]. The results of this study imply a  
50 heritable basis to cognitive traits, and that their expression can carry a fitness cost in some  
51 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

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 S1a). 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  $\pm$  s.d.  
181 of  $2.8 \pm 1.0$  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 ( $\pm$  SE) of  $13.0 (\pm 1.2)$  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

206 (f) Heritability: *in vitro* fertilisations

207 The heritability of learning accuracy was measured using a North Carolina Type II breeding  
208 design using *in vitro* fertilizations (IVF) to generate a series of replicated half-sib families  
209 [29]. Eight blocks, each with a set of  $2 \times 2$ , male  $\times$  female, factorial crosses were conducted  
210 using fish from the same stock. Within each block, both males were crossed with both  
211 females, with a replicate of each cross. This design generated 2 replicates of 4 families of  
212 maternal and paternal half-siblings, in each of eight blocks, with a total of 64 replicated  
213 families in the final combined design. A comparable design was used successfully in previous  
214 heritability studies with *R. ocellatus* [30], and permits the relative contribution of additive and  
215 nonadditive genetic effects for a trait of interest to be measured, and to identify maternal and  
216 paternal contributions to additive genetic variance.

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

231

232 (g) Heritability: offspring learning accuracy

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

239 Learning accuracy was measured in a series of dichotomous choice chambers. These  
240 comprised a glass aquarium measuring 300 (length) x 200 (width) x 220 (depth) mm  
241 containing 7 L of fresh water. Halfway along the aquarium there was a sliding partition that  
242 retained the test fish in the rear portion of the aquarium (figure S1c). The front of the  
243 aquarium was partitioned into two 70 x 100 mm choice chambers, with 40 mm openings at  
244 each side. A single 30 mm diameter Petri dish was situated immediately inside each choice  
245 chamber, such that they could not be seen from outside the chamber. A red and blue plastic  
246 marker, measuring 5 x 5 mm, was attached to the front of the aquarium, so that it was visible  
247 to the test fish from the rear of the aquarium as landmarks for navigation. The side of the  
248 aquarium on which the blue or red markers were attached was randomised. Test fish were  
249 randomly assigned to either the red or blue chamber as a reward chamber.

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

256 To measure learning accuracy, fish were initially given a familiarity trial. Test fish  
257 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  $\mu_i$ , 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

$$286 \quad accuracy_i \sim Poisson(\mu_i)$$

$$287 \quad E(accuracy_i) = \mu_i \text{ and } var(accuracy_i) = \mu_i$$

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

$$289 \quad \log(\mu_i) = \eta_i$$

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

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

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

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

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

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

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

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

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.  $\pi_{ij}$  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.  $\varepsilon_{ij}$  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  $2 \times 2$  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

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

326

327  $\mu$  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 
$$\sigma_z^2 = \sigma_s^2 + \sigma_d^2 + \sigma_I^2 + \sigma_e^2$$

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 ( $\pm$  s.e.) learning accuracy score for males was 8.6 ( $\pm$  0.74) errors and for females 11.0  
347 ( $\pm$  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  $\times$  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.

445 **Ethic statement.** All work was approved by the ethical committees of the IVB (No. 163-12)  
446 and complies with the legal regulations of the Czech Republic.

447 **Data accessibility.** The data associated with this paper are available on dryad  
448 (doi:10.5061/dryad.hs31q).

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453 **Author contributions.** M.R. and C.S. conceived and designed the experiments, conducted  
454 data analyses and wrote the paper. A.P. and C.S. performed the experiments. M.R. conducted  
455 genetic analyses. All authors reviewed and approved the paper.

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458 **Conflict of interest.** We have no competing interests.

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  $N_{\text{obs}} = 32$ .  
 570

Model parameter	Estimate	s.e.	<i>z</i>	<i>P</i>
Intercept	1.510	0.591	2.55	0.011
<i>sl</i>	0.014	0.010	1.36	0.171
<i>emg</i>	-0.001	0.001	-0.32	0.750
<i>sex</i> <sub>(female)</sub>	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.  $N_{\text{obs}} = 30$ .

577

Model parameter	Estimate	s.e.	<i>z</i>	<i>P</i>
Intercept	-0.223	0.297	-0.77	0.441
<i>role</i> <sub>(guarder)</sub>	1.054	0.327	3.23	0.001
<i>accuracy</i>	-0.739	0.372	-1.97	0.047
<i>sl</i>	0.560	0.307	1.82	0.068
<i>mussel</i>	0.434	0.200	2.17	0.030
<i>role</i> <sub>(guarder)</sub> × <i>accuracy</i>	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	<i>F</i>	<i>p</i>	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 ( $\pm$  s.e.).

Figure 1

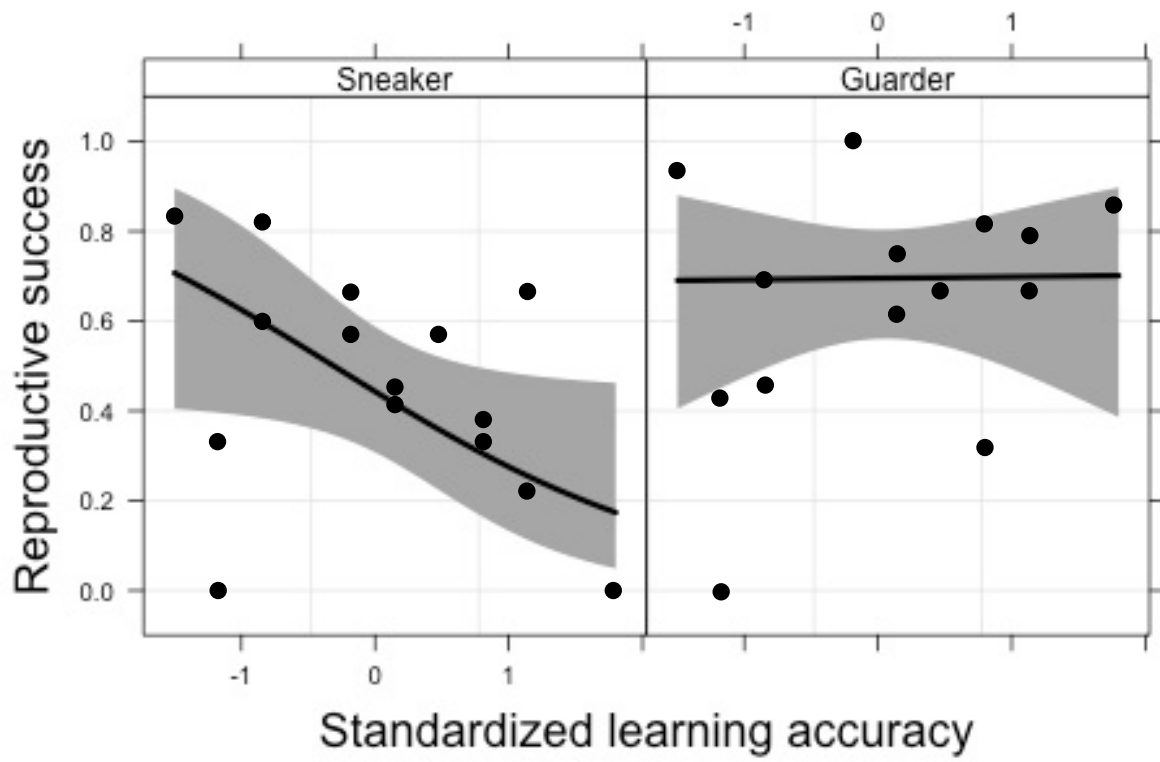
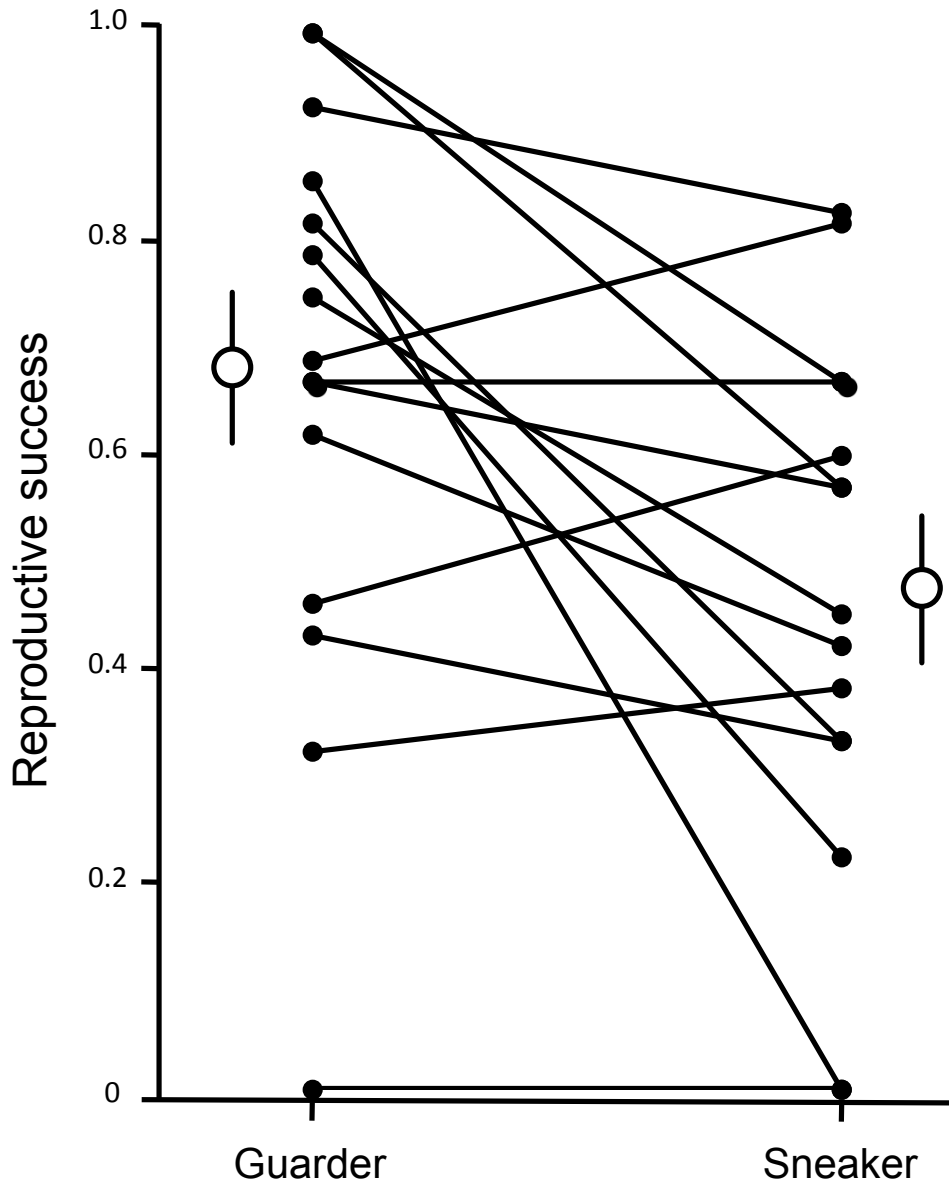


Figure 2



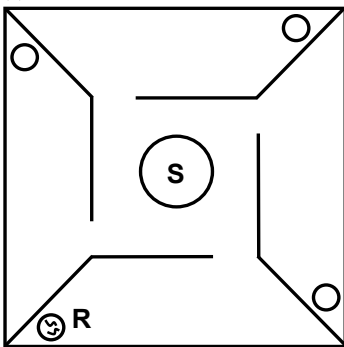
# Cognitive ability is heritable and predicts the success of an alternative mating tactic

C. Smith *et al.* Online Appendix

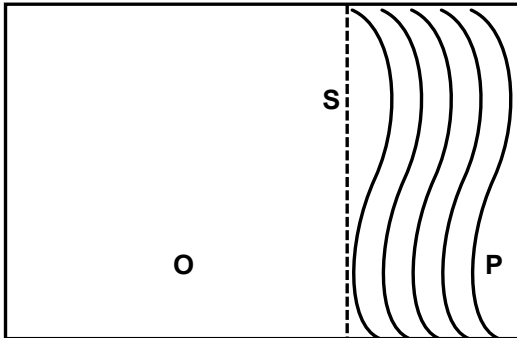
## 1. Supplementary figures

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)

