Paternity analysis reveals sexual selection on cognitive performance

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Abstract

In many animal species, cognitive abilities are under strong natural selection because decisions about foraging, habitat choice, and predator avoidance affect fecundity and survival. But how has sexual selection, which is usually stronger on males than females, shaped the evolution of cognitive abilities that influence success when competing for mates or fertilizations? No study has yet linked individual differences in male cognitive performance to variation in paternity arising solely from sexual selection. We therefore ran four standard cognitive assays to quantify five measures of cognitive performance by male mosquitofish (*Gambusia holbrooki*). Males were then assigned to 11 outdoor ponds where they could compete for females. Females mate multiply, which leads to intense sperm competition and broods with mixed paternity. We genotyped 2430 offspring to identify their fathers. Males with greater inhibitory control and better spatial learning abilities sired significantly more offspring, while males with better initial impulse control sired significantly fewer offspring. Associative and reversal learning did not predict a male's share of paternity. In sum, there was sexual selection on several, but not all, aspects of male cognitive performance.

Introduction

Cognitive adaptations are traits that allow animals to acquire, store, process and act upon information obtained from the environment[1]. Although greater cognitive ability should improve decision making and executive functions, there are potential trades off with allocation of resources to other life-history traits[2]. As a result, cognitive abilities vary widely among taxa and within species. This variation across species is often, but controversially, linked to differences in brain size[3,4]. To demonstrate that cognition has evolved under selection, cognitive performance must be heritable, and it must affect fitness[5,6]. There is evidence from many species that cognitive abilities are heritable[7], including in humans[8]. There is, however, far less evidence linking cognitive traits to fitness[3]. Most of the available studies focus on naturally selected components of fitness, such as survival (e.g.[5,9,10]) or its proxies, like growth, foraging ability, and anti-predator skills (e.g. [11–13]); and these studies involve only a few taxa, with a strong bias towards birds.

Sexual selection for cognitive abilities is even less well documented than natural selection for cognitive abilities[3]. This is an oversight as greater cognitive abilities should provide a competitive advantage in gaining access to mates. More appropriate and timely decisions can lead to better mating opportunities (e.g. locating or preferentially approaching more sexually receptive mates). Superior cognition can also enhance traits favoured by mate choice. For example, male songbirds with larger song repertoires are often more attractive to females, and better cognition should improve the ability to learn songs[14]. Evolution of cognition is therefore likely to be driven by both natural and sexual selection. Some researchers have attributed sex differences in brain structure and cognition to sexual selection based on which sex searches for mates[15]. For example, it has been suggested that sexual selection has driven a greater ability of males than females to solve spatial problems (e.g. rodents[16] and gobies[17]). Controversially, it has even been argued that female mate choice for intelligent males selected for enlarged brain size in humans[18]. More convincing, albeit indirect, evidence comes from two experimental evolution studies of insects that reported improved cognition by individuals from polygamous lines than by those from monogamous lines where sexual selection is absent[19,20] (see also ref.[21]).

Ideally, we need to quantify the link between the cognitive abilities of individuals and fitness. Individual fitness is challenging to estimate and includes the reproductive value of offspring and kin, but counts of offspring production (ideally lifetime) are often the best proxies for fitness[22]. Most of the relevant studies that relate individual variation in cognition to measures of annual or shorter-term offspring production (e.g. clutch size or fledglings) are on females or mean cognition of breeding pairs[23–26]. In these studies, the benefits of improved cognition are likely to reflect natural selection on parents (e.g., better foraging or parental ability). Data on males, the sex usually under stronger sexual selection, is rare.

To date, studies have provided limited evidence for sexual selection on cognition in males. Some studies have reported a relationship between variation in male cognitive ability and sexual attractiveness[27,28] or mating success[29–34]. Six studies have even tested for a relationship between male cognitive ability and offspring production (e.g., clutch size, number of fledglings[25,29,35–38]; see also ref.[39]). However, the role of natural and sexual selection is conflated in these studies because parental ability, survival, and mating or fertilization success could all influence offspring production. In addition, these studies have other limitations that include using only a single cognitive assay[25,35,36,38], a very small sample size[37], a laboratory-based measure of breeding success[29,38], or assuming that offspring in a nest are sired by the social mate[35,37]. Our study of a fish species without male parental care deals with all these limitations. We measured five cognitive traits of potential sires and determined paternity for

>2400 offspring from 11 unique sets of competing males, with each set assigned to a different pond. Crucially, we show that sexual rather than natural selection on cognition is likely to have determined most of the non-random variation in these males' share of paternity.

It is challenging to tease apart natural selection (e.g. for greater survival or parental ability), and sexual selection (e.g. for improved mating or fertilisation success) on cognition. One option is to run experiments where mortality is minimal so that non-random variation in paternity reflects differences among males in their ability to acquire mates, copulate and/or fertilise eggs (i.e. sexual selection). In addition, it is preferable to use species where males do not provide parental care that might affects offspring counts (e.g. refs.[40,41] documented sexual selection on inbreeding status in mosquitofish using this approach). This is the approach that we take.

Individuals can vary in their relative ability to perform different cognitive tasks, perhaps because these tasks tap into distinct cognitive domains[42]. It is therefore preferable to run several assays to characterise an individual's cognitive abilities (e.g.[5,31,43]). It is noteworthy that most studies that have related male cognition to offspring production used only a single cognitive assay (which might tap into several cognitive abilities)[25,35,36,38]. Performance across tasks is sometimes highly correlated (e.g.[44]), especially in humans, leading to the notion of 'general intelligence' or *g*[45]. A recent meta-analysis of 11 species of birds and mammals reported that the mean correlation is weak, but still likely to be biologically significant (r = 0.185; 95% CI: 0.087–0.287)[46]. When there is little evidence for general intelligence, however, it is important to test how performance in different assays relates to estimates of fitness (e.g.[4,9]).

Poeciliid fishes lack male parental care and are extensively used to study both sexual selection and cognition[47]. We quantified individual variation in cognition in male mosquitofish *Gambusia holbrooki* (Poeciliidae) with four commonly used assays for vertebrates (e.g.[4,23,44]). First, in an inhibitory control assay we measured a male's ability to suppress an impulse to swim through a transparent barrier to reach females, and instead to detour around it[48,49,50]. We differentiated between how long a male spent trying to swim through the transparent barrier on his initial attempt ('initial impulsiveness'), and his subsequent average time at the barrier once he had opportunities to learn that it was not possible to swim through it ('inhibitory control'). This provided two measures of cognitive performance from one assay (see *Methods*). Second, in a spatial learning assay we measured the ability to navigate a maze to reach females[51,52]. Third, in an associative learning assay we measured the ability to learn which of two visual cues in a T-maze identified the location of females. Fourth, in a reversal learning assay we switched the cues and tested the ability to unlearn the old association and learn the new one[53,54]. In addition, in the inhibitory control assay, we quantified boldness which is a repeatable 'personality' trait in *G. holbrooki*[55,56]. A recent meta-analysis suggests that, aside from boldness, most personality traits are uncorrelated with cognitive performance[57]. We therefore controlled for any effect of boldness by including it as a covariate when quantifying the relationship between cognition and fitness.

Once cognitive performance was quantified, we transferred males to 11 outdoor ponds where they could freely compete to mate and fertilise females at population densities that are within the range that we observe in the field. We then genotyped offspring to measure male reproductive success (Fig. 1). We also quantified male heterozygosity as inbreeding has been linked to lower male reproductive success in *G. holbrooki*[40] (but see ref.[58]); and we measured body size as larger males tend to have greater reproductive success[59–61].

We had three key aims: (1) to test whether male performance improved over successive trials or was better than expected by chance in each cognitive assay; (2) to determine the correlations between performance in different cognitive assays and other potential predictors of paternity (boldness, heterozygosity, body size); (3) to test if cognitive performance is positively related

to paternity (i.e. sexually selected variation in male reproductive success). For Aim 3 we took two approaches: (a) running univariate tests for each of the five cognitive performance measures; and (b) conducting a multiple regression (generalised linear mixed model, GLMM) that accounted for covariation between our cognitive measures as well as three other potential predictors of male reproductive success (boldness, heterozygosity, body size). To avoid bias, we preregistered our statistical analysis on the Open Science Framework before running tests (https://osf.io/xg86s).

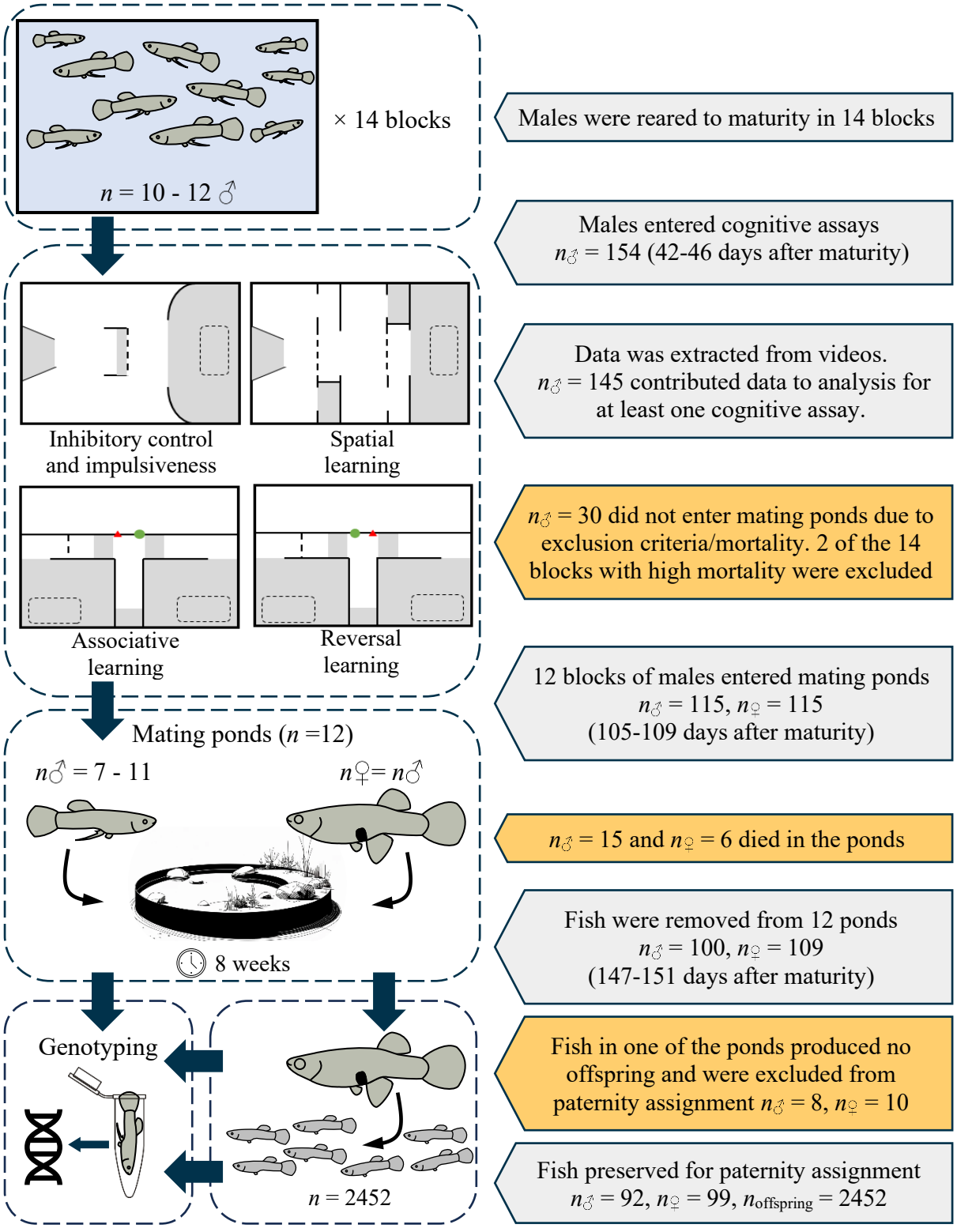

Figure 1. Timeline of study and sample sizes from cognitive assays, to introduction of male and female mosquitofish, *Gambusia holbrooki,* to ponds to compete for mates and fertilization to genotyping of offspring to assign paternity.

Results

Evidence for Non-random Variation in Male Performance in the Cognitive Assays (Aim 1)

There was evidence in all four assays that either (a) on average, males improved in performance over successive trials; or (b) the number of males that reached the performance threshold was greater than expected by chance alone.

In the inhibitory control assay males were more likely to detour around the barrier rather than try to swim through it as the number of trials they had completed increased (GLMM, $Z = 3.08$, $P = 0.002$). In later trials a male that tried to swim through the transparent barrier also spent less time doing so (GLMM, $Z = 5.55$, $P < 0.001$). Both findings are consistent with improved cognitive performance. In later trials, males did not reach the goal zone more often (GLMM, $F = 0.35$, $P = 0.51$). This is unsurprising, however, as only 3% of trials were not solved within the allotted 5 minutes. Males eventually reached the goal zone in 97% of trials, but the time taken did not decrease over consecutive trials (GLMM, $F = 0.20$, $P = 0.65$). Time spent at the barrier on the first occasion that a male tried to swim through the transparent barrier, which was not always during his first trial ('initial impulsiveness'), was shorter if the barrier was encountered in a later trial (LM, $F = 5.75$, $P = 0.02$). Initial impulsiveness was also greater than the mean inhibitory control time in subsequent trials (paired t-test, $P < 0.0001$; mean difference = 36.0 s, 95% CI: 48.0 to 23.9), again strongly suggestive of males being less persistent in trying to swim through the barrier with experience.

In the spatial learning assay males made fewer errors in the maze than expected by chance (Chi-squared goodness of fit to 1:2:1 ratio, $\chi^2 = 43.7$, df = 2, $P < 0.001$; expected: = 1 error/trial, observed: mean for trial 1 = 0.97 error/trial, mean for trial 8 = 0.67 error/trial). The number of errors over successive trials decreased (GLMM, Z = 1.81, $P = 0.07$) and the likelihood that a male solved the task increased (GLMM, Z = 1.60, $P = 0.11$). Both trends are non-significant, but in the direction expected if males gain information that improved their ability to navigate the maze. The time taken to solve the task did not decrease significantly over successive trials ($t = 0.46$, $P = 0.65$). This is inconsistent with better performance, but we suggest that solving time is the least useful measure of cognition because it is confounded by variation among males in their speed of movement (see *Discussion*).

In both the associative and the reversal learning assays more males met the learning criterion than expected by chance (70 versus 30.2 and 37 versus 14.6 fish, respectively; one-sided Exact Poisson tests, both $P < 0.001$).

Correlation in Performance Across Different Cognitive Assays (Aim 2)

There was little evidence for general intelligence, *g*. We calculated pairwise correlations among the five measures of cognition (Fig. 2; n = 68-130 males per correlation). The mean correlation was $r_s = 0.14$, and there was only one significant positive correlation (associative and reversal learning). There was a non-random correlational matrix (Fig. S1) and PC1 explained 37% (95% CI: 29.6-45.7) of the observed variation in cognitive performance. Some researchers use the criteria that there is evidence for general intelligence (*g*) if PC1 explains at least 30% of variation and all loadings are positive (e.g.[31,33,43]). In our study, however, only associative and reversal learning had a statistically significant positive loading on PC1[46,62] (Tables S1, S2). We therefore analysed the relationship of each measure of cognition with paternity separately as we had only weak evidence for *g*. But to allow comparison with other approaches, we also

calculated a composite cognition score (CCS) that gives equal weighting to all assays used (*Methods* and ref.[5]). CCS is highly correlated with PC1 ($r = 0.99$, $n = 68$ males; Fig. S2).

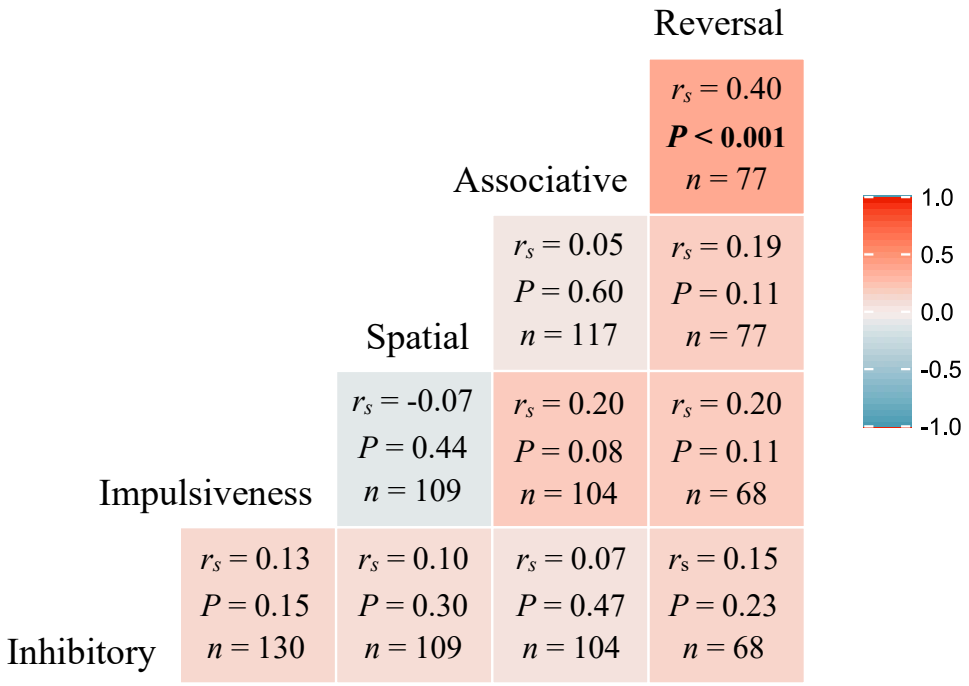

Figure 2. Correlation matrix of cognitive scores. The matrix includes five measures of cognition: inhibitory control, initial impulsiveness, spatial ability, associative learning, and reversal discriminative learning. Each tile shows the pairwise Spearman correlation coefficient (r_s), *P*-value, and sample size. Significant correlations are emboldened.

Relationships Between Cognition and Boldness, Body size and Heterozygosity (Aim 2)

Male boldness did not significantly correlate with any of the five measures of cognition, nor with the CCS (Table 1). It is worth noting that our measure of boldness can also be interpreted as a measure of motivation as it is equivalent to 'latency to approach a task' (e.g., ref[49]).

Male boldness was defined as the time taken to leave the start zone in the inhibitory control assay. In the trial where a male first encountered the barrier, the time he spent there (i.e. his initial impulsiveness) was uncorrelated with his boldness (GLMM, $F = 0.006$, $P = 0.937$). In subsequent trials, bolder males were more likely to approach the barrier (GLMM, $Z = 3.130$, $P = 0.002$), and then spent more time trying to swim through it (i.e. had lower inhibitory control) (GLMM, $Z = 2.222$, $P = 0.026$), but boldness was unrelated to whether a male reached the females in the goal zone during a trial (GLMM, $Z = 0.665$, $P = 0.506$). Of those males that reached the goal zone during a trial, bolder males had a faster solving time (time from leaving the start zone to reaching the goal zone) ($F = 9.664$, $P = 0.002$), suggesting that some males tended to move more or faster than others. This makes solving time a weak index to measure cognition (see *Discussion*).

In the spatial learning assay, a male's average boldness was unrelated to how often he reached the goal zone in the allotted time (GLMM, $Z = 1.703$, $P = 0.089$), or how fast he reached it (GLMM, $t = 1.745$, $P = 0.084$). Bolder males made marginally, but non-significantly, more errors in the maze (GLMM, $t = 1.914$, $P = 0.057$). Finally, only one of the 12 tested correlations

between the five measures of cognition or CCS and male body length or heterozygosity was significant (Table 1).

Table 1. Spearman's correlations (r_s) between the five cognitive measures, the composite cognitive score CCS, boldness, body length, and heterozygosity of male mosquitofish.

Predictor	Boldness		Body length		Heterozygosity	
	Spearman's *r* (n)	*P*	Spearman's *r* (n)	*P*	Spearman's *r* (n)	*P*
CCS	-0.02 (130)	0.82	0.11 (112)	0.27	-0.03 (90)	0.81
Inhibitory	-0.14 (130)	0.11	-0.07 (102)	0.48	-0.06 (82)	0.60
Impulsiveness	-0.10 (130)	0.25	-0.07 (102)	0.50	-0.12 (81)	0.27
Spatial	0.18 (109)	0.06	-0.02 (97)	0.83	**-0.21 (90)**	**0.049**
Associative	0.01 (104)	0.88	-0.18 (96)	0.09	0.17 (90)	0.11
Reversal	-0.15 (69)	0.22	0.19 (68)	0.12	0.04 (65)	0.74
Boldness	-	-	-0.14 (102)	0.16	-0.10 (82)	0.35
Body length	-	-	-	-	-0.01 (88)	0.94

Relationship between Cognition and Paternity (Aim 3)

Mortality, hence natural selection on traits that affect survival, explained almost none of the variation in reproductive success among the males that we genotyped. These males, who did not die during the 8-week mating period (n = 92 of 105), sired >99% of the offspring genotyped in the 11 ponds (n = 2430 of the 2452). In total, 40 of these 92 potential sires gained paternity, while 72 of 99 females gave birth (median: 27 offspring/female). As in most species, male reproductive success was more variable than that of females[63] (see Fig. S3).

On average, males with greater inhibitory control and better spatial learning ability gained a significantly higher share of paternity ($P = 0.008$, $P = 0.002$, respectively: t-tests of mean r_s from $n = 10$ or 11 ponds; Table 2; Fig. 3). Contrary to our prediction, more impulsive males that initially spent longer at the barrier gained a significantly *higher* share of paternity (Table 2; Fig. 3; for P-value assignment see *Method*s and ref.[64]). Males with a higher composite cognitive score (CCS) gained a greater share of paternity, but the effect was not statistically significant ($P = 0.09$). Neither larger nor bolder males gained a higher share of paternity (Table 2, Fig. 3).

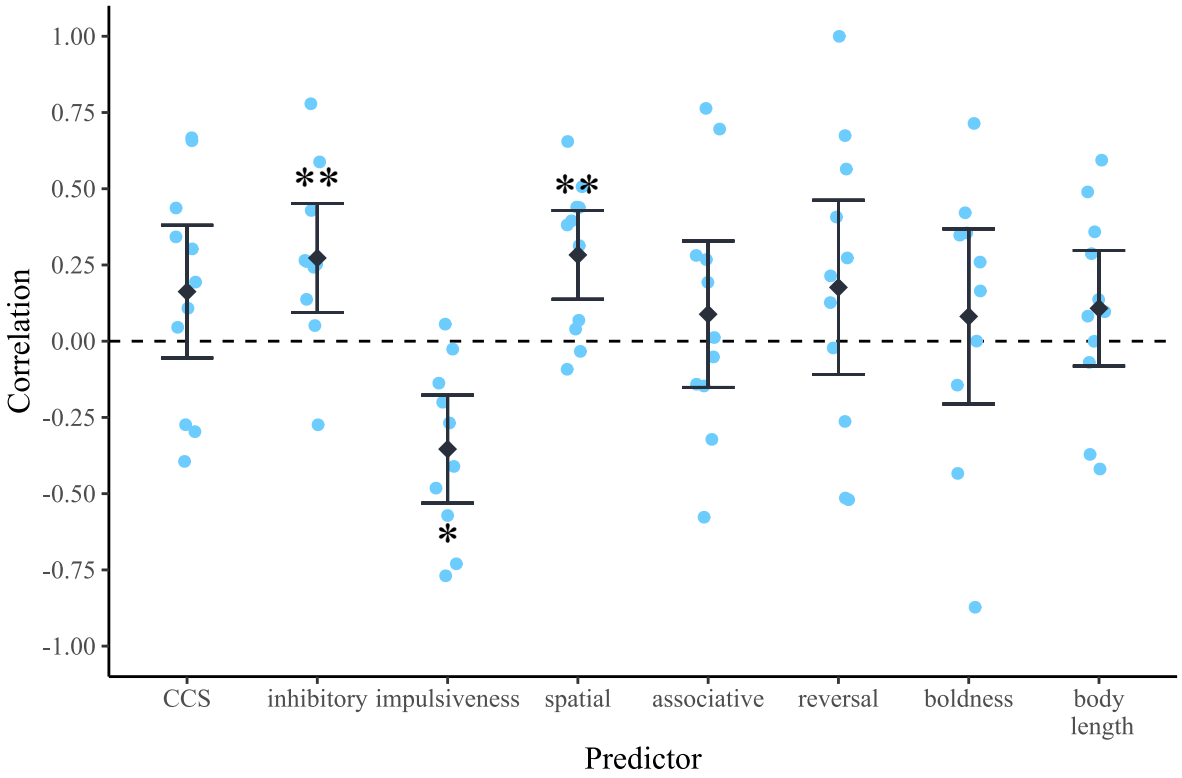

Figure 3. Spearman's correlations between share of paternity and five cognitive traits, a composite measure of cognition (CCS), boldness, and body size of male mosquitofish *Gambusia holbrooki*. Each blue point represents the correlation in a pond ($n = 11$ ponds, except for inhibitory control, impulsiveness, and boldness where $n = 10$: see *Methods*). The black diamond indicates the mean correlation and its 95% confidence interval. Significant means are marked with stars (see text for details).

Table 2. The mean correlation between share of paternity and male traits for 10 or 11 ponds for male mosquitofish *Gambusia holbrooki*. The traits are a composite cognitive score (CCS), five measures of cognition, boldness, and standard body length. The table shows the mean correlation and its 95% confidence intervals, *t* statistics and *P*-values from directional tests. Predictors in bold are significant (see *Methods*).

Paternity predictor	Mean r_s	95% CI	*t*	df	*P*
CCS	0.16	-0.06, 0.38	1.46	10	0.09
Inhibitory	**0.27**	**0.09, 0.45**	**2.99**	**9**	**0.008**
Impulsiveness	**-0.35**	**-0.53, -0.18**	**3.92**	**9**	**< 0.05***
Spatial	**0.28**	**0.14, 0.43**	**3.81**	**10**	**0.002**
Associative	0.09	-0.15, 0.33	0.72	10	0.24
Reversal	0.18	-0.11, 0.46	1.21	10	0.13
Boldness	0.08	-0.21, 0.37	0.55	9	0.30
Body length	0.11	-0.08, 0.30	1.12	10	0.15

* unexpected negative correlation, *P*-value from a directional *t*-test, see *Methods* and ref.[64]

Finally, we ran a multiple regression to test how variation in a male's share of paternity in a pond was explained by the five cognitive measures, body size, and heterozygosity after controlling for their covariation (Table 3). Males with better spatial ability and greater inhibitory control than their rivals gained a significantly higher share of paternity ($P = 0.002$; $P = 0.03$), while more impulsive males also had a significantly higher share of paternity ($P = 0.02$). The other two cognitive measures, body length, and heterozygosity were unrelated to a male's share of paternity. This result confirms the robustness of the univariate tests (Table 2).

Table 3. Results of a linear mixed effects model to explain variation in paternity in mosquitofish *Gambusia holbrooki.* Log-transformed offspring number is the response variable. The fixed factors are five cognitive measures, heterozygosity, and body size (all standardised within each pond as traits contributing to a male's reproductive success are only biologically relevant when compared to those of rival males in the same pond). Pond identity was included as a random factor. Parameter estimates and standard errors (SE) are from the model summary, with *F* statistics and *P*-values (two-tailed) from type-III ANOVA. Significant results are emboldened.

Paternity predictor	Estimate	SE	*F*	*P*
Inhibitory	**0.58**	**0.26**	**4.82**	**0.03**
Impulsiveness	**-0.68**	**0.28**	**6.04**	**0.02**
Spatial	**0.88**	**0.27**	**10.52**	**0.002**
Associative	-0.04	0.39	0.01	0.91
Reversal	-0.26	0.29	0.78	0.38
Boldness	0.03	0.27	0.02	0.90
Body length	0.38	0.26	2.12	0.15
Heterozygosity	-3.01	9.87	0.09	0.76

Random effects	Variance	SD
Intercept: Pond	0.57	0.75
Residual	2.63	1.62

Discussion

Our findings indicate that sexual selection on males has potentially favoured the evolution of specific cognitive traits in *G. holbrooki*. We draw this conclusion because our study minimized the role of natural selection (e.g., mortality, male parental care) in determining variation in reproductive success among putative sires. We ran four different assays to quantify five measures of cognitive performance and a personality trait ('boldness') of male mosquitofish. We then tested if these measures were related to a male's share of paternity when males competed for mates and fertilizations. There was very weak evidence for general intelligence, *g*. We therefore separately investigated the effect of each cognitive trait on reproductive success. Males with greater inhibitory control and better spatial learning performance had significantly higher reproductive success, but more impulsive males also had significantly higher reproductive success. Neither boldness nor heterozygosity nor body size predicted male reproductive success, nor were they correlated with any measures of cognitive performance.

Sexual Selection on Cognition

The cognitive traits measured in our assays have plausible behavioural links to mechanisms that could improve male reproductive success in mosquitofish. Females shoal to reduce male harassment[65], which suggest that males who navigate efficiently and choose which shoals to target might mate more often. This is a testable prediction (e.g. does male cognitive performance predict joining shoals with a better ratio of females to males?). We also found that males with stronger inhibitory control, but who were initially more impulsive (i.e., how soon they detour around the barrier after they first encountered it), had higher reproductive success. These results seem contradictory, but we speculate that being more impulsive could benefit males when they first encounter a female and attempt to mate, whereas greater self-control might be more effective when a male repeatedly tries to copulate with the same female. Male mosquitofish often pursue a female for a prolonged period[65]. A shift from initial repeated attempts to copulate to then wait for an opportunity to approach a female without being attacked by her might increase a male's net reproductive success. Again, this generates testable prediction (e.g. does male cognitive performance predict the rate of successful mating attempts?). These explanations imply that three of five cognitive traits that we measured in male mosquitofish are under direct, rather than correlational, sexual selection.

Our study has four advantages over previous studies testing for sexual selection on cognition. First, the effect of natural selection on a male's share of paternity in our study is likely to be minimal. There was ready access to food and no predators were present, hence male mortality was low. In addition, our selection analysis only included males that survived until the end of the mating trial. It is noteworthy, however, that despite prolonged sperm storage by female *G. holbrooki*, these males sired >99% of offspring. Second, we genotyped offspring to confirm paternity, rather than assuming social mates sired offspring in a nest. Third, we quantified five distinct cognitive traits. Fourth, we calculated relationships in 11 independent populations (i.e. ponds), and we also genotyped >2450 offspring. These are far larger sample sizes than in previous studies. As in any selection analysis it is, however, possible that focal traits are correlates and not causes of higher fitness[66]. Cognitive traits might simply covary with some of the previously described mating behaviours that affect copulation rates, or even covary with sperm competitiveness[67]. For example, positive covariation might arise if better body condition elevates both cognitive abilities and mating behaviours, which are often condition-dependent traits[68]. We did, however, control for covariation with body size, heterozygosity and boldness (Table 3). Nonetheless, there will still be sexual selection *of*, if not *for*, cognitive ability[69,70] (i.e. if it is under indirect/correlation selection because it is correlated with traits that have a

causal effect on fitness). A standard approach to determine causality is to manipulate a trait experimentally. This is feasible for some male sexual traits (e.g. tail length in birds), but there are huge challenges to manipulating cognitive abilities (e.g. via diet or pharmacologically) without affecting other fitness-enhancing traits.

Neither associative nor reversal learning were correlated with male reproductive success. Although some males learned that a colour-shape cue indicated the location of females, associative learning might not be useful in the wild as shoals of females constantly move. This might explain why there was no evidence that associative or reversal learning ability was sexually selected. Associative learning might be more relevant for fish that court or have ornamental traits, allowing for easier identification of individuals, such as guppies[71–73].

Effect of Boldness on Male Reproductive Success and Cognition

In our study the boldness of male *G. holbrooki* was uncorrelated with reproductive success. In contrast, studies on other fish report a positive correlation (e.g. zebrafish, *Danio rerio*[74,75]; the African cichlid, *Pelvicachromis pulcher*[76]). There was also little evidence that a male's boldness is correlated with any of the five cognitive traits that we measured. The strongest relationship was with spatial learning ($r = 0.18$, $P = 0.06$).

For a given trial in the inhibitory control assay, the time a male spent at the transparent barrier (i.e. inhibitory control or initial impulsiveness) was uncorrelated with how soon he left the start zone (i.e. boldness), but males that left the start zone sooner reached the reward significantly faster (Note: this 'solving time' excludes time in the start zone so there is no statistical autocorrelation). Faster movement could potentially obscure any causal link between the measure of cognition (i.e. time at the barrier) and time to reach the goal zone, which is why we do not interpret solving time as an indicator of cognitive ability (see *Methods*).

No Effect of Heterozygosity on Male Reproductive Success or Cognition

Lower heterozygosity, as occurs with inbreeding, is often associated with weaker expression of fitness-enhancing traits[77]. We found no evidence that heterozygosity affected male reproductive success. In past studies of *G. holbrooki* from the same population, lower heterozygosity was associated with reduced male reproductive success[40,78,79] (but see ref.[58]). The reason for these differences among studies is currently unclear. In the present study there was no evidence that lower heterozygosity is linked to poorer cognitive abilities. Instead, males with lower heterozygosity had better spatial learning ability (Table 1), while there was no significant correlation with the other four measures of cognitive ability.

Studies reporting a link between heterozygosity and cognitive abilities in wild animals are rare[79,80], but lower cognitive abilities of inbred individuals have been documented in humans[81,82] and laboratory rodents[83–86]. Long-term population studies that genotype individuals to determine paternity (hence allow estimates of heterozygosity)[87] often quantify behaviours that, while not formal assays of cognition, can be used to infer cognitive abilities (e.g., recovery of stored food). These data could be used to test for a link between heterozygosity and cognitive ability. To the best of our knowledge, however, our study is the first to test for a link between heterozygosity and assay-based measures of cognition in a wild animal. Many more studies are needed given conservation concerns that declining populations become inbred[88,89], and that lower cognitive abilities elevate mortality[1].

Evidence for General Intelligence

Male performance in all four assays either improved over consecutive trials or exceeded null expectations for correct decisions. This is consistent with male *G. holbrooki* learning to solve tasks. But there was little evidence for general intelligence, *g*. Of 10 pairwise correlations, while 9 were positive, only that between associative and reversal learning was significant. Our finding of a lack of evidence for *g* agrees with a recent, multi-taxa meta-analysis[46], and the only equivalent study on another fish[90].

The weak correlations in performance across the cognitive assays suggest that they measure different cognitive domains[90,91,92]. Even measures of inhibitory control and impulsiveness, which both relied on performance near a transparent barrier, were not strongly correlated. Domain specific cognition might be an adaptive specialization to environmental challenges[91] that vary among individuals (e.g., smaller males tend to avoid fights). In Poecilid fishes, the evolution of cognition is often discussed with reference to highly divergent behaviour between the sexes, as well as variation in male mating strategies[93]. For example, male *G. holbrookii* fight for access to mates[94–96], harass females to mate, and attempt sneaky copulations[97], while females attack males, and actively resist male mating attempts. Differences in the fitness gains from specific behaviours may lead to phenotypic plasticity in cognition that is sex and/or size-dependent (e.g.[48]).

Conclusions

Our study provides the most conclusive evidence to date for sexual selection of cognitive abilities. There was a significant correlation between male reproductive success and three of five cognitive performance measures. Reproductive success was recorded in a context where natural selection could not readily explain variation in a male's share of paternity. Inhibitory control, initial impulse control and spatial learning were all significantly correlated with male reproductive success, while associative and reversal learning were not. There was little evidence for general intelligence. This suggests that different cognitive domains vary in how they evolve in response to sexual selection on males. The next challenge is to identify the proximate mechanisms that link specific cognitive abilities to male reproductive success to test whether these relationships are causal or correlational.

Methods

Origin and maintenance of focal individuals

Juvenile mosquitofish were caught in streams in Canberra, Australia. They were then raised to maturity in the laboratory under a 14 L:10 D cycle at 28 ± 1°C. Fish were fed twice daily on commercial fish flake and *Artemia* nauplii and held at densities of 50 fish per 90*l* aquarium. We separated males and females before they matured. We then transferred sets of adult virgins to mixed-sex aquaria (15 females: 30 males) for a period of 20 days. Females mate multiply and most broods are of mixed paternity[98]. For logistical reasons we set up new mating tanks every two weeks from June to September 2020 to stagger the birth of offspring. After 20 days, the females were individually isolated in 4*l* tanks that we checked twice daily for newborn offspring.

A maximum of five fry per brood were transferred to a 90*l* aquarium tank and raised to adulthood to produce focal males. Each tank was stocked with up to 60 fry collected over two weeks. As offspring matured, fish were sexed and males were transferred to male-only aquaria

in groups of 10-12 individuals. Each aquarium became the source of an experimental block of males (maximum age difference: 14 days). Two weeks prior to starting the cognition assays, we marked males with a unique position/colour combination of Visible Implant Elastomer tag (Northwest Marine Technology Inc., USA) to individually identify them throughout the study. In total we set up 154 males in 14 blocks (n = 10-12/block) for cognition assays (Fig. 1).

Measurements of cognition

At 42-46 days after maturation, we began to test each block of males in four cognition assays (inhibitory control, spatial learning, associative learning, and discrimination reversal learning). There was at least a five-day break between assays. We randomised the order of the assays across males, except that the reversal learning assay always had to follow the associative learning assay. The protocol for each assay is described below. Between assays, males were returned to their 90*l* communal holding aquarium where they could continue to interact.

Each of the four test arenas was set up inside a 90*l* tank (60x42 cm) lined with white Corflute ® to shield the focal male from external movements. The day before testing, focal males were transferred to individual 1*l* tanks and held overnight. The following morning, we fed males *Artemia* nauplii, and began the cognition trials >30 min after feeding. We re-fed males in their individual tanks throughout the day, but always at least 30 min prior to testing, to standardize their food satiation.

The reward stimulus in all four assays was female conspecifics in a transparent plastic tank. Conspecifics trigger schooling in mosquitofish and are often used as a social reward in cognition studies (e.g.,[48,99,100]). Each day a set of five females were randomly drawn from our stock tanks. A given set of five females was used to test a single male in a single cognitive assay within a day. All females were returned to stock tanks at the end of the day. There was no olfactory exchange between the females and males. We used a social rather than food reward to: (a) avoid any decline in motivation due to males being fed to satiation; (b) take advantage of the natural schooling tendency of mosquitofish; (c) take advantage of the fact that males constantly attempt to mate and are strongly motivated to approach females[101]. If a male appeared unhealthy (i.e. swam slowly or erratically) he was excluded from further cognition assays and subsequent mating trials (see below). At the start of the trial sequence in a cognition assay, each male was transferred from his holding tank to the start zone of the test arena. All trials were run between 07:30-15:30 and were video-recorded for data extraction and independent verification of the data. We made recordings using a CCTV kit with four 2MP indoor security cameras (one per test arena) connected to a 4-Channel DVR with 2TB HDD. Video recordings of the inhibitory control assay from one block (n = 11 males) were lost due to hardware failure.

Inhibitory control and initial impulsiveness assays

In this 'detour test', a male had to inhibit his impulse to swim directly towards the reward group of five females. The test arena was divided into three chambers (Fig. S4). The first was the start zone, with an opening into the main chamber. The third chamber (goal zone) contained the females, who were visible from the start zone. The main chamber contained a U-shaped transparent barrier (15 cm long) with solid sidewalls (2.5 cm). This barrier was positioned directly between the start zone and the females. The U-shape of the barrier meant that the focal male had to inhibit its impulse to swim through the transparent barrier and instead detour around it, briefly moving away from the females, to reach them. Males that solved the task then spent 5 minutes adjacent to the females. In some studies trials are first run using an opaque barrier so that the test subjects learn a route to the reward (e.g., refs [102,103]). We did not use an

opaque barrier because (1) it could lead the male to favour a familiar route and (2) we needed to assess the initial naïve response to a transparent barrier for our measure of impulsiveness (for justification and examples see refs.[23,104, 105]).

Each male was tested in 10 consecutive trials. On leaving the start zone, the male had 5 min to reach the females in the goal zone. To control for how many positively reinforced trials each male experienced, males that did not solve the task within 5 minutes were gently guided to the goal zone using a small aquarium net. In all trials, after the male has spent 5 min in the goal zone, he was returned to the start zone and could then start the next trial. For each trial we recorded: (1) the time taken to leave the start zone as a measure of boldness; (2) the time between leaving the start zone and reaching the goal zone (henceforth, 'solving time'; maximum = 300 seconds); (3) whether or not a male tried to swim through the barrier; and (4) the time spent trying to swim through the transparent barrier (henceforth, 'inhibitory control'). We defined a male as having left the start zone when his body fully left the start zone. We defined a male as having reached the goal zone when the tip of his head entered the goal zone. We defined 'time spent at the barrier' as the time any part of the male's body was within the 15 cm x 2.5 cm area behind the barrier.

When a male approached the transparent barrier for the first time (which was not necessarily during his first trial), we referred to the time at the barrier as 'initial impulsiveness'. This was a separate response variable in our data analysis. This initial time at the barrier was excluded from our measure of inhibitory control, which was the mean time at the barrier in all subsequent trials. This distinction separated the initial ability to control an impulse from any subsequent learned responses after having encountered the transparent barrier; it also eliminated statistical auto-correlation between these two parameters. Most studies do not distinguish between the first and subsequent response to a transparent barrier (e.g.,[106], but see ref.[107]), but we suggest that it is biologically relevant, especially in *Gambusia holbrooki*. Males did not know that they could not swim through the barrier at the start of their first encounter. This information only became available during the trial. The initial time at the barrier was therefore due to persistence (i.e., impulsiveness). In each subsequent trial, however, males had the potential to use their experience of the barrier to moderate their behaviour. It is noteworthy that time at the barrier in the first trial and mean time at the barrier in subsequent trials had significant but opposite effects on paternity (Tables 2 and 3).

Another potential index of cognitive performance is the proportion of trials after an initial encounter with the barrier where the male bypassed the barrier and swam to the females. One problem with this third index is that some males might have been alerted to the existence of the barrier without physically encountering it (i.e. visual cues detected from >2.5cm from the barrier); another problem is that a first encounter with the barrier in a later trial would result in a proportion based on a small sample size.

Researchers vary in how they measure performance in an inhibitory control assay. This is why we have reported several metrics: time spent trying to swim through the transparent barrier on an initial attempt (i.e. impulsiveness); subsequent mean time at the barrier; likelihood to detour/approach a barrier in each trial; and the time taken to reach the social reward zone. Crucially, however, to test for the relationship between cognitive performance and male reproductive success, we made an *a priori* decision to use 'impulsiveness' and 'mean time interacting with the barrier' (see *Statistical Analysis*). The latter is a standard measure of inhibitory control in fishes[48,105,108].

Spatial ability assay

Our spatial ability apparatus was a modification of similar designs[52,109]. In our assay a male had to navigate a maze requiring one left and one right turn to reach females in the goal zone. The test arena had four chambers (Fig. S5). The first contained the start zone with an opening into the main chamber. The fourth chamber contained the goal zone housing five females who were visible to the male from the start zone. The remainder of the apparatus contained two sets of walls, each with a central transparent segment so that the male could see the females in the goal zone. Each wall offered the opportunity to enter the next section through an opening on the right or left. One opening was a dead end, but this was only apparent to a male once he had started to move through the opening. The second wall had the opening on the opposite side to that on the first wall. A male had to enter both correct openings to reach the goal zone. We used two sets of apparatus: one with a 'left-right' and the other with a 'right-left' sequence of openings. We randomly assigned half of the males to each sequence type.

Each male was tested in eight consecutive trials, with five minutes to solve the maze after he left the start zone. Once in the goal zone, he was given a 5 min reward period to be adjacent to the females. To control for how many positively reinforced trials each male experienced, males that did not solve the task within 5 minutes were gently guided to the goal zone using a small net. In all trials, after the male has spent 5 min in the goal zone he was returned to the start zone for the next trial. From the recordings we extracted: (1) time to complete the maze (time from leaving the start zone to reaching the goal zone; maximum = 300 s), (2) the number of errors (i.e., entering dead end zones B_1 and/or B_2) (henceforth, 'spatial learning').

Associative and Discrimination Reversal Learning Assay

Associative and discrimination reversal learning were measured in a T-junction maze with a choice between a correct and an incorrect direction (Fig. S6). In the associative learning assay one arm of the T-junction was marked with a green circle and the other with a red triangle. The male had to learn to associate one of the coloured shapes with the arm that led to females. The other arm was a dead end. To avoid any bias associated with learning a particular colour/shape, for 50% of the males the green circle indicated the arm leading to the females; and for the other 50% of males the red triangle indicated the arm leading to the females. The apparatus had detachable walls so that the open-end of the maze could be switched between trials with minimal disturbance. The coloured indicators were attached to the arena walls using Velcro ® stickers and could also be switched between trials with minimal disturbance to match a pre-determined sequence of correct turn directions on that day. Each day a new trial sequence of correct-choice directions (left or right) was generated by tossing a coin, and the same sequence was then used for all males on that day. We ensured that no sequence had four or more consecutive correct turns in the same direction to reduce the likelihood that a male associated the females with the turn direction[100] rather than the colour/shape cue. We used a new sequence each day to test the ability of males to associate the colour/shape cue with the reward rather than to memorize a spatial sequence. Males could not see females at the T-junction and had to decide which arm to swim down based solely on the colour/shape cue.

Males were given 8 consecutive trials per day for a maximum of 10 consecutive days. At the start of his daily eight trial sequence, a male was placed at the base of the T-junction. We oriented the male to initially face away from the T junction to avoid any bias in his release position relative to the correct choice. Males had to rotate 180º before starting the task. In each trial a male had one minute to enter the left or right arm of the T-junction.

Males were deemed to have chosen when the anterior tip of their head entered an arm of the 'T' (Fig. S6, areas B_1 and B_2). We scored their choice as either correct (1) or incorrect (0). If a male did not reach the females within 1 min, this was also scored as an incorrect choice. Males that did not reach the females were guided to the correct coloured shape and down the associated arm of the maze to the females. This ensured that all males experienced the same number of positively reinforced trials per day. The learning criterion was that a male made 7 or more correct choices over the 8 trials on a given day, excluding the first day of testing ($p = 0.035$ that a male makes 7 or 8 correct choices on a given day by chance). Male performance was quantified as the days taken to reach the learning criterion. Males that did not achieve the criterion within 10 days were deemed to have failed to learn, given the lowest score for learning performance (i.e. 11 days; note we have ranked order correlation tests so any number greater than 10 produces the same outcome), and did not participate in the discrimination reversal learning assay.

In the discrimination reversal assay males that had learned to associate the green circle with access to females now had to learn to associate this reward with the red triangle, or vice versa. Males were again given 8 consecutive trials per day for up to 10 consecutive days and the same learning criterion (7 or more correct choices) was applied.

Mating ponds setup

After a block of males had completed the suite of cognition assays, they were placed in their communal aquarium for three days (Fig. 1). Males were 105-109 days old (since birth). They were then transferred to a 1m diameter, outdoor pond. The ponds had a gravel substrate, rocks and artificial plants to provide natural shelter and structure, and the water depth was 15 cm. These ponds mimicked the edges of the natural waterways from which we collected fish. All ponds were covered with bird netting to exclude predators, and one half was shaded.

For each block, we introduced all males (n = 7-11 per block) and the same number of virgin females to a pond. Two of the 14 blocks had only 5 surviving males and we therefore did not place them in mating ponds because of the low sample size. In total, we set up 12 ponds that contained 115 focal males and 115 females. The fish were in the pond for 8 weeks during which time females could mate, produce a first brood, and by week 8 would have started to gestate a second or third brood (the typical gestation period for *G. holbrooki* is at least 21 days). We could not genotype every brood a female produced to calculate each male's lifetime reproductive success because females need to be housed individually until they give birth to obtain newborn fry. We therefore only genotyped offspring from females removed from the ponds after 8 weeks. This means, however, that males had more time to vary in their ability to gain access to females, reducing chance events affecting paternity in early broods. It is important to note that females can store sperm for prolonged periods so that later broods are better measures of long-term male success at mating females and fertilizing eggs. Fish were fed twice daily with *Artemia* nauplii and experienced the natural light cycle. Fish were in ponds from December 2020 – March 2021 (Australian summer). After 8 weeks all surviving fish were collected. Male survival was 87% (100 of 115). In 9 ponds only one male died, in two ponds two males died, and in one pond four males died. All surviving males were returned to their respective 90*l* communal aquaria and later photographed for size measurements, euthanised in *Aqui-S* solution, and preserved in 100% ethanol for paternity tests. Female survival was 95% (109 of 115 survived).

We placed females in individual 4*l* tanks and checked twice daily for up to 30 days for newborn fry. As soon as a female gave birth, she and the fry were euthanised in *Aqui*-S solution and preserved in 100% ethanol for paternity testing. In one pond none of the females gave birth;

we therefore excluded surviving males (n = 8) and females (n = 10) from this pond from genotyping. In the other 11 ponds, 72 of 99 females produced 2452 offspring that were preserved for genotyping. These offspring were potentially sired by the 92 males that were also genotyped (n = 5-11 males/pond).

Paternity Assignment

We preserved tail muscle of putative sires (n = 92), females that gave birth (n = 72) and the bodies of their fry (n = 2452). We used the commercial genotyping service Diversity Arrays Technology (DArT) to genotype single-nucleotide polymorphisms (SNPs) for the females, offspring, and their putative sires using DArTseq[110]. We have successfully used this method to assign paternity in six previous studies[40,41,58,78,111,112]. Each offspring was lined up against the potential sires (i.e. all males in the same block, hence pond), and the Hamming Distance values calculated (> 4000 SNP loci). The male with the lowest value was considered the sire, but only if two additional conservative criteria were fulfilled: (a) the percentage difference in the Hamming Distance from that of the next closest matching male was >10%; and (b) if the absolute distance was <0.25. Following these criteria, we were unable to assign paternity for 14 offspring, and an additional 8 offspring did not provide usable genetic data. In total we therefore assigned paternity to 99.1% of the 2452 fry (i.e., 2430 fry). Inspection of the Hamming Distances confirmed that offspring were correctly assigned to females and that males from other ponds did not have lower Hamming Distances than assigned sires (i.e., specimens were correctly labelled). We calculated the proportion of heterozygous loci for the 92 putative sires using the SNP data, defined as the number of heterozygous loci divided by the total number of successfully classified loci (see ref.[56]).

Statistical Analyses

We preregistered our statistical analysis on the Open Science Framework (OSF, https://osf.io/xg86s). Any deviations are stated and explained (see Supplementary Material). Deviations were always made prior to analysis and never driven by inspection of the outcome of statistical tests. The sole exception is that we made a *post hoc* decision about how to *present* a strong result in the unanticipated direction from a one-tailed *t*-test. We used the approach of ref.[61] that, in hindsight, we should have deployed for all one-tailed tests (see below).

We ran analysis in R version 4.2.2[113] using the packages *glmmTMB*[114] and *lme4*[115], to run linear models. We used *DHARMa*[116] and *performance*[117] to run diagnostics and check the appropriateness of the models. We used the *PCAtest*[118] package to run permutation-based tests to evaluate the significance of the principal component analysis.

Fish performance in cognitive tests: evidence for learning

For the spatial learning assays we ran generalized linear mixed models (GLMM) with trial number (trials 2-8) as a fixed covariate, average male boldness (assigned from the inhibitory control assay) as a covariate, and fish ID and block ID as random factors. We ran separate models for: whether a fish solved the trial (binomial error), the total solving time if they did (log-normal error), and the average error rate across trials 2-8 (log-normal error) to test if performance improved over the trials.

We ran a chi-squared test to determine if the number of errors made differed from that expected if males randomly chose openings in the maze. Given two choices per trial, males are expected to make 0, 1 or 2 errors per trial with probabilities of 0.25, 0.5 and 0.25 respectively. We compared the observed number of mistakes to the expected ratio of 1:2:1.

For the associative and reversal learning assays we ran exact one-sided Poisson tests to determine if more fish meet the learning criteria per day (excluding day 1) than expected by chance given how many males were tested each day. With an equal likelihood of choosing the correct stimulus in each trial, a male had a 0.0352 probability of meeting the learning criteria by making the correct choice in 7 or 8 trials per day by chance alone.

For the inhibitory control assay we ran GLMM models with trial number (trials 2-10) as a fixed covariate, and fish ID and block ID as random factors. We ran separate models for: whether a male solved a trial in the available time (binomial error), and total solving time and inhibitory control time (both log-normal error) if he did. We were again interested in testing whether performance improved over the trials.

We also investigated whether 'initial impulsiveness' depended on which trial (1-10) the male initially spent time at the barrier. Note that some males only approached the barrier in a later trial. In sum, 85% of males approached the barrier on their first or second trial, 7% on their third trial, and 8% on a later trial. We also ran a paired *t*-test to see if initial impulse time is longer than the average inhibitory control time across trials 2-10 to test if males modified their behaviour at the barrier.

Correlations across cognitive assays

To test for correlations between male performance on the different cognitive assays, we first assigned each male a single score for each of the five cognitive measures: (1) *initial impulsiveness* was the time spent trying to swim through a transparent barrier when it was initially encountered (not necessarily in the first trial), and a higher value equates to being more impulsive, (2) *inhibitory control* was the mean time spent trying to swim through a transparent barrier in trials 2-10 after excluding the first encounter, and a higher values equates to lower inhibitory control, (3) *spatial ability* was the mean number of errors per trial in the spatial assay across trials 2-8, (4) *associative learning* was the number of days it took a fish to meet the learning criteria (i.e. $\geq$ 7/8 correct choices in a day), and (5) *reversal discriminative learning* was the number of days it took a fish to meet the re-learning criteria (i.e. $\geq$ 7/8 correct choices in a day). Each cognitive score was log-transformed, scaled between 0 and 1, and inverted so that higher values represent better performance (i.e. faster learning, fewer errors and greater inhibitory control)[5]. All cognitive scores were approximately normally distributed, and uniformly scaled.

We calculated a composite cognitive score (CCS) from the mean of four of the standardized cognitive scores: (1) spatial learning, (2) associative learning, (3) reversal learning, and (4) the combined mean of initial impulsiveness and inhibitory control (see ref.[5] for justification of CCS). We used the mean of initial impulse and inhibitory control (scaled between 0 and 1) to be conservative as both measures were extracted from the same assay.

We calculated Spearman's correlations for performance based on the five cognitive measures (n = 10 pairwise tests). We also conducted a *post hoc* exploratory principal component analysis (PCA) using the five measures of cognition to reduce the dimensionality of the dataset. We decided to conduct a PCA to test the robustness of our Spearman's correlation analysis. If cognitive measures are strongly correlated, we expect PC1 (and possibly PC2) to explain a large amount of variation in the original data set. We ran a permutation test to evaluate whether there is a significant correlation structure among cognitive measures. It is important to determine whether the observed correlation structure differs from that expected by chance alone before interpreting the results of a PCA. This step is often overlooked in empirical studies (reviewed in refs.[62,118]). In addition, it is also necessary to test whether individual loadings are

significant or not, rather than simply assume that a high proportion of positive loadings means that individual traits are truly associated with general intelligence[118].

Correlations between boldness, body size, heterozygosity, and cognition

We assigned boldness to each male as the mean time to emerge from the start zone in trials 1-8 in the inhibitory control assay. We then ran a Spearman's correlation test between boldness and the five measures of cognitive performance and CCS. We measured the standard body length of focal males using *ImageJ*[119]. We ran a Spearman's correlation between body length and performance for each of the five cognitive measures, CCS, and boldness. Similarly, we ran Spearman's correlations between heterozygosity, the five cognitive measures, CCS and boldness.

The relationship between cognition and paternity

For each of the 11 ponds with offspring, we calculated separate Spearman's correlations between male paternity and: the five cognitive scores, CCS, boldness, and standard body length (all pond standardized). We then ran one-sample, one-tailed *t*-tests to test whether the mean correlation from the 11 ponds was significantly greater than zero. In addition, as part of our *post hoc* exploratory analysis, we ran the same tests between paternity and PC1 from the PCA (see Supplementary information). The one-tailed *t*-tests revealed one very strong result in the unanticipated direction. To illustrate this, we assigned a new asymmetric set of rejection regions for the null hypothesis: $P \leq 0.04$ and $P \geq 0.990$ (i.e. 80% of the zone to reject the null hypothesis of a zero effect is in the predicted direction). This approach was not initially specified in our pre-registration on OSF. In hindsight, this was an oversight. Incorporating this method to handle unexpected results in a one-tailed *t*-test reduces the likelihood of Type II error by increasing the sensitivity of the test to findings in the alternative direction[64].

Finally, to test the robustness of our reported findings for univariate relationships between the five cognitive performance measures and paternity (see *Results*), we ran a linear mixed effect model with the number of offspring sired as the response variable and heterozygosity, body length, and measures of cognition (all standardised within ponds) as fixed covariates, and pond identity as a random factor. We used Type III sum of squares. This model controls for covariation between the predictor variables to test whether each cognitive trait is still correlated with paternity after controlling for effects of other cognitive traits, body length, and heterozygosity.

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Author Contributions

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Supplementary Materials

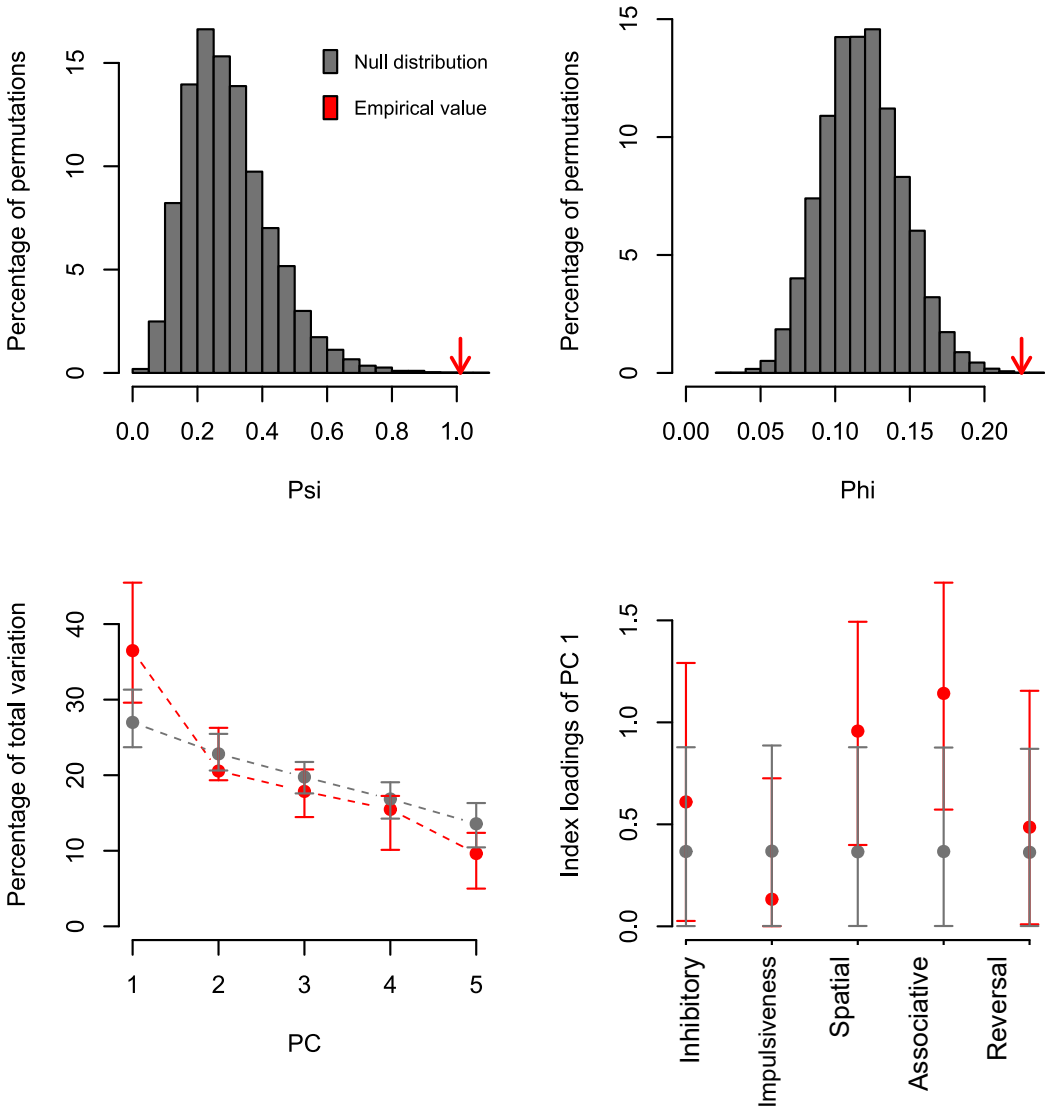

Figure S1. Null PCA distributions calculated from 10 000 simulated data sets using *PCAtest* package in R[118] and empirical statistics from measurements of cognitive abilities in mosquitofish. Grey and red points represent random permutations and mean observed values, respectively, with error-bars showing 95% confidence intervals (CI).

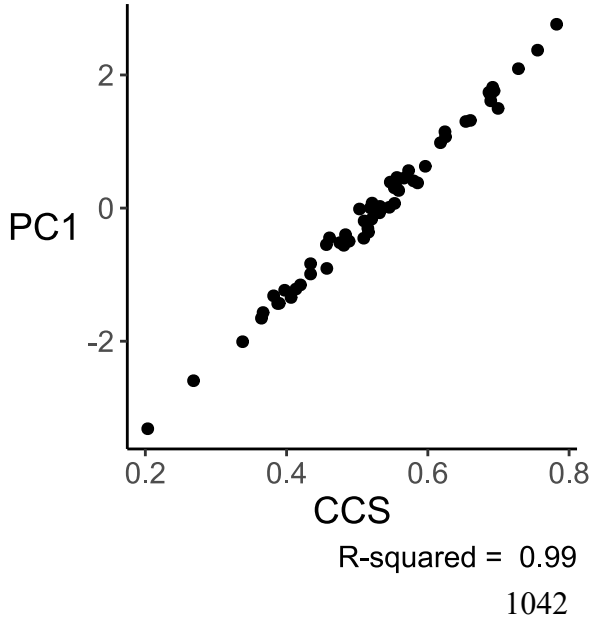

Figure S2. Linear relationship between principal component 1 (PC1) and the composite cognitive score (CCS) for cognitive measures in male mosquitofish *Gambusia holbrooki*. CCS was calculated as the mean of the standardised values for spatial learning, associative learning, reversal learning, and a standardised combined mean of inhibitory control and initial impulse time.

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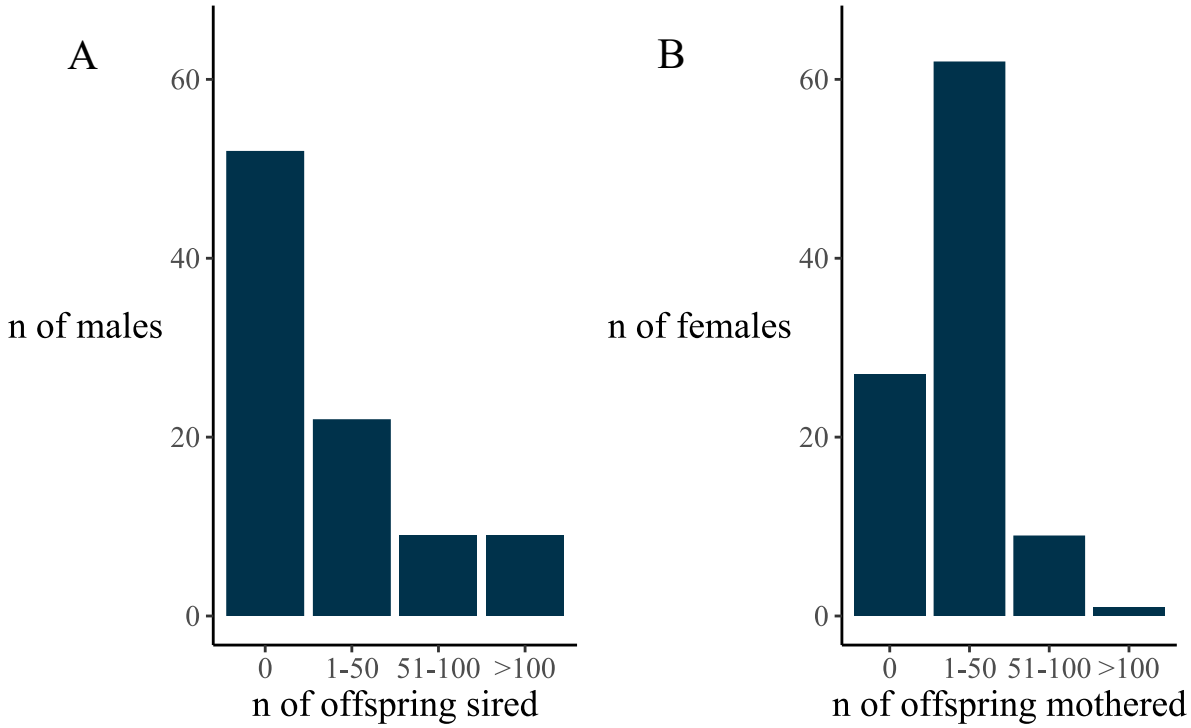

1043
1044 Figure S3. Histogram of the number of offspring sired per male (A) and produced per female
1045 (B) by mosquitofish *Gambusia holbrooki* in 11 mating ponds. Each pond initially had 7-11
1046 males and an equal number of virgin females.
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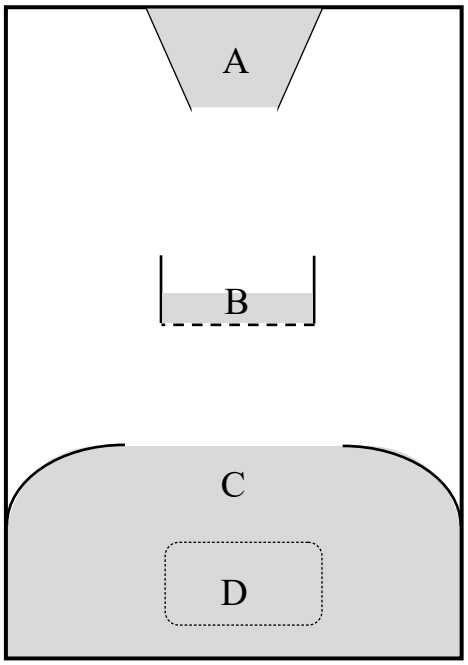

Figure S4. Diagram of the inhibitory control test apparatus inside a glass tank (60 × 42 cm). The water depth was 10 cm. Solid and dotted lines indicate opaque and transparent walls, respectively. Each focal male *G. holbrooki* began its trial in the start zone (A). The time taken to fully leave the start zone was our measure of 'boldness'. A small transparent plastic tank (D; 30 × 19 cm) containing five females was located opposite the start zone. Between the females and the start zone there was a 15 cm wide transparent barrier with opaque walls on either side (B). The total time a fish spent within 2.5 cm of the barrier (on the start zone side) was our measure of 'inhibitory control'. The time it took a male to reach the goal zone (C) after leaving the start zone was our measure of 'solving time'.

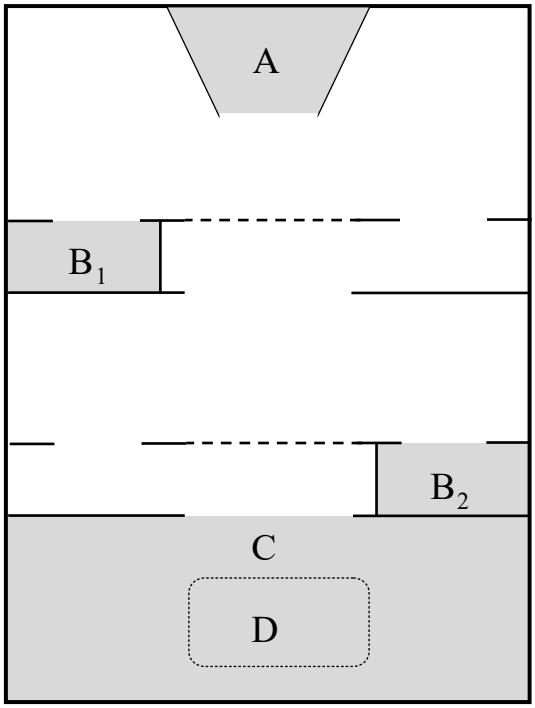

Figure S5. The spatial learning test apparatus inside a glass tank (60 × 42 cm). The water depth was 10 cm. Solid and dotted lines indicate opaque and transparent walls, respectively. Each focal male started his trial in the start zone (A). A small transparent plastic tank (D; 30 × 19 cm) containing five females and was located opposite the start zone, behind two transparent barriers that had a thin white mesh net over them to prevent males from trying to swim through the barrier. The male had to avoid the dead-end zones (B_1 and B_2) to complete the task without making an error. A male was considered to have made an error and entered a dead-end zone if any part of his body entered the grey zone in B_1 or B_2. The number of errors per trial was our measure of spatial learning (0, 1, 2). The time it took a male to reach the goal zone (C) after leaving the start zone was our measure of 'solving time'.

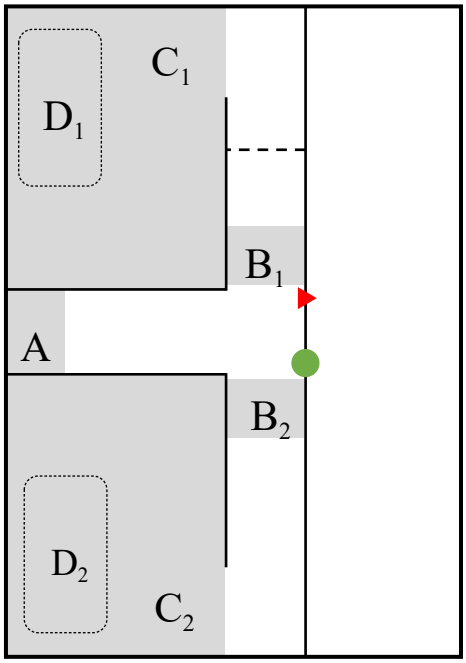

Figure S6. The associative and reversal learning test apparatus inside a glass tank (60 × 42 cm). The water depth was 10 cm. Solid and dotted lines indicate opaque and transparent walls, respectively. Each focal male began his trial in the start zone (A). Two small transparent plastic tanks (D1 and D2; 30 × 19 cm) containing five females were placed in the corners of the tank in zones C1 and C2. The focal male had to swim to the T intersection and then chose to move towards either C1 or C2. At the start of each trial, one arm was blocked with a transparent barrier while the other arm was open. One arm was marked with a red triangle and the other arm with a green circle. If the anterior tip of the male's head entered the grey region (B1 or B2) it was considered a 'correct' or 'incorrect' choice if it entered the open or closed arm respectively. The position of the transparent barrier and the appropriate coloured shape cue for each trial was allocated based on a pre-determined random sequence that changed daily (see text). For half the males the green circle was always associated with the open arm of the maze, and for the other males the red triangle was always associated with the open arm of the maze. Once a male reached the criteria for learning (at least 7 of 8 correct choices within a day), the cue associated with the open end of the maze was switched and the process then repeated to assay his reversal learning ability.

Supplementary Tables

Table S1. Principle component analysis of cognitive measures. The table shows the eigenvalue for PC1, the percent of variation explained, and the loadings of the five cognitive measures.

Cognitive measure	PC1
Eigenvalue	1.82
% variation explained	37
Inhibitory control	0.43
Initial impulsiveness	0.38
Spatial learning	0.20
Associative learning	0.54
Reversal learning	0.59

Table S2. Raw output from the test of PCA significance using the PCAtest package in R[118] performed on 68 observations of 5 variable: inhibitory control, initial impulse, spatial learning, associative learning, and reversal learning. Null values are based on 10 000 bootstrap replicates and 10 000 random permutations.

	Empirical	Max null	*P*
Psi	1.0113	1.0600	**< 0.001**
Phi		0.2302	**< 0.001**
Eigenvalues			
PC1	1.82415	1.80062	**< 0.001**
PC2	1.02778	1.43532	0.979
PC3	0.89268	1.18469	0.9556
PC4	0.77357	1.01759	0.861
PC5	0.48183	0.90774	0.9933