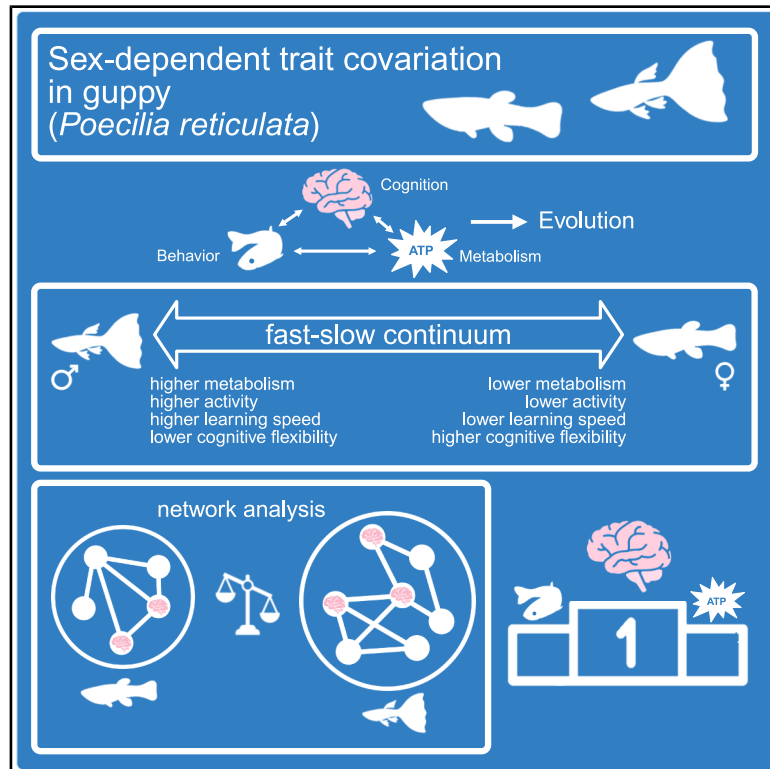


Sex affects the network of covariances between cognitive, behavioral, and physiological traits in guppies

Graphical abstract



Authors

Gaia De Russi, Elia Gatto, Cristiano Bertolucci, Tyrone Lucon-Xiccato

Correspondence

gaia.derussi@unife.it

In brief

Biological sciences; Zoology; Evolutionary biology

Highlights

- We tested whether sex affects the covariations involving cognitive traits in guppies
- Guppies showed sex differences along a fast-slow continuum
- Males exhibited more and stronger covariations between traits than females
- Cognitive traits formed sex-specific covariation networks with other traits



Article

Sex affects the network of covariances between cognitive, behavioral, and physiological traits in guppies

Gaia De Russi,^{1,3,4,*} Elia Gatto,^{1,2,3} Cristiano Bertolucci,¹ and Tyrone Lucon-Xiccato¹¹Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy²Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy³These authors contributed equally⁴Lead contact*Correspondence: gaia.derussi@unife.it<https://doi.org/10.1016/j.isci.2025.112487>

SUMMARY

Cognitive variance has been documented across many species. It may stem from constraints imposed by the covariation among different traits. We hypothesized that sex could contribute to cognitive variance by affecting the traits' covariation. By applying a network analysis in the guppy (*Poecilia reticulata*), a fish with marked sexual dimorphism, we found that males and females differed along a fast-slow continuum, with males exhibiting higher metabolism, higher activity levels, faster learning, and lower cognitive flexibility. Moreover, we found more covarying traits in males compared to females (12 versus 7). Pairwise associations involving cognitive traits significantly differed in the covariance networks of the two sexes and were stronger in males. Various traits, including learning and cognitive flexibility, had different influences in the covariation network between males and females. Cognitive traits are embedded in a complex, sex-dependent web of covariation, which may be important for the evolution and maintenance of cognitive variance.

INTRODUCTION

Cognitive variance, historically considered a hallmark of human psychology,^{1–3} has now been extensively documented across a wide range of vertebrates, including mammals,^{4–6} birds,^{6,7} non-avian reptiles,⁸ and teleost fish,⁹ as well as in invertebrates.¹⁰ This variability in cognitive abilities is remarkably pronounced in certain species. For example, in the guppy, *Poecilia reticulata*, a small teleost fish, some individuals achieved scores in an inhibitory control task that were 200 times higher than those of others.¹¹ Such variance is certainly critical for individual fitness^{12–17} and cognitive evolution,^{6,18} yet how it arises and is maintained remains unclear.

In many cases, individuals' scores in different cognitive traits have been reported to be correlated.^{19–21} This can be due to underlying cognitive processes, trade-offs, and the involvement of the same neural substrates, and it certainly has effects on determining cognitive variance. Moreover, part of the observed phenotypic variance in cognition has been linked to covariation with traits from other domains. Animals often exhibit relatively stable individual differences in behavioral traits, often referred to as personalities,²² and in physiological traits.^{23,24} Variation within populations in behavior and physiology is thought to be maintained through underlying life-history trade-offs^{25,26} (see also Pace-of-Life hypothesis).^{27,28} Evidence suggests that behavior^{29–31} and, to a lesser extent, physiology^{32,33} covary with cognition. For example, more active and bolder individuals

have been reported to be faster learners in various species.^{34,35} Several studies have found significant correlations between the way information processing is divided between the two brain hemispheres (e.g., cerebral lateralization) and various behavioral traits.^{36–39} In addition to these correlations, life-history trade-offs may also directly influence cognitive variation due to the high metabolic costs of neural structures.^{40,41} Due to these direct and indirect links with life-history variation within a species, cognitive variation might be affected by factors impacting life histories.

One of the factors determining large differences in life histories is the sex,⁴² and evidence suggests that individual differences in behavior and physiology are often sex-dependent,^{43,44} as well as their covariation.^{45–48} Average differences in cognitive traits between the sexes are also frequently documented in the literature.^{49–52} We therefore hypothesize that sex could be a factor contributing to cognitive variation by influencing the structure of covariation of cognition with behavior and physiology. Here we tested this hypothesis in guppies, a species known for its pronounced sexual dimorphism^{53–55} and significant cognitive variation.¹¹ To this goal, we analyzed in both male and female guppies the covariation pattern of five cognitive traits (cerebral lateralization, visual discrimination learning, spatial discrimination learning, and cognitive flexibility in both visual and spatial discrimination contexts,^{39,56–60} two physiological traits (basal and stress metabolism^{61–63}), and a set of behavioral traits (activity, swimming velocity, exploration, shyness, and sociability)^{64–66}



by using a network analysis. The network analysis is a multivariate approach that analyses associations considering not only specific pairs of traits but simultaneously controlling for all the other traits and their relationships, thereby comprehensively describing the structure of the system.⁶⁷

RESULTS

Physiological traits

As physiological traits, we assessed two aspects of metabolism, one representing the basal metabolism and the other representing the metabolism under stress.^{61–63,68,69} These traits were estimated using ventilation rate as a proxy.^{70–73} The basal ventilation rate in our experimental subjects was 194.80 ± 36.13 bpm. The stress ventilation rate was significantly higher than the basal ventilation rate (348.62 ± 71.63 bpm; paired *t* test: $t_{44} = 16.664$, $p < 0.001$). The observed ventilation rates were similar with those reported for other poeciliid fish, although slightly higher perhaps due to higher metabolism or to the fact that our subjects were tested in a novel environment.^{70,74,75} Sex had a significant influence on the basal ventilation rate index, with males having higher basal ventilation rates (two-sample *t* test: $t_{42} = 3.160$, $p = 0.003$; Figure 1A), but not on the stress ventilation rate ($t_{43} = 1.486$, $p = 0.145$; Figure 1B).

Behavioral traits

We assessed guppies' behavior in three well-established tests: exploration, scototaxis, and sociability test.^{64,65} We also checked for the repeatability of the behavioral traits by observing each subject in each the test twice. Significant repeatability is usually considered evidence that the trait is a personality dimension.⁷⁶ In our exploration test, the activity of the subjects (measured as the distance swam) significantly decreased in the second trial compared to the first trial (trial 1: 284.26 ± 81.73 cm; trial 2: 250.48 ± 106.33 cm; $t_{47} = 2.307$, $p = 0.026$). Subjects decreased their activity during testing ($F_{1,1869} = 617.214$, $p < 0.001$) with a steeper decrease in the first trial (interaction time \times trial: $F_{1,1869} = 12.733$, $p < 0.001$; Figure S3A). Activity was significantly repeatable between trials (repeatability estimation via parametric bootstrapping: $R = 0.388$ [0.093; 0.606], $p = 0.011$). On average, males exhibited higher activity ($t_{46} = 2.147$, $p = 0.037$; Figure 1C). The second variable obtained from the exploration test, the swimming velocity, decreased in the second trial (trial 1: 4.79 ± 1.38 cm/s; trial 2: 4.21 ± 1.83 cm/s; $t_{47} = 2.269$, $p = 0.028$), with temporal trend similar to that detected for the activity (time: $F_{1,1869} = 597.322$, $p < 0.001$; interaction time \times trial: $F_{1,1869} = 12.061$, $p < 0.001$; Figure S3B). Subjects showed significant repeatability across trials ($R = 0.344$ [0.065; 0.576], $p = 0.016$). Significant sex differences emerged for this trait, indicating faster swimming in males compared to females ($t_{46} = 2.337$, $p = 0.024$; Figure 1D). Swimming velocity resulted strongly correlated to activity ($\rho > 0.99$, $p < 0.001$), suggesting that these two variables described the same behavioral trait.

Regarding the spatial behavior of the subjects in the exploration test, they showed a significant preference for the edges of the experimental arena (time spent in the edge: 0.94 ± 0.03 ; $t_{47} = 104.000$, $p < 0.001$), demonstrating thigmotaxis, a behavior

considered an indicator of traits such as shyness and anxiety.^{65,77,78} Thigmotaxis decreased between the first and the second trial (trial 1: $0.96\% \pm 0.03\%$; trial 2: $0.93\% \pm 0.05\%$; $t_{47} = 3.943$, $p < 0.001$), and also showed different temporal patterns between the trials (interaction time \times trial: $F_{1,1869} = 7.859$, $p = 0.005$; Figure S3C). This trait was not significantly repeatable ($R = 0.114$ [0; 0.375], $p = 0.207$). There was no significant sex difference in thigmotaxis ($t_{46} = 0.285$, $p = 0.777$; Figure 1E). The last variable collected in the first behavioral test was the proportion of area covered in the arena, which indicated exploration tendency. We found that subjects explored most of the arena (proportion of area covered: 0.65 ± 0.08 ; $t_{47} = 13.193$, $p < 0.001$), with a significant exploration increase in the second trial (trial 1: $0.60\% \pm 0.10\%$; trial 2: $0.69\% \pm 0.11\%$; $t_{47} = 4.523$, $p < 0.001$). Subjects were also faster to explore over testing time, especially in the second trial (interaction time \times trial: $F_{1,1869} = 54.793$, $p < 0.001$; Figure S3D). Exploration was not significantly repeatable ($R = 0.069$ [0; 0.334], $p = 0.322$) and did not differ between the sex ($t_{46} = 1.023$, $p = 0.312$; Figure 1F). The exploration variable (i.e., proportion of area covered) was likely affected by the same behavioral trait of thigmotaxis (e.g., shyness and/or anxiety), as they negatively correlated ($\rho = -0.79$, $p < 0.001$).

In the scototaxis test, the subjects displayed the expected significant preference for the dark sector of the arena (0.57 ± 0.12 , $t_{47} = 4.473$, $p < 0.001$), a preference usually associated with shyness. Scototaxis was significantly repeatable ($R = 0.589$ [0.366; 0.757], $p = 0.001$), with no significant difference between trials (trial 1: 0.58 ± 0.14 ; trial 2: 0.56 ± 0.12 ; $t_{47} = 0.639$, $p = 0.526$). Scototaxis response changed during testing time ($F_{1,1869} = 37.641$, $p < 0.001$) with a steeper increase in the second trial (interaction time \times trial: $F_{1,1869} = 7.737$, $p = 0.005$; Figure S3E). We did not find a significant difference between sexes ($t_{46} = 1.010$, $p = 0.318$; Figure 1G).

In the sociability test, the guppies displayed the expected preference for the stimulus shoal (one sample *t* test: 0.95 ± 0.06 , $t_{47} = 48.443$, $p < 0.001$). However, the sociability variable was not significantly repeatable ($R < 0.001$ [0; 0.275], $p > 0.999$). Subjects' sociability score did not significantly differ between trials (trial 1: 0.94 ± 0.09 ; trial 2: 0.95 ± 0.10 ; $t_{47} = 0.549$, $p = 0.586$), but different temporal trends were found between them (interaction time \times trial: $F_{1,1853} = 11.478$, $p < 0.001$; Figure S3F). No differences were found between sexes ($t_{46} = 0.585$, $p = 0.561$; Figure 1H).

In the attempt to reduce the number of behavioral variables for the following analyses, we performed an explorative principal-components analysis (PCA). Several adequacy measures suggested that our dataset was hardly suitable for the analysis (Kaiser-MO: overall 0.572; low internal consistency $\alpha = 0.12$; α changed if single-item deleted < 0.70). The parallel analysis suggested considering only the first two components (eigenvalues significantly greater than 1). The PCA (Table S1) showed that both activity and swimming velocity cross-loaded the two significant principal components (loading factors > 0.40), while scototaxis and sociability traits failed to load a component (loading factors < 0.40). Although the remaining two variables (thigmotaxis and area covered) separately loaded the two components, these eventually resulted as an artifact as they captured individual differences in the same behavioral dimension. Overall, these

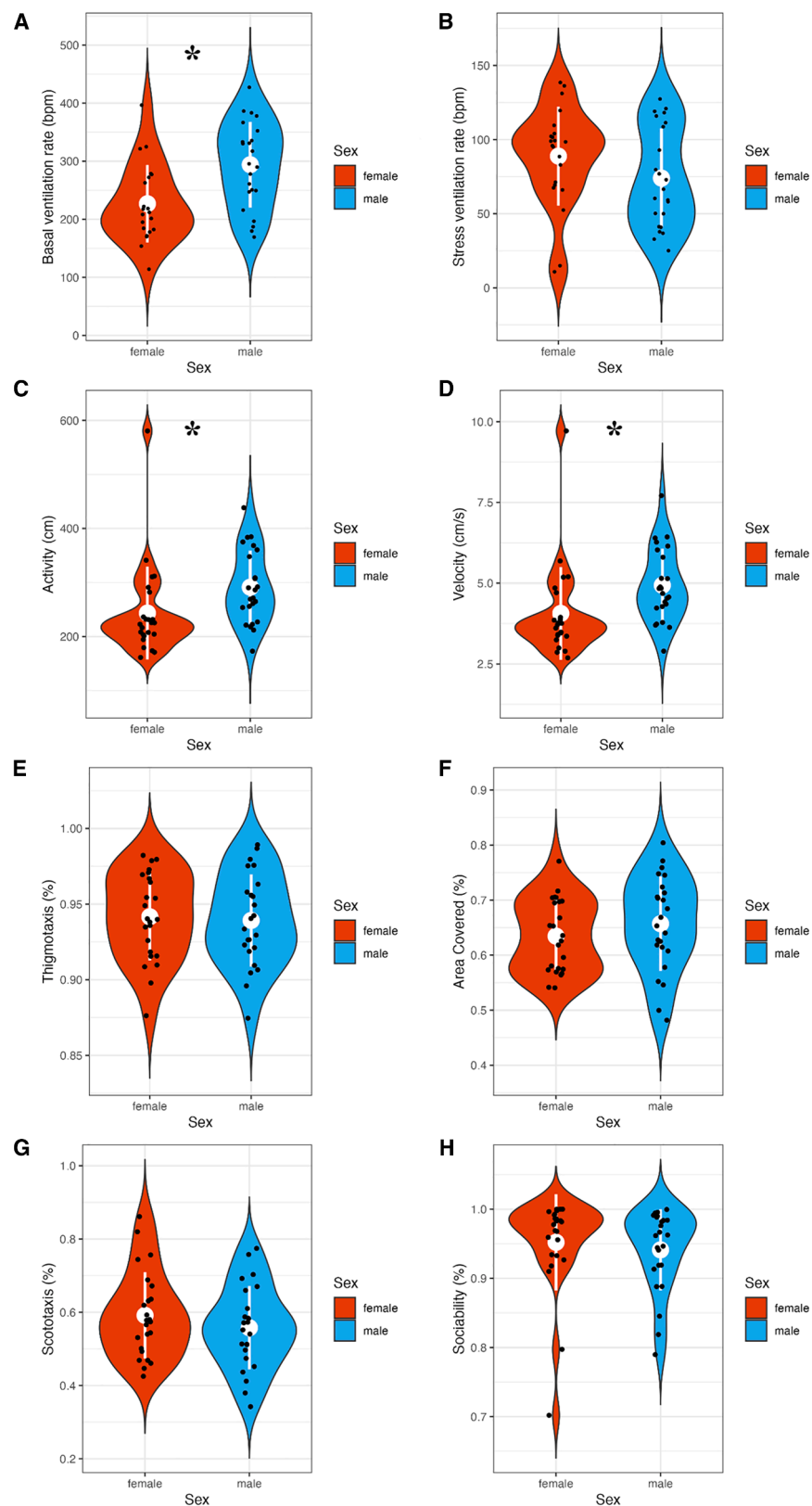


Figure 1. Sex differences in physiology and behavior

Violin plots with individual data points comparing average scores of male and female guppies for (A) basal ventilation rate, (B) stress ventilation rate, (C) activity, (D) velocity, (E) thigmotaxis, (F) area covered, (G) scototaxis, and (H) sociability. Asterisks indicate significant differences. White point and error bars represent mean and SEM.

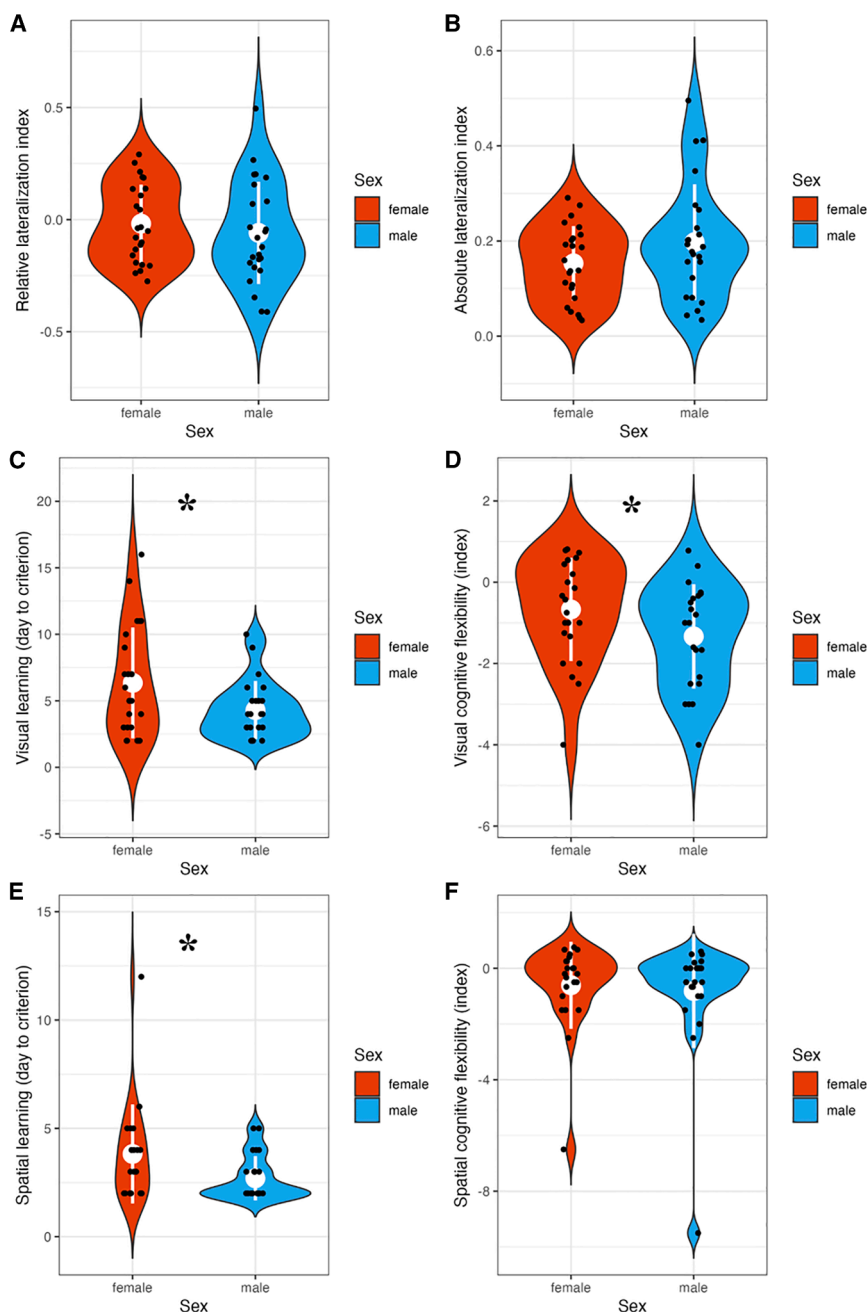


Figure 2. Sex differences in cognitive traits

Violin plots with individual data points comparing average scores of male and female guppies for (A) relative lateralization index, (B) absolute lateralization index, (C) visual learning, (D) visual cognitive flexibility, (E) spatial learning, and (F) spatial cognitive flexibility. Asterisks indicate significant differences. White points and error bars represent mean and SEM.

dex, which accounts only for the strength of lateralization regardless of directionality, was 0.18 ± 0.10 and was significantly greater than zero ($t_{47} = 11.724$, $p < 0.001$). Both these lateralization indices were not significantly repeatable across trials (relative lateralization: $R = 0.091$ [0; 0.364], $p = 0.310$; absolute lateralization: $R < 0.001$ [0; 0.272], $p > 0.999$). The sex did not significantly affect neither the relative ($t_{46} = 0.707$, $p = 0.483$; Figure 2A) nor the absolute lateralization index ($t_{46} = 1.512$, $p = 0.137$; Figure 2B).

To assess visual learning abilities, we used a color discrimination task where the guppies were rewarded with food for selecting a predetermined color from two options.^{59,86} All the guppies participating to the visual learning task (46 out of 46; 100%) reached the learning criterion. The average number of days to reach the criterion was 5.32 ± 3.45 . After the subjects learned the criterion, we conducted a reversal learning test to measure cognitive flexibility by switching the reward contingency between the two colors.^{58,59} All the subjects tested (45 out of 45; 100%) solved the visual reversal learning task in 7.20 ± 2.84 days. Simulation analyses showed that the occurrence of subjects reaching the learning criterion in both the visual learning and visual reversal learning task was not due to chance (simulated subjects reaching the criterion in the learning task: $1.11\% \pm$

analyses suggest that principal components were hardly suitable for describing behavioral variances on our population^{79–83}; thus, we used separate and distinct traits in the following analyses.

Cognitive traits

As the first cognitive trait, we measured lateralization, a trait related to how individuals split information processing between the two hemispheres.⁸⁴ We used a test based on the response to a social stimulus.^{11,36,39,56,85} The relative lateralization index in the population, which considered directionality of hemispheric preferences, was -0.04 ± 0.20 and did not significantly diverge from zero ($t_{47} = 1.329$, $p = 0.190$). The absolute lateralization in-

1.54% , $p < 0.001$; in the reversal learning task: $0.96\% \pm 1.44\%$, $p < 0.001$). Males reached the criterion of the visual learning task significantly faster than females ($t_{44} = 2.085$, $p = 0.043$; Figure 2C). The analyses of a cognitive flexibility index corrected for individuals' learning performance (i.e., positive values indicate high flexibility) showed that females reached the criterion of the visual reversal learning task significantly faster than males ($t_{42} = 2.289$, $p = 0.027$; Figure 2D).

We further characterized cognitive abilities with a spatial learning task where the subjects had to learn to select the correct arm of a T-maze.^{57,60,87} The spatial learning task was followed by a spatial reversal learning task to assess cognitive flexibility.⁵⁷ In

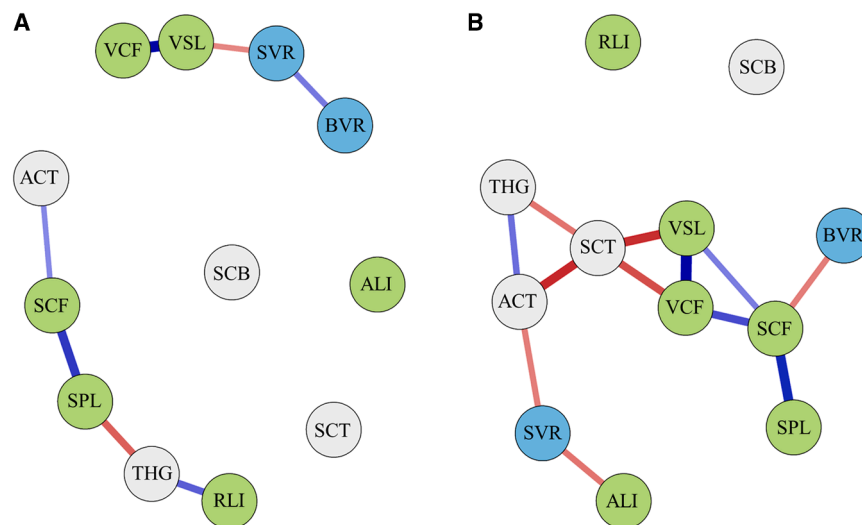


Figure 3. Network structure in male and female guppies

Network plot with significant correlations of (A) females and (B) males. The traits are represented by nodes, and their association by the edges connecting the nodes. The thickness of an edge represents the strength of the correlation between nodes, while the color of edge represented whether the correlation was either negative (red) or positive (blue). The three domains were differentiated by nodes' coloring, i.e., physiology (blue), behavior (light gray), and cognition (green). VCF = visual cognitive flexibility; VSL: visual learning; SCF: spatial cognitive flexibility; SPL: spatial learning; THG: thigmotaxis; ACT: activity; SVR: stress ventilation rate; SCT: scototaxis; BVR: basal ventilation rate; RLI: relative lateralization index; ALI: absolute lateralization index; SCB: sociability.

the spatial tasks, 45 out of 45 participating guppies (100%) reached the criterion both in the learning and reversal learning phase, taking 3.24 ± 1.82 days and 4.31 ± 3.36 days, respectively. The simulation demonstrated that this level of performance was not due to chance (simulated subjects reaching the criterion in the learning task: $0.81\% \pm 1.33\%$, $p < 0.001$; in the reversal learning task $1.48\% \pm 1.82\%$, $p < 0.001$). Males were significantly faster in learning ($t_{43} = 2.147$, $p = 0.037$; Figure 2E), although this effect was only marginal after removing an outlier subject ($t_{42} = 1.998$, $p = 0.052$). There was no sex difference in the cognitive flexibility performance of the spatial task ($t_{43} = 0.380$, $p = 0.706$; Figure 2F).

Network analysis

We investigated covariation among traits using a network approach.^{67,88} The network analysis relied on the correlation coefficients calculated between each pair of traits and produced a series of metrics corrected for the other pairwise relationships.^{89,90} Therefore, the network analysis offers an overall view of the association between a set of traits, potentially providing novel insights on intricate relationships. The results of the network analysis revealed a complex covariation pattern among traits and across different domains, with marked differences between the two sexes (Figure 3). In males, almost all traits were linked in a single, complex cluster (Figure 3A); conversely, in females, associations derived from two linear clusters (Figure 3B). Inspection of the correlation matrixes underlying the networks (Figures 4A and 4B) indicated that females displayed 7 significant correlations overall, 6 of which involving cognitive traits (relative lateralization and thigmotaxis; visual learning and stress metabolism; visual learning and visual cognitive flexibility; spatial learning and thigmotaxis; spatial learning and spatial cognitive flexibility; spatial cognitive flexibility and activity; Figure 4C). Males showed 12 significant correlations, 8 of which involving cognitive traits (spatial learning and spatial cognitive flexibility; spatial cognitive flexibility and basal metabolism; spatial cognitive flexibility and visual cognitive flexibility;

spatial cognitive flexibility and visual learning; visual learning and visual cognitive flexibility; visual learning and scototaxis; visual cognitive flexibility and scototaxis; absolute lateralization and stress metabolism; Figure 4D). A stability permutation analysis suggested that most of the associations between traits identified in the network were highly robust (Figure S4), with the exception of two in the males' network (visual learning versus spatial cognitive flexibility; and basal ventilation rate versus spatial cognitive flexibility) and one in the females' network (activity versus spatial cognitive flexibility).

A comparison between unweighted networks (i.e., without considering the strength of the associations) revealed that males and females showed a similar number of connections per trait when considering all traits ($t_{22} = 1.758$, $p = 0.093$), while males tended to show more associations per trait when considering only traits with at least one association in the network ($t_{17} = 1.982$, $p = 0.064$). In the weighted networks, including all possible relationships between traits, females and males showed similar average pair weights, and thus, a similar strength of association between pairs of traits (considering directionality of the relationship: $t_{130} = 0.033$, $p = 0.974$; considering the absolute value of the relationship: $t_{130} = 1.149$, $p = 0.253$). This conclusion was supported by a permutation analysis on the whole network (global strength invariance test: $S = 2.314$, $p = 0.222$). There was a positive correlation between the weight of the traits of the two sexes (Pearson's product moment correlation; considering directionality of the relationship: $r = 0.245$, $t_{64} = 2.021$, $p = 0.048$; considering the absolute value: $r = 0.300$; $t_{64} = 2.520$, $p = 0.014$). A permutation analysis on the weighted networks did not reveal a significant sex difference considering the whole network (network invariance test: $M = 0.911$, $p = 0.099$).

Divergences between the sexes in network parameters emerged when considering specific traits, specific pairwise relationships, and specific domains. The weights of the traits were significantly affected by the interaction between sex and domain (ANOVA with Satterthwaite's method: $F_{4,60} = 2.897$, $p = 0.029$). Post hoc analysis revealed that males showed stronger

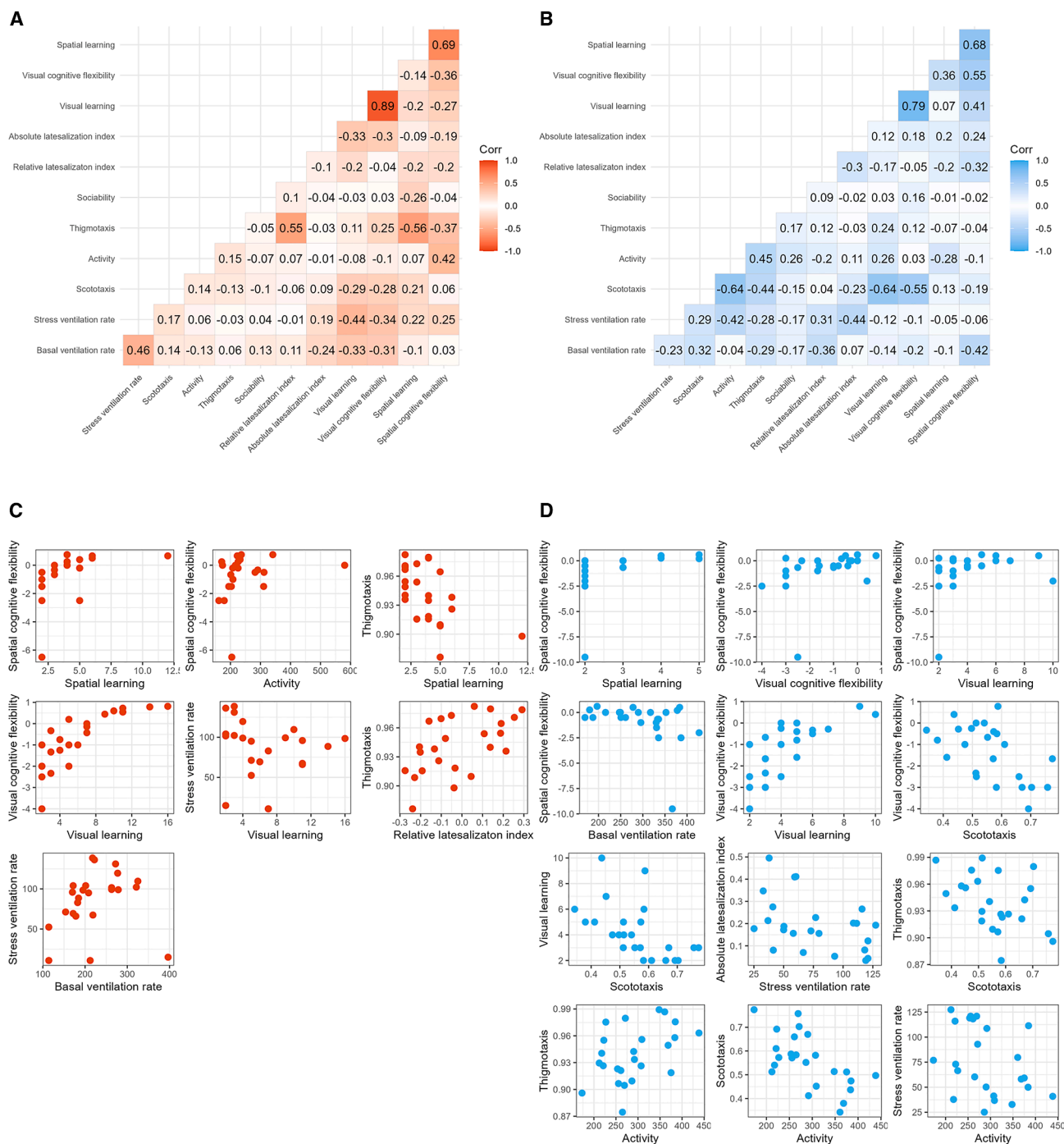


Figure 4. Comparison between female and male guppies in the correlations between traits

Correlation matrix reporting Spearman's ρ for (A) females and (B) males. Scatterplot of significant correlations detected in (C) females and (D) males.

connections between pairs of cognitive traits compared to females ($t_{60} = 2.991$, $p = 0.004$; Figure 5A). Permutation analyses identified five edges (i.e., pairwise associations between traits) that significantly differed between males and females, with an additional four showing marginal differences (Table 1). Of these edges with some degree of sex difference, 7 involved at least

one cognitive trait (77.78%). A similar pattern was observed when running the permutation analysis on the network based on the first 10 min (4 significant and 4 marginals, of whom 6 involved a cognitive trait) or the last 10 min of behavioral testing (6 significant and 5 marginals, of whom 9 involved a cognitive trait), suggesting consistency in covariation differences between

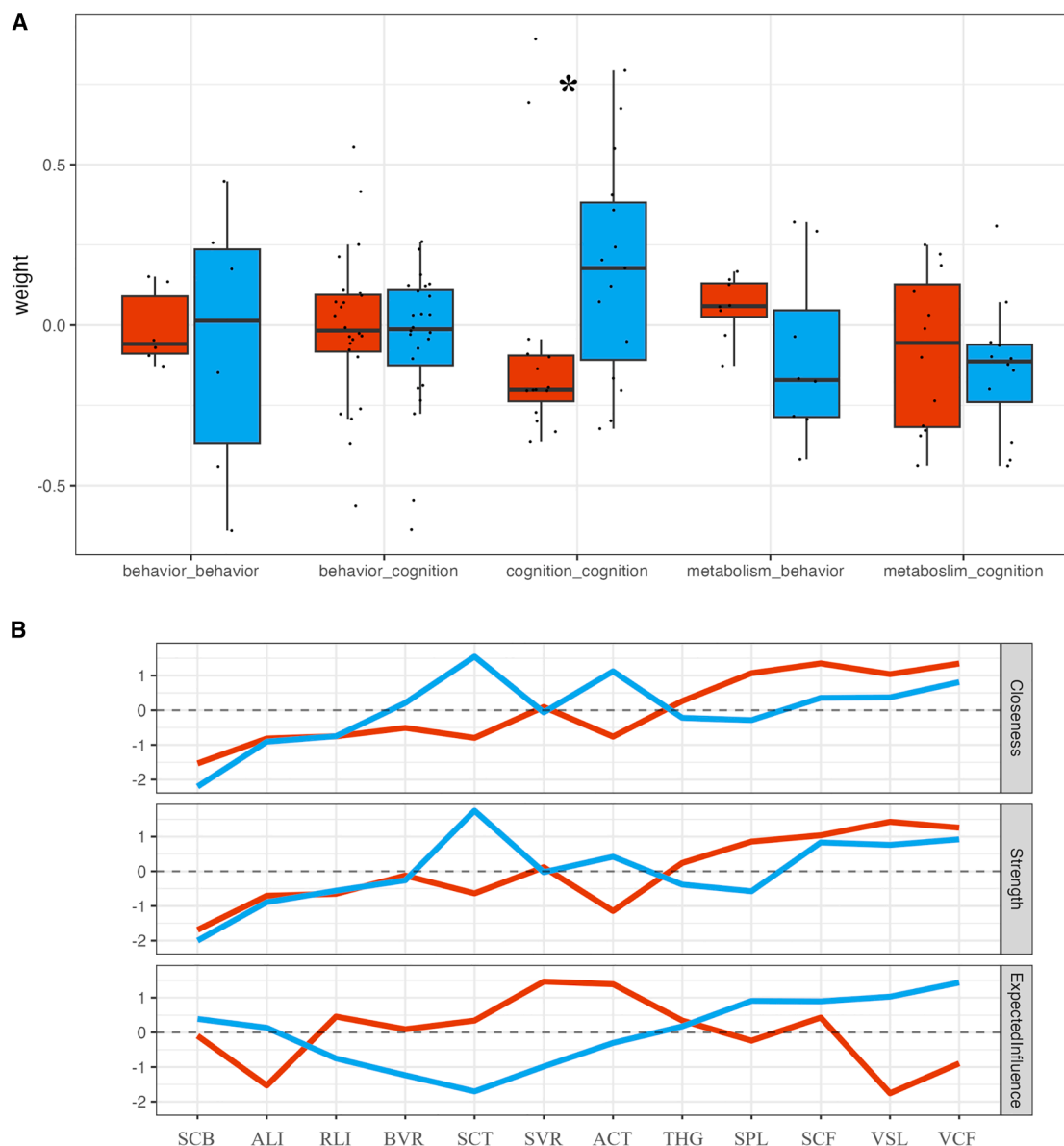


Figure 5. Differences between males and females in edge weight and centrality measures of networks

(A) Relative weight of associations grouped by domain in the male (blue) and female (red) networks. Data are represented as median and upper and lower quartiles.

(B) Centrality plot represented the three centrality measures describing the influence of each node in the female (red) and male (blue) network: "closeness" indicated the proximity of each nodes to the others by summing all nodes' distance; "strength" quantify how a node is strongly connected to other nodes by summing all absolute edge weights from a node; "expected influence" described the relative importance of a node in a network by summing all positive and negative edges extending from the certain node. VCF = visual cognitive flexibility; VSL: visual learning; SCF: spatial cognitive flexibility; SPL: spatial learning; THG: thigmotaxis; ACT: activity; SVR: stress ventilation rate; SCT: scototaxis; BVR: basal ventilation rate; RLI: relative lateralization index; ALI: absolute lateralization index; SCB: sociability.

sex. By considering the centrality measures describing the network structure on the overall dataset, scototaxis and activity had significant differences in strength (i.e., absolute importance of a certain trait) and closeness (i.e., proximity of each trait to the other traits in the network) in the networks of the two sexes (Figure 5B; Table 2). Last, basal metabolism, scototaxis, visual learning, and visual cognitive flexibility had different expected in-

fluences (i.e., relative importance of a certain trait in the network) in the networks of males and females (Figure 5B; Table 2).

DISCUSSION

Phenotypic variance in cognitive traits, whose evolutionary causes are not fully understood, is often associated with

Table 1. Results of the edge invariance test

Pair of traits (dominion)		Edge invariance test
Basal ventilation rate (metabolism)	Stress ventilation rate (metabolism)	E = 0.693, $p = 0.023$
Basal ventilation rate (metabolism)	Relative lateralization index (cognition)	E = 0.472, $p = 0.093$
Absolute lateralization index (cognition)	Stress ventilation rate (metabolism)	E = 0.624, $p = 0.035$
Activity (behavior)	Scototaxis (behavior)	E = 0.775, $p = 0.010$
Activity (behavior)	Spatial cognitive flexibility (cognition)	E = 0.520, $p = 0.060$
Thigmotaxis (behavior)	Spatial learning (cognition)	E = 0.491, $p = 0.078$
Visual cognitive flexibility (cognition)	Spatial learning (cognition)	E = 0.495, $p = 0.078$
Visual learning (cognition)	Spatial cognitive flexibility (cognition)	E = 0.677, $p = 0.035$
Visual cognitive flexibility (cognition)	Spatial cognitive flexibility (cognition)	E = 0.911, $p = 0.006$

Comparison of the strength of the relationships between pairs of traits between male and female guppies. The table reports significant and marginally significant differences. The full list of invariance edge strength tests is reported in the ESM (Table S1).

individual differences in behavioral and physiological traits, potentially due to the underlying effects of intraspecific life-history trade-offs. Given that sexual selection induces significant life-history differences between the sexes, and may directly affect cognition, behavior, and physiology, we predicted that the structure of covariation between traits from these domains would differ between the sexes, particularly in highly sexually dimorphic species. Our study in guppies substantially supports this prediction.

The comparison between male and female guppies revealed several sex differences in the average value of the traits. Males exhibited higher basal metabolism and, consistent with earlier studies, greater activity levels compared to females.^{91,92} Additionally, males learned faster in a spatial discrimination task, as reported by Lucon-Xiccato and Bisazza,⁹³ and in a visual discrimination task, an effect not previously documented in the literature to the best of our knowledge.⁹⁴ Conversely, evidence suggested that females were more efficient at reversing a learned association, supporting the hypothesis of greater cognitive flexibility in female guppies.⁵² While part of the cognitive sex differences could be due to sex differences in motivation, this cannot be the only factor involved, as the two sexes performed differently in the visual learning and reversal learning task, which exploited the same reward (i.e., food). Overall, the sex differences align with predictions from two evolutionary hypotheses, which suggest that variation in the relationships between physiology and behavior²⁷ and between cognition and behavior³¹ may align along a fast-slow continuum. In this specific case, males, with higher metabolism and activity, faster learning, and lower flexibility, would represent the fast side of the continuum. Interestingly, a recent study characterizing phenotypic covariation in the European eel, *Anguilla anguilla*, did not find data supporting the slow-fast continuum across individuals for cognition, behavior, and physiology.³³ There has been ongoing debate over the generality of the fast-slow continuum hypothesis, as evidence both supporting and contradicting it exists.⁹⁵ Notably, the eels in De Russi et al.³³ study were tested at an early developmental stage (glass eel), where sex differentiation had not yet occurred. We propose that at least part of the contrasting findings regarding the fast-slow continuum may be attributable to the effects of sex, as sex differences can indeed magnify alignment in trait covariation.

When we looked at the covariation between traits, we found several significant relationships, in line with previous studies in this species.^{35,38,39,96–98} It is worth noting that lateralization and two behavioral traits were not significantly repeatable. This outcome is often observed for lateralization, but less common in behavioral traits.⁹⁹ The lack of repeatability may be ascribed to several factors, including methodological ones.⁹⁹ For example, the repeatability test for sociability was influenced by low variance across subjects, likely due to the pronounced difference between stimuli that elicited a strong social attraction. It is also possible that some of the behavioral traits measured were not aspects of personality in our population. We cannot exclude that modeling the non-repeatable traits could have contributed to an underestimation of trait covariation.

Critically, our analysis on the effects of sex on traits' covariation revealed several differences between males and females. Males displayed more significant correlations (12 versus 7), also considering correlations involving cognitive traits. When considering all the traits in the networks, we did not detect significant differences between males and females in number of connections per trait and the average trait weights. A potential trend for more connections in male traits, albeit not significant, has emerged after removing the traits without at least one connection. Moreover, the covariations between cognitive traits were significantly stronger in males, suggesting more marked individual differences in cognition in this sex. Specific associations of cognitive traits with behavior and physiology traits significantly differed between sexes in the network, as did the influence of certain traits, such as visual learning and cognitive flexibility. All these findings overall suggest that cognition covaries with traits from other domains in a different way in males and females.

The conclusion of our analyses holds important evolutionary implications. When selection acts on a trait involved in the observed covariations, evolutionary changes in that trait might also indirectly influence the other traits involved in the relationship. This scenario might be crucial for maintaining cognitive variance because it enables indirect effects. For example, fluctuating selection acting on personality¹⁰⁰ and physiology¹⁰¹ may indirectly affect covarying cognitive traits. Our study suggests that such indirect selection differs between males and females. Certainly, there is another side to the story. Covarying traits may exert contrasting effects on the evolution of the focal trait

Table 2. Results of the centrality invariance test

Trait (dominion)	Expected influence	Closeness	Strength
Basal ventilation rate (metabolism)	$C = 1.383, p = 0.123$	$C = 0.006, p = 0.175$	$C = 0.313, p = 0.709$
Stress ventilation rate (metabolism)	$C = 1.852, p = 0.032$	$C = 0.003, p = 0.595$	$C = 0.283, p = 0.756$
Scototaxis (behavior)	$C = 2.015, p = 0.018$	$C = 0.012, p = 0.014$	$C = 1.955, p = 0.014$
Activity (behavior)	$C = 1.088, p = 0.148$	$C = 0.010, p = 0.015$	$C = 1.492, p = 0.015$
Thigmotaxis (behavior)	$C = 0.016, p = 0.984$	$C = 0.002, p = 0.681$	$C = 0.033, p = 0.966$
Sociability (behavior)	$C = 0.451, p = 0.583$	$C = 0.002, p = 0.622$	$C = 0.368, p = 0.421$
Relative lateralization index (cognition)	$C = 1.063, p = 0.119$	$C = 0.004, p = 0.391$	$C = 0.510, p = 0.458$
Absolute lateralization index (cognition)	$C = 0.948, p = 0.293$	$C = 0.003, p = 0.398$	$C = 0.344, p = 0.583$
Visual learning (cognition)	$C = 2.023, p = 0.008$	$C = 0.001, p = 0.796$	$C = 0.184, p = 0.741$
Visual cognitive flexibility (cognition)	$C = 1.995, p = 0.006$	$C = 0.001, p = 0.721$	$C = 0.041, p = 0.948$
Spatial learning (cognition)	$C = 1.080, p = 0.199$	$C = 0.001, p = 0.736$	$C = 0.606, p = 0.346$
Spatial cognitive flexibility (cognition)	$C = 0.710, p = 0.318$	$C < 0.001, p = 0.961$	$C = 0.146, p = 0.834$

Comparison of expected influence, closeness, and strength of each trait in the network between males and females. In bold are reported significant differences.

due to co-occurring selective pressures. Our work suggests that these constraints are potentially influenced by sex differences in the structure of the covariation. The role of indirect selection and constraints in maintaining variance has been extensively studied in behavioral traits.¹⁰² More attention should also be directed toward understanding this in the context of cognitive traits.

Notably, the mechanism generating sex-specific covariation patterns between cognition and other traits remains unclear. One possibility is that cognitive variance is ultimately due to life-history variance. Indeed, neural tissues, being among the most energetically costly in the animal body,⁴⁰ are likely subject to significant energy trade-offs.⁴¹ Given that life-history variance also affects behavior and physiology, and considering the often-divergent life histories between sexes, this could explain why traits from these domains covary. An alternative hypothesis, currently underexplored, is that sexual selection directly influences the links involving cognition. Evidence suggests that when only one sex is required to solve a specific cognitive task, selection may favor the evolution of cognitive sex differences.⁵¹ For example, in the polygynous vole *Microtus pennsylvanicus*, males have larger territories and better spatial abilities than females, a difference not observed in monogamous voles where the two sexes share the same territory.^{103,104} In guppies, several studies have highlighted that the tasks typically faced by the two sexes can be distinct, with females spending most of their effort foraging and males attempting to obtain mates.^{54,55,105} Sexual selection could drive the evolution of specific cognition-cognition, cognition-behavior, or cognition-physiology covariations in one sex due to fitness advantages. This latter possibility is particularly intriguing because correlational selection is thought to be highly effective in maintaining the genetic architectures that determine trait covariation.¹⁰⁶

Individual cognition appears to be embedded in a complex network of relationships with traits from other domains, which might work as constraints and indirect pressures in cognitive evolution. The present study suggests that sex plays a major role in the structure of this covariation among traits, emphasizing the critical direct and indirect role of sexual selection in cognitive

evolution. It is important to note that the focal species of this work is characterized by higher-than-average sexual dimorphism and likely under strong sexual selection.⁵³ Investigating species with varying levels of these two factors is essential to assess the relative importance of sex in cognitive covariations.

Limitations of the study

Our sample size is relatively large compared to typical animal cognition studies; however, it may be insufficient for reliably estimating whether the investigated behaviors should be considered personalities (e.g., consistent behavioral tendencies). This limitation could explain the lack of repeatability observed in certain traits. Additionally, our sociability test yielded very similar scores across individuals, indicating that it may not be robust enough to effectively assess individual differences. Repeatability was not assessed for the physiological traits, although these were expected to be quite consistent in this species.^{107,108} Lastly, the study might underestimate individual differences and covariation as it uses laboratory-raised individuals. While these fish were primarily expected to exhibit individual differences driven by genetic factors, the controlled environment and uniform experiences may have reduced individual differences arising from phenotypic plasticity.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Gaia De Russi (gaia.derussi@unife.it).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Datasets are available as supplementary materials ([Data S2](#)).
- R Code is available as supplementary material ([Data S1](#)).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) (G.D.R.) upon request.

ACKNOWLEDGMENTS

We thank Andrea Margutti for help in building the apparatuses and Nicola Faroli and Fabio Tarantelli for helping in the maintenance and testing of the subjects. Graphical abstract was created with BioRender.com. Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3—Call for tender no. 341 of 15/03/2022 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU award number: project code PE0000006, concession decree no. 1553 of 11/10/2022 adopted by the Italian Ministry of University and Research, CUP D93C22000930002, “A multiscale integrated approach to the study of the nervous system in health and disease” (MNESYS).

AUTHOR CONTRIBUTIONS

G.D.R.: conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft, writing – review and editing; E.G.: data curation, formal analysis, writing – original draft, writing – review and editing; C. B.: conceptualization, methodology, resources, writing – review and editing, funding acquisition; T.L.-X.: conceptualization, methodology, formal analysis, resources, data curation, writing – original draft, writing – review and editing, funding acquisition. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

DECLARATION OF INTERESTS

The authors declare they have no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS**
 - Subjects
 - Ethics
- **METHOD DETAILS**
 - Experimental design
 - Housing conditions for the experiments
 - Physiological traits
 - Behavioral traits
 - Cognitive traits
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2025.112487>.

Received: October 14, 2024

Revised: February 10, 2025

Accepted: April 16, 2025

Published: April 19, 2025

REFERENCES

1. Deary, I.J. (2001). Human intelligence differences: a recent history. *Trends Cogn. Sci.* 5, 127–130. [https://doi.org/10.1016/S1364-6613\(00\)01621-1](https://doi.org/10.1016/S1364-6613(00)01621-1).
2. Galton, F. (1870). *Hereditary Genius: An Inquiry into its Laws and Consequences* (Appleton & Co).
3. Thorndike, E.L. (1918). Individual differences. *Psychol. Bull.* 15, 148–159. <https://doi.org/10.1037/h0070314>.
4. Fernandes, H.B.F., Woodley, M.A., and te Nijenhuis, J. (2014). Differences in cognitive abilities among primates are concentrated on G: Phenotypic and phylogenetic comparisons with two meta-analytical databases. *Intelligence* 46, 311–322. <https://doi.org/10.1016/j.intell.2014.07.007>.
5. Matzel, L.D., and Sauce, B. (2017). Individual differences: Case studies of rodent and primate intelligence. *J. Exp. Psychol. Anim. L.* 43, 325–340. <https://doi.org/10.1037/xan0000152>.
6. Thornton, A., and Lukas, D. (2012). Individual variation in cognitive performance: developmental and evolutionary perspectives. *Philos. T. Roy. Soc. B* 367, 2773–2783. <https://doi.org/10.1098/rstb.2012.0214>.
7. Cussen, V.A. (2017). Psittacine cognition: Individual differences and sources of variation. *Behav. Process.* 134, 103–109. <https://doi.org/10.1016/j.beproc.2016.11.008>.
8. Matsubara, S., Deeming, D.C., and Wilkinson, A. (2017). Cold-blooded cognition: new directions in reptile cognition. *Curr. Opin. Behav. Sci.* 16, 126–130. <https://doi.org/10.1016/j.cobeha.2017.06.006>.
9. Lucon-Xiccato, T., and Bisazza, A. (2017). Individual differences in cognition among teleost fishes. *Behav. Processes* 141, 184–195. <https://doi.org/10.1016/j.beproc.2017.01.015>.
10. Lucon-Xiccato, T., Carere, C., and Baracchi, D. (2024). Intraspecific variation in invertebrate cognition: a review. *Behav. Ecol. Sociobiol.* 78, 1. <https://doi.org/10.1007/s00265-023-03413-8>.
11. Lucon-Xiccato, T., Bisazza, A., and Bertolucci, C. (2020). Guppies show sex and individual differences in the ability to inhibit behaviour. *Anim. Cogn.* 23, 535–543. <https://doi.org/10.1007/s10071-020-01357-4>.
12. Cole, E.F., and Quinn, J.L. (2012). Personality and problem-solving performance explain competitive ability in the wild. *Proc. Biol. Sci.* 279, 1168–1175. <https://doi.org/10.1098/rspb.2011.1539>.
13. Cole, E.F., Morand-Ferron, J., Hinks, A.E., and Quinn, J.L. (2012). Cognitive ability influences reproductive life history variation in the wild. *Curr. Biol.* 22, 1808–1812. <https://doi.org/10.1016/j.cub.2012.07.051>.
14. Fichtel, C., Henke-von der Malsburg, J., and Kappeler, P.M. (2023). Cognitive performance is linked to fitness in a wild primate. *Sci. Adv.* 9, ead9365. <https://doi.org/10.1126/sciadv.adf9365>.
15. Huebner, F., Fichtel, C., and Kappeler, P.M. (2018). Linking cognition with fitness in a wild primate: fitness correlates of problem-solving performance and spatial learning ability. *Philos. T. Roy. Soc. B* 373, 20170295. <https://doi.org/10.1098/rstb.2017.0295>.
16. Rochais, C., Schradin, C., and Pillay, N. (2023). Cognitive performance is linked to survival in free-living African striped mice. *Proc. Biol. Sci.* 290, 20230205. <https://doi.org/10.1098/rspb.2023.0205>.
17. Smith, C., Philips, A., and Reichard, M. (2015). Cognitive ability is heritable and predicts the success of an alternative mating tactic. *Proc. Biol. Sci.* 282, 20151046. <https://doi.org/10.1098/rspb.2015.1046>.
18. Rowe, C., and Healy, S.D. (2014). Measuring variation in cognition. *Behav. Ecol.* 25, 1287–1292. <https://doi.org/10.1093/beheco/aru090>.
19. Bebus, S.E., Small, T.W., Jones, B.C., Elderbrock, E.K., and Schoech, S. J. (2016). Associative learning is inversely related to reversal learning and varies with nestling corticosterone exposure. *Anim. Behav.* 111, 251–260. <https://doi.org/10.1016/j.anbehav.2015.10.027>.
20. Beran, M.J., and Hopkins, W.D. (2018). 2018 Self-control in chimpanzees relates to general intelligence. *Curr. Biol.* 28, 574–579.e3. <https://doi.org/10.1016/j.cub.2017.12.043>.
21. Lucon-Xiccato, T., and Dadda, M. (2017). Individual guppies differ in quantity discrimination performance across antipredator and foraging contexts. *Behav. Ecol. Sociobiol.* 71, 13–19. <https://doi.org/10.1007/s00265-016-2231-y>.
22. Wolf, M., and Weissing, F.J. (2012). Animal personalities: consequences for ecology and evolution. *Trends Ecol. Evol.* 27, 452–461. <https://doi.org/10.1016/j.tree.2012.05.001>.
23. Biro, P.A., and Stamps, J.A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* 25, 653–659. <https://doi.org/10.1016/j.tree.2010.08.003>.

24. Speakman, J.R., Król, E., and Johnson, M.S. (2004). The functional significance of individual variation in basal metabolic rate. *Physiol. Biochem. Zool.* 77, 900–915. <https://doi.org/10.1086/427059>.
25. Biro, P.A., and Stamps, J.A. (2008). Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.* 23, 361–368. <https://doi.org/10.1016/j.tree.2008.04.003>.
26. Stamps, J.A. (2007). Growth-mortality tradeoffs and ‘personality traits’ in animals. *Ecol. Lett.* 10, 355–363. <https://doi.org/10.1111/j.1461-0248.2007.01034.x>.
27. Dammhahn, M., Dingemanse, N.J., Niemelä, P.T., and Réale, D. (2018). Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. *Behav. Ecol. Sociobiol.* 72, 1–8. <https://doi.org/10.1007/s00265-018-2473-y>.
28. Montiglio, P.O., Dammhahn, M., Dubuc Messier, G., and Réale, D. (2018). The pace-of-life syndrome revisited: the role of ecological conditions and natural history on the slow-fast continuum. *Behav. Ecol. Sociobiol.* 72, 116–119. <https://doi.org/10.1007/s00265-018-2526-2>.
29. Carere, C., and Locurto, C. (2011). Interaction between animal personality and animal cognition. *Curr. Zool.* 57, 491–498. <https://doi.org/10.1093/czoolo/57.4.491>.
30. Griffin, A.S., Guille, L.M., and Healy, S.D. (2015). Cognition and personality: an analysis of an emerging field. *Trends Ecol. Evol.* 30, 207–214. <https://doi.org/10.1016/j.tree.2015.01.012>.
31. Sih, A., and Del, G.M. (2012). Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philos. T. Roy. Soc. B* 367, 2762–2772. <https://doi.org/10.1098/rstb.2012.0216>.
32. Cortese, D., Munson, A., Storm, Z., Jones, N.A.R., and Killen, S.S. (2024). The effect of metabolism on cognitive performance varies with task complexity in common minnows, *Phoxinus phoxinus*. *Anim. Behav.* 214, 95–110. <https://doi.org/10.1016/j.anbehav.2024.06.003>.
33. De Russi, G., Lanzoni, M., Bisazza, A., Domenici, P., Castaldelli, G., Bertolucci, C., and Lucon-Xiccato, T. (2024). Eels’ individual migratory behaviour stems from a complex syndrome involving cognition, behaviour, physiology and life-history. *Proc. Natl. Acad. Sci. USA* 121, e2407804121. <https://doi.org/10.1073/pnas.2407804121>.
34. Guenther, A., Brust, V., Dersen, M., and Trillmich, F. (2014). Learning and personality types are related in caviés (*Cavia aperea*). *J. Comp. Psychol.* 128, 74–81. <https://doi.org/10.1037/a0033678>.
35. Savaşçı, B.B., Lucon-Xiccato, T., and Bisazza, A. (2021). Ontogeny and personality affect inhibitory control in guppies, *Poecilia reticulata*. *Anim. Behav.* 180, 111–121. <https://doi.org/10.1016/j.anbehav.2021.08.013>.
36. Berlinghieri, F., Jansen, N., Riedstra, B., Brown, C., and Groothuis, T.G. G. (2024). The effect of light during embryonic development on laterality and exploration in Western Rainbowfish. *Laterality* 29, 1–18. <https://doi.org/10.1080/1357650X.2023.2252567>.
37. Gourso, C., Düpjan, S., Kanitz, E., Tuchscherer, A., Puppe, B., and Leliveld, L.M.C. (2019). Assessing animal individuality: links between personality and laterality in pigs. *Curr. Zool.* 65, 541–551. <https://doi.org/10.1093/cz/zoy071>.
38. Irving, E., and Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *J. Fish. Biol.* 83, 311–325. <https://doi.org/10.1111/jfb.12165>.
39. Panizzon, P., Gismann, J., Riedstra, B., Nicolaus, M., Brown, C., and Groothuis, T. (2024). Effects of early predation and social cues on the relationship between laterality and personality. *Behav. Ecol.* 35, arae012. <https://doi.org/10.1093/beheco/arae012>.
40. Aiello, L.C., and Wheeler, P. (1995). The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Curr. Anthropol.* 36, 199–221. <https://doi.org/10.1086/204350>.
41. Köttschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A.A., and Kolm, N. (2013). Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Curr. Biol.* 23, 168–171. <https://doi.org/10.1016/j.cub.2012.11.058>.
42. Bonduriansky, R., Maklakov, A., Zajitschek, F., and Brooks, R. (2008). Sexual selection, sexual conflict and the evolution of ageing and life span. *Funct. Ecol.* 22, 443–453. <https://doi.org/10.1111/j.1365-2435.2008.01417.x>.
43. Schuett, W., Tregenza, T., and Dall, S.R.X. (2010). Sexual selection and animal personality. *Biol. Rev.* 85, 217–246. <https://doi.org/10.1111/j.1469-185X.2009.00101.x>.
44. Somjee, U., Shankar, A., and Falk, J.J. (2022). Can sex-specific metabolic rates provide insight into patterns of metabolic scaling? *Integr. Comp. Biol.* 62, icac135–1470. <https://doi.org/10.1093/icb/icac135>.
45. Hämäläinen, A., Immonen, E., Tarka, M., and Schuett, W. (2018). Evolution of sex-specific pace-of-life syndromes: causes and consequences. *Behav. Ecol. Sociobiol.* 72, 1–15. <https://doi.org/10.1007/s00265-018-2466-x>.
46. Immonen, E., Hämäläinen, A., Schuett, W., and Tarka, M. (2018). Evolution of sex-specific pace-of-life syndromes: genetic architecture and physiological mechanisms. *Behav. Ecol. Sociobiol.* 72, 1–23. <https://doi.org/10.1007/s00265-018-2462-1>.
47. Moschilla, J.A., Tomkins, J.L., and Simmons, L.W. (2019). Sex-specific pace-of-life syndromes. *Behav. Ecol.* 30, 1096–1105. <https://doi.org/10.1093/beheco/arz055>.
48. Tarka, M., Guenther, A., Niemelä, P.T., Nakagawa, S., and Noble, D.W. (2018). Sex differences in life history, behavior, and physiology along a slow-fast continuum: a meta-analysis. *Behav. Ecol. Sociobiol.* 72, 1–13. <https://doi.org/10.1007/s00265-018-2534-2>.
49. Cummings, M.E. (2018). Sexual conflict and sexually dimorphic cognition—reviewing their relationship in poeciliid fishes. *Behav. Ecol. Sociobiol.* 72, 73. <https://doi.org/10.1007/s00265-018-2483-9>.
50. Jonasson, Z. (2005). Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. *Neurosci. Biobehav. Rev.* 28, 811–825. <https://doi.org/10.1016/j.neubiorev.2004.10.006>.
51. Jones, C.M., Braithwaite, V.A., and Healy, S.D. (2003). The evolution of sex differences in spatial ability. *Behav. Neurosci.* 117, 403–411. <https://doi.org/10.1037/0735-7044.117.3.403>.
52. Lucon-Xiccato, T. (2022). The contribution of executive functions to sex differences in animal cognition. *Neurosci. Biobehav. Rev.* 138, 104705. <https://doi.org/10.1016/j.neubiorev.2022.104705>.
53. Houde, A.E. (2019). *Sex, Color, and Mate Choice in Guppies* (Princeton University Press).
54. Magurran, A.E., and Garcia, C.M. (2000). Sex differences in behaviour as an indirect consequence of mating system. *J. Fish. Biol.* 57, 839–857. <https://doi.org/10.1111/j.1095-8649.2000.tb02196.x>.
55. Magurran, A.E., and Seghers, B.H. (1994). A cost of sexual harassment in the guppy, *Poecilia reticulata*. *Proc. R. Soc. Lond. B Biol. Sci.* 258, 89–92. <https://doi.org/10.1098/rspb.1994.0147>.
56. Dadda, M., Agrillo, C., Bisazza, A., and Brown, C. (2015). Laterality enhances numerical skills in the guppy, *Poecilia reticulata*. *Front. Behav. Neurosci.* 9, 285. <https://doi.org/10.3389/fnbeh.2015.00285>.
57. De Russi, G., Montalbano, G., Gatto, E., Maggi, E., Cannicci, S., Bertolucci, C., and Lucon-Xiccato, T. (2024). Differential impact of artificial light at night on cognitive flexibility in visual and spatial reversal learning tasks. *Anim. Behav.* 218, 173–183. <https://doi.org/10.1016/j.anbehav.2024.10.008>.
58. Lucon-Xiccato, T., and Bisazza, A. (2014). Discrimination reversal learning reveals greater female behavioural flexibility in guppies. *Biol. Lett.* 10, 20140206. <https://doi.org/10.1098/rsbl.2014.0206>.
59. Lucon-Xiccato, T., Montalbano, G., and Bertolucci, C. (2023). Adaptive phenotypic plasticity induces individual variability along a cognitive trade-off. *Proc. Biol. Sci.* 290, 20230350. <https://doi.org/10.1098/rspb.2023.0350>.

60. Wang, S., Qiu, J., Zhao, M., Li, F., Yu, R., and Li, A. (2020). Accumulation and distribution of neurotoxin BMAA in aquatic animals and effect on the behavior of zebrafish in a T-maze test. *Toxicol.* 173, 39–47. <https://doi.org/10.1016/j.toxicol.2019.11.005>.
61. Handelsman, C.A., Broder, E.D., Dalton, C.M., Ruell, E.W., Myrick, C.A., Reznick, D.N., and Ghalambor, C.K. (2013). Predator-induced phenotypic plasticity in metabolism and rate of growth: rapid adaptation to a novel environment. *Integr. Comp. Biol.* 53, 975–988. <https://doi.org/10.1093/icb/ict057>.
62. Killen, S.S., Marras, S., Ryan, M.R., Domenici, P., and McKenzie, D.J. (2012). A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Funct. Ecol.* 26, 134–143. <https://doi.org/10.1111/j.1365-2435.2011.01920.x>.
63. Metcalfe, N.B., Van Leeuwen, T.E., and Killen, S.S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *J. Fish. Biol.* 88, 298–321. <https://doi.org/10.1111/jfb.12699>.
64. Brown, C., and Irving, E. (2014). Individual personality traits influence group exploration in a feral guppy population. *Behav. Ecol.* 25, 95–101. <https://doi.org/10.1093/beheco/art090>.
65. Dos Santos, C.P., de Oliveira, M.N., Silva, P.F., and Luchiari, A.C. (2023). Relationship between boldness and exploratory behavior in adult zebrafish. *Behav. Processes* 209, 104885. <https://doi.org/10.1016/j.beproc.2023.104885>.
66. Smith, B.R., and Blumstein, D.T. (2010). Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). *Behav. Ecol.* 21, 919–926. <https://doi.org/10.1093/beheco/arq084>.
67. Borsboom, D., Deserno, M.K., Rhemtulla, M., Epskamp, S., Fried, E.I., McNally, R.J., Robinaugh, D.J., Perugini, M., Dalege, J., Costantini, G., et al. (2021). Network analysis of multivariate data in psychological science. *Nat. Rev. Methods Primers* 1, 58. <https://doi.org/10.1038/s43586-021-00055-w>.
68. Killen, S.S., Croft, D.P., Salin, K., and Darden, S.K. (2016). Male sexually coercive behaviour drives increased swimming efficiency in female guppies. *Funct. Ecol.* 30, 576–583. <https://doi.org/10.1111/1365-2435.12527>.
69. Norin, T., and Clark, T.D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *J. Fish. Biol.* 88, 122–151. <https://doi.org/10.1111/jfb.12796>.
70. Brown, C., Gardner, C., and Braithwaite, V.A. (2005). Differential stress responses in fish from areas of high-and low-predation pressure. *J. Comp. Physiol. B* 175, 305–312. <https://doi.org/10.1007/s00360-005-0486-0>.
71. Frisk, M., Skov, P.V., and Steffensen, J.F. (2012). Thermal optimum for pikeperch (*Sander lucioperca*) and the use of ventilation frequency as a predictor of metabolic rate. *Aquaculture* 324–325, 151–157. <https://doi.org/10.1016/j.aquaculture.2011.10.024>.
72. Millidine, K.J., Metcalfe, N.B., and Armstrong, J.D. (2008). The use of ventilation frequency as an accurate indicator of metabolic rate in juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 65, 2081–2087. <https://doi.org/10.1139/F08-118>.
73. Queiroz, H., and Magurran, A.E. (2005). Safety in numbers? Shoaling behaviour of the Amazonian red-bellied piranha. *Biol. Lett.* 1, 155–157. <https://doi.org/10.1098/rsbl.2004.0267>.
74. Dadda, M., Zandonà, E., and Bisazza, A. (2007). Emotional responsiveness in fish from lines artificially selected for a high or low degree of laterality. *Physiol. Behav.* 92, 764–772. <https://doi.org/10.1016/j.physbeh.2007.06.001>.
75. Köhler, A., Hildenbrand, P., Schleucher, E., Riesch, R., Arias-Rodriguez, L., Streit, B., and Plath, M. (2011). Effects of male sexual harassment on female time budgets, feeding behavior, and metabolic rates in a tropical livebearing fish (*Poecilia mexicana*). *Behav. Ecol. Sociobiol.* 65, 1513–1523. <https://doi.org/10.1007/s00265-011-1161-y>.
76. Bell, A.M., Hankison, S.J., and Laskowski, K.L. (2009). The repeatability of behaviour: a meta-analysis. *Anim. Behav.* 77, 771–783. <https://doi.org/10.1016/j.anbehav.2008.12.022>.
77. Lucon-Xiccato, T., Loosli, F., Conti, F., Foulkes, N.S., and Bertolucci, C. (2022a). Comparison of anxiety-like and social behaviour in medaka and zebrafish. *Sci. Rep.* 12, 10926. <https://doi.org/10.1038/s41598-022-14978-1>.
78. Simon, P., Dupuis, R., and Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav. Brain Res.* 61, 59–64. [https://doi.org/10.1016/0166-4328\(94\)90008-6](https://doi.org/10.1016/0166-4328(94)90008-6).
79. Comrey, A.L., and Lee, H.B. (2013). *A First Course in Factor Analysis* (Psychology press).
80. Dziuban, C.D., and Shirkey, E.C. (1974). When is a correlation matrix appropriate for factor analysis? Some decision rules. *Psychol. Bull.* 81, 358–361. <https://doi.org/10.1037/h0036316>.
81. Hucheson, G.D., and Sofroniou, N. (1999). *The Multivariate Social Scientist: Introductory Statistics Using Generalized Linear Models* (New York: Sage).
82. Kaiser, H.F. (1960). The application of electronic computers to factor analysis. *Educ. Psychol. Meas.* 20, 141–151. <https://doi.org/10.1177/001316446002000>.
83. Price, P.C., Jhangiani, R.S., Chiang, I.A., Leighton, D.C., and Cuttler, C. (2017). *Research Methods in Psychology, 3rd american edition* (Press-BooksPublications).
84. Bisazza, A., and Brown, C. (2011). Lateralization of cognitive functions in fish. *Fish Cogn. Behav.* 2, 298–324.
85. Brown, C., Western, J., and Braithwaite, V.A. (2007). The influence of early experience on, and inheritance of, cerebral lateralization. *Anim. Behav.* 74, 231–238. <https://doi.org/10.1016/j.anbehav.2006.08.014>.
86. Montalbano, G., Bertolucci, C., and Lucon-Xiccato, T. (2022). Cognitive phenotypic plasticity: environmental enrichment affects learning but not executive functions in a teleost fish, *Poecilia reticulata*. *Biology* 11, 64. <https://doi.org/10.3390/biology11010064>.
87. Lucon-Xiccato, T., Montalbano, G., Gatto, E., Frigato, E., D'Aniello, S., and Bertolucci, C. (2022b). Individual differences and knockout in zebrafish reveal similar cognitive effects of BDNF between teleosts and mammals. *Proc. Biol. Sci.* 289, 20222036. <https://doi.org/10.1098/rspb.2022.2036>.
88. Hevey, D. (2018). Network analysis: a brief overview and tutorial. *Health Psychol. Behav. Med.* 6, 301–328. <https://doi.org/10.1080/21642850.2018.1521283>.
89. Epskamp, S., and Fried, E.I. (2018). A tutorial on regularized partial correlation networks. *Psychol. Methods* 23, 617–634. <https://doi.org/10.1037/met0000167>.
90. Epskamp, S., Borsboom, D., and Fried, E.I. (2018). Estimating psychological networks and their accuracy: A tutorial paper. *Behav. Res. Methods* 50, 195–212. <https://doi.org/10.3758/s13428-017-0862-1>.
91. Madenjian, C.P., Rediske, R.R., Krabbenhoft, D.P., Stapanian, M.A., Chernyak, S.M., and O'Keefe, J.P. (2016). Sex differences in contaminant concentrations of fish: a synthesis. *Biol. Sex Differ.* 7, 42. <https://doi.org/10.1186/s13293-016-0090-x>.
92. Piyapong, C., Krause, J., Chapman, B.B., Ramnarine, I.W., Louca, V., and Croft, D.P. (2010). Sex matters: a social context to boldness in guppies (*Poecilia reticulata*). *Behav. Ecol.* 21, 3–8. <https://doi.org/10.1093/beheco/arp142>.
93. Lucon-Xiccato, T., and Bisazza, A. (2017). Sex differences in spatial abilities and cognitive flexibility in the guppy. *Anim. Behav.* 123, 53–60. <https://doi.org/10.1016/j.anbehav.2016.10.026>.
94. Fuss, T., and Witte, K. (2019). Sex differences in color discrimination and serial reversal learning in mollies and guppies. *Curr. Zool.* 65, 323–332. <https://doi.org/10.1093/cz/zoz029>.
95. Van de Walle, J., Fay, R., Gaillard, J.M., Pelletier, F., Hamel, S., Gamelon, M., Barbraud, C., Blanchet, F.G., Blumstein, D.T., Charmantier, A.,

- Delord, K., et al. (2023). Individual life histories: neither slow nor fast, just diverse. *Proc. Biol. Sci.* 290, 20230511. <https://doi.org/10.1098/rspb.2023.0511>.
96. Gatto, E., Agrillo, C., Brown, C., and Dadda, M. (2019). Individual differences in numerical skills are influenced by brain lateralization in guppies (*Poecilia reticulata*). *Intelligence* 74, 12–17. <https://doi.org/10.1016/j.intell.2019.04.004>.
97. Godin, J.G.J., Le Roy, A., Burns, A.L., Seebacher, F., and Ward, A.J.W. (2022). Pace-of-life syndrome: linking personality, metabolism and colour ornamentation in male guppies. *Anim. Behav.* 194, 13–33. <https://doi.org/10.1016/j.anbehav.2022.09.012>.
98. Trompf, L., and Brown, C. (2014). Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. *Anim. Behav.* 88, 99–106. <https://doi.org/10.1016/j.anbehav.2013.11.022>.
99. Penry-Williams, I.L., Brown, C., and Ioannou, C.C. (2022). 2022 Detecting behavioural lateralisation in *Poecilia reticulata* is strongly dependent on experimental design. *Behav. Ecol. Sociobiol.* 76, 25. <https://doi.org/10.1007/s00265-022-03135-3>.
100. Dingemanse, N.J., Both, C., Drent, P.J., and Tinbergen, J.M. (2004). 2004 Fitness consequences of avian personalities in a fluctuating environment. *Proc. Biol. Sci.* 271, 847–852. <https://doi.org/10.1098/rspb.2004.2680>.
101. Nilsson, J.F., and Nilsson, J.Å. (2016). Fluctuating selection on basal metabolic rate. *Ecol. Evol.* 6, 1197–1202. <https://doi.org/10.1002/ecs3.1954>.
102. Sih, A., Bell, A.M., Johnson, J.C., and Ziemba, R.E. (2004). Behavioral syndromes: an integrative overview. *Q. Rev. Biol.* 79, 241–277. <https://doi.org/10.1086/422893>.
103. Gaulin, S.J., and FitzGerald, R.W. (1986). Sex differences in spatial ability: an evolutionary hypothesis and test. *Am. Nat.* 127, 74–88. <https://doi.org/10.1086/284468>.
104. Gaulin, S.J.C., and FitzGerald, R.W. (1989). Sexual selection for spatial-learning ability. *Anim. Behav.* 37, 322–331. [https://doi.org/10.1016/0003-3472\(89\)90121-8](https://doi.org/10.1016/0003-3472(89)90121-8).
105. Magurran, A.E., and Seghers, B.H. (1994). Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. Lond. B Biol. Sci.* 255, 31–36. <https://doi.org/10.1098/rspb.1994.0005>.
106. Sinervo, B., and Svensson, E. (2002). 2002 Correlational selection and the evolution of genomic architecture. *Heredity* 89, 329–338. <https://doi.org/10.1038/sj.hdy.6800148>.
107. Oufiero, C.E., and Garland, T., Jr. (2009). Repeatability and correlation of swimming performances and size over varying time-scales in the guppy (*Poecilia reticulata*). *Funct. Ecol.* 23, 969–978. <https://doi.org/10.1111/j.1365-2435.2009.01571.x>.
108. White, S.J., Kells, T.J., and Wilson, A.J. (2016). Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulata*. *Beyond Behav.* 153, 1517–1543. <https://doi.org/10.1163/1568539X-00003375>.
109. Bischoff, R.J., Gould, J.L., and Rubenstein, D.I. (1985). Tail size and female choice in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* 17, 253–255. <https://doi.org/10.1007/BF00300143>.
110. Bell, A. (2013). Randomized or fixed order for studies of behavioral syndromes? *Behav. Ecol.* 24, 16–20. <https://doi.org/10.1093/beheco/ars148>.
111. Voutilainen, A., Seppänen, E., and Huuskonen, H. (2011). A methodological approach to measuring the oxygen consumption profile of six freshwater fish species: implications for determination of the standard metabolic rate. *Mar. Fresh. Behav. Phys.* 44, 239–250. <https://doi.org/10.1080/10236244.2011.622090>.
112. Beukeboom, R., and Benhaim, D. (2024). How to measure exploration: A combined estimation method. *Behav. Process.* 216, 104998. <https://doi.org/10.1016/j.beproc.2024.104998>.
113. Mair, A., Lucon-Xiccato, T., and Bisazza, A. (2021). Guppies in the puzzle box: innovative problem-solving by a teleost fish. *Behav. Ecol. Sociobiol.* 75, 17. <https://doi.org/10.1007/s00265-020-02953-7>.
114. Maximino, C., Marques de Brito, T., Dias, C.A.G.D.M., Gouveia, A., Jr., and Morato, S. (2010). Scototaxis as anxiety-like behavior in fish. *Nat. Protoc.* 5, 209–216. <https://doi.org/10.1038/nprot.2009.225>.
115. Cote, J., Fogarty, S., and Sih, A. (2012). Individual sociability and choosiness between shoal types. *Anim. Behav.* 83, 1469–1476. <https://doi.org/10.1016/j.anbehav.2012.03.019>.
116. Moretz, J.A., Martins, E.P., and Robison, B.D. (2007). The effects of early and adult social environment on zebrafish (*Danio rerio*) behavior. *Environ. Biol. Fishes* 80, 91–101. <https://doi.org/10.1007/s10641-006-9122-4>.
117. Bisazza, A., and Vallortigara, G. (1997). Rotational swimming preferences in mosquitofish: evidence for brain lateralization? *Physiol. Behav.* 62, 1405–1407. [https://doi.org/10.1016/S0031-9384\(97\)00230-8](https://doi.org/10.1016/S0031-9384(97)00230-8).
118. Bisazza, A., Cantalupo, C., Capocchiano, M., and Vallortigara, G. (2000). Population lateralisation and social behaviour: a study with 16 species of fish. *Laterality* 5, 269–284. <https://doi.org/10.1080/713754381>.
119. Cantalupo, C., Bisazza, A., and Vallortigara, G. (1995). Lateralization of predator-evasion response in a teleost fish (*Girardinus falcatus*). *Neuropsychologia* 33, 1637–1646. [https://doi.org/10.1016/0028-3932\(95\)00043-7](https://doi.org/10.1016/0028-3932(95)00043-7).
120. Faul, F., Erdfelder, E., Lang, A.G., and Buchner, A. (2007). G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. <https://doi.org/10.3758/BF03193146>.
121. Nakagawa, S., and Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.* 85, 935–956. <https://doi.org/10.1111/j.1469-185X.2010.00141.x>.
122. Buuren, S.V., and Groothuis-Oudshoorn, K. (2011). 2011 mice: Multivariate imputation by chained equations in R. *J. Stat. Softw.* 45, 1–67. <https://doi.org/10.18637/jss.v045.i03>.
123. Nakagawa, S., and Freckleton, R.P. (2008). Missing inaction: the dangers of ignoring missing data. *Trends Ecol. Evol.* 23, 592–596. <https://doi.org/10.1016/j.tree.2008.06.014>.
124. White, I.R., Royston, P., and Wood, A.M. (2011). Multiple imputation using chained equations: issues and guidance for practice. *Stat. Med.* 30, 377–399. <https://doi.org/10.1002/sim.4067>.
125. Williams, D.R., Rhemtulla, M., Wysocki, A.C., and Rast, P. (2019). On Nonregularized Estimation of Psychological Networks. *Multivar. Behav. Res.* 54, 719–750. <https://doi.org/10.1080/00273171.2019.1575716>.
126. Wysocki, A.C., and Rhemtulla, M. (2021). On Penalty Parameter Selection for Estimating Network Models. *Multivar. Behav. Res.* 56, 288–302. <https://doi.org/10.1080/00273171.2019.1672516>.
127. Epskamp, S., Cramer, A.O.J., Waldorp, L.J., Schmittmann, V.D., and Borsboom, D. (2012). 2012 qgraph: Network visualizations of relationships in psychometric data. *J. Stat. Softw.* 48, 1–18. <https://doi.org/10.18637/jss.v048.i04>.
128. van Borkulo, C.D., van Bork, R., Boschloo, L., Kossakowski, J.J., Tio, P., Schoevers, R.A., Borsboom, D., and Waldorp, L.J. (2023). Comparing network structures on three aspects: A permutation test. *Psychol. Methods* 28, 1273–1285. <https://doi.org/10.1037/met0000476>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
<i>Poecilia reticulata</i>	University of Ferrara	N/A
Software and algorithms		
CiclicTimer	N/A	N/A
ImageJ	ImageJ	https://imagej.net/ij/
RStudio	RStudio	https://cloud.r-project.org/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Subjects

The subjects belonged to a laboratory population originated in 2012 from guppies bought in a local shop. This guppy population is maintained in large groups (approximately 100 individuals) housed in 400-L aquaria enriched with natural gravel bottom and plants (*Echinodorus bleheri* and *Taxiphyllum barbieri*). To reduce inbreeding, at least once per year several guppies were moved across the different aquaria and new fish were brought from the shop. Fluorescence lamps (Sylvania GRO-LUX, 15 W, Milano, Italy; 12 h:12 h light: dark photoperiod), aerators, and mechanical, chemical and biological filters were also provided. Water was kept as follows: temperature $27 \pm 1^\circ\text{C}$, conductivity $606.7 \pm 60.18 \mu\text{S/cm}$, nitrite levels $<0.1 \text{ mg/L}$ and nitrate levels $<50 \text{ mg/L}$. The guppies were fed twice per day with food flakes (Vipan Nature, Sera, Germany) and brine shrimps (*Artemia salina*).

The subjects were randomly selected from the maintenance aquaria. All the subjects were experimentally naïve and after testing were returned to the maintenance aquaria in the facility. At the end of the experiments, each subject was moved to a cup for weighing (300 C, Precisa, Switzerland) and then moved into a narrow transparent tank with a reference background where it was photographed. Using ImageJ (<https://imagej.net/>), we measured the standard length of the subjects.¹⁰⁹ The length and weight in the experimental subjects were $3.33 \pm 0.26 \text{ cm}$ and $0.79 \pm 0.18 \text{ g}$ (mean \pm standard deviation), respectively. Females were significantly larger than males considering both traits (length: males $3.18 \pm 0.21 \text{ cm}$, females $3.50 \pm 0.21 \text{ cm}$, $t_{42} = -5.065$, $p < 0.001$; weight: males: $0.73 \pm 0.15 \text{ g}$, females: 0.85 ± 0.18 , $t_{42} = 3.160$, $p = 0.003$).

Ethics

The experimental protocols have been revised and approved by University of Ferrara's Ethical Committee (OPBA UNIFE, permit TLX-2020_1 - TLX-2022_1).

METHOD DETAILS

Experimental design

The study was conducted in 2023 in the laboratories of the University of Ferrara. We tested 48 naïve adult guppies, 24 females and 24 males. For the entire study (approximately 2 months), the subjects were housed in separate housing apparatuses to allow individual recognition and testing. The learning and flexibility tests were conducted in this housing apparatus. The physiology tests, behavioural tests, and one cognitive test (lateralisation test) were conducted by moving the subject into different apparatuses, and then moving it back to the housing apparatus. The order of the tests was fixed to favour detection of individual differences.¹¹⁰

Housing conditions for the experiments

To allow individual testing, we moved each guppy into a grey plastic housing apparatus ($30 \times 40 \times 22 \text{ cm}$; water level 18 cm). Each subject remained in this housing apparatus for approximately 60 days, although the exact period was affected by the length of the cognitive testing (described in the main text). The housing apparatuses were kept in a laboratory room separated from the facility to reduce external interferences. The room's light was kept off, and illumination for the subjects was provided by cold white a LED strip (TMR, ELCART, Italy; 1180 lux , 9.322 W/m^2) placed directly above the apparatus. The testing rooms and water was maintained at $27 \pm 1^\circ\text{C}$, and the water was partially changed once a week and replenished when necessary. To lessen stress in subjects, experimental tanks were provided with gravel on the bottom and an aerator, while a net was placed over them at night breaks to keep the fish from jumping out. Net and aerators were removed an hour before testing to allow the subjects to settle. Guppies received food randomly twice a day, unless they were participating in the visual and spatial discrimination tasks, where they were fed *Artemia salina* as reward.

Physiological traits

The ventilation measurements used to estimate metabolism were conducted upon moving the subjects to an unfamiliar environment,¹¹¹ a white plastic chamber (5 × 5 cm; water level 4 cm). The chamber was illuminated from above by cold white LEDs (TMR, distributed by ELCART, Italy). A HD camera (HC-VX980, Panasonic, Japan) recorded the subject from above for two minutes to measure ventilation rate under stress. Subsequently, another recording was conducted after one hour (minutes 60–62) to obtain our measure of basal ventilation rate.¹¹¹ We score the ventilation rate as number of operculum openings per minute (bpm) from the recordings played back on a computer using VLC media player (<https://www.videolan.org/>). A different blind experimenter confirmed the adequacy of measures by manually scoring the operculum openings from the video-recording. We considered as the basal metabolism the basal ventilation rate normalised by the subject's weight and the stress metabolism as the ventilation increase from the basal to the stress situation: [(stress ventilation rate – basal ventilation rate)/ basal ventilation rate].

Behavioral traits

The behavioural tests were conducted without acclimatisation to observe the response to the novel environment. Each trait was assessed twice to investigate repeatability. The interval between the two trials was relatively short (i.e., 10 days) to avoid marked changes in the size of the subjects due to growth. Moreover, the testing conditions of the two trials (e.g., temperature, illumination, water parameters) were kept as constant as possible. During the interval between trials, the fish were not subjected to other tests and were individually maintained following the housing schedule.

Exploration test

We first conducted a novel environment exploration test.^{35,64,97} We moved the subject to an unfamiliar, empty arena made of white plastic (40 × 40 × 15 cm, water level 5 cm; [Figure S1A](#)). The testing arenas were placed on a backlight table illuminated from below with infrared LEDs ($\lambda > 980$ nm; Noldus Information Technology, Wageningen, The Netherlands). An infrared-sensitive camera (Monochrome GigE camera, Basler, Germany; resolution: 1280 × 1024) was placed 1 m above the open field arena to record fish behaviour. The testing arena was illuminated from above by cold white LEDs (TMR, distributed by ELCART, Italy). A software (Ethovision, Noldus, The Netherlands) tracked the position of the subject for 20 minutes, allowing us to calculate distance moved and swimming velocity as a proxy of general activity,⁶⁶ the proportion of time spent in the edge of the arena (at least 5 cm from the edge) as a proxy of shyness,⁶⁵ and the proportion of arena covered.¹¹² These two latter indices had range 0–1 and were higher and lower for shier individuals, respectively.

Scototaxis test

After the completion of the exploration test, we moved the subject into a half white and half black apparatus (56 × 15 × 32 cm; water level 5 cm; [Figure S1B](#)) illuminated by cold white neon tubes placed above each sector (15 W, Sylvania GRO-LUX, Milano, Italy). This apparatus was used to obtain a measure of shyness based on scototaxis, the tendency of shy individuals to avoid white environments.^{113,114} From a 20-minutes recording performed with a camera on the ceiling (HDR-CX405, SONY, Japan), we obtained the time spent in the black sector and the time spent in the white sector. These time variables were scored using a custom software (CiclicTimer) with multiple stopwatches activated via a computer keyboard. The scototaxis variable was then computed as: time spent in the black sector (in seconds) / total testing time (e.g., 1200 s). This scototaxis index had range 0–1 and was higher for shier individuals.

Sociability test

After the scototaxis test, the subject was moved into a three-chamber apparatus (60 × 20 × 36 cm; water level 5 cm; [Figure S1C](#)) for the sociability test.^{64,115} The two lateral chambers (10 cm), illuminated by cold white neon tubes (15 W, Sylvania GRO-LUX, Milano, Italy), either contained a group of 4 guppies (2 males and 2 females), acting as a social stimulus, or was empty. Subjects' behaviour was video recorded (HC-VX980, Panasonic, Japan or HDR-CX405, SONY, Japan) from above for 20 minutes and analysed with the Ethovision software to obtain the time spent in close proximity (i.e., within 4 cm) to the social stimulus and the empty sector. All the subjects visited both sectors, and spent at least 94% of time in these sectors. Sociability was calculated as: time spent near the social stimulus/(time spent near the social stimulus + time spent near the empty sector).^{115,116} This sociability index had range 0–1 and was higher for more social individuals.

Cognitive traits

Lateralization test

The subject was moved into an apparatus consisting of an octagonal arena (each side: 15 cm, water level: 5 cm; [Figure S2A](#)) with internal walls covered by mirrors. LEDs provided illumination (cold white light, TMR, distributed by ELCART, Italy) and a video camera (HC-VX980, Panasonic, Japan or HDR-CX405, SONY, Japan) recorded the subjects for 20 minutes. The lateralisation trial was conducted twice, with a 10-day interval, as described for the behavioural tests. From the recordings played back on a computer, the experimenter operating the CiclicTimer software scored the position of the subject to obtain the time spent close to the mirror (i.e., 5 cm from the mirrors) swimming in clockwise direction and the time spent close to the mirror swimming in counter-clockwise direction. Fish processing social information with, for instance, the left hemisphere, were expected to look the social stimulus (i.e., their mirror image), preferentially with the right eyes, thereby swimming mostly in counter-clockwise direction. From the data, we calculated two commonly used indices of lateralization^{117–119}: the relative lateralization index as [(Time spent swimming clockwise – Time spent swimming counter-clockwise) / (Time spent swimming clockwise + Time spent swimming counter-clockwise)]; and the

absolute lateralisation index as the absolute value of the relative lateralisation index. The relative lateralisation index had range -1 to +1: subjects that preferred to swim clockwise had positive scores, and vice versa.

Visual learning test

The day before the beginning of this test, two trapezoidal-shaped transparent plastic structures were inserted into the housing apparatus, delimiting two main sectors linked by a central corridor (Figure S2B). The test lasted multiple days, according to subjects' performance. Each day, the experimenter administered to the subject 12 learning trials, split into two sessions (i.e., morning and afternoon) separated by a 3-hour interval. Consecutive trials within session were separated by a 10-minute interval. In each trial, the experimenter simultaneously presented two visual stimuli: a yellow disc and a red disc ($\varnothing = 1.0$ cm) mounted on a white card (4×4 cm) at the end of a transparent plastic stick (26×2 cm). The stick allowed to fix the stimuli in correspondence of the corners of a short wall of the apparatus.

One rewarded colour (i.e., either the yellow or the red) was assigned to each subject with a pseudo-random scheme, with care to counterbalance the number of subjects assigned to each colour between the two sexes. The assigned rewarded colour did not significantly affect subjects' learning performance (Wilcoxon rank sum test: learning: $W = 309.000$, $p = 0.329$). Before the start of the learning trials, subjects were allowed to familiarise with the rewarded colour for two days. In the first day of familiarisation, the experimenter presented only the rewarded colour stimulus for 12 times and rewarded the subject when it approached the stimulus (i.e., within less than 1 body length) with 3-4 *A. salina* nauplii delivered with a Pasteur pipette. On the second day of familiarisation, the non-rewarded colour was presented in at the same time as the rewarded one. Subjects were allowed to investigate both stimuli but were rewarded only when they approached the correct one. After a correct choice, the rewarded stimulus was left in the apparatus for 5 minutes, while the unrewarded stimulus was removed. After this familiarisation phase, the experimenter began presenting both stimuli, but rewarding the subject only if it first approached the correct stimulus within 15 minutes. After a correct choice, the rewarded stimulus was left in the apparatus for 5 minutes, while the unrewarded stimulus was immediately removed. In case of an incorrect choice, the experimenter immediately removed both stimuli. The learning task continued until the subject reached a learning criterion of $> 80\%$ correct responses in two consecutive days (20 correct choices out of 24 trials). This criterion corresponded to a statistically significant preference for the correct stimulus (binomial test: $p = 0.002$) and was thus less likely to be achieved by subjects making random choices.

Cognitive flexibility in visual discrimination

To conduct the reversal learning task and assess cognitive flexibility, the experimenter switched the reward between the two colours and trained the subject until it reached the same criterion described above. This procedure started the day after a subject reached the learning criterion. As a variable for the analyses, we used an index controlled for learning ability calculated as: $-(\text{number of days to criterion in the visual task} - \text{number of days to criterion in the reversal task}) / \text{number of days to criterion in the visual task}$. Higher values of the index indicated greater cognitive flexibility. One subject was excluded in this task because it died during testing (final sample $N = 45$).

Spatial learning test

The spatial learning test started two days after the end of the colour discrimination test. The day before the start of the spatial learning test, the transparent structures used for the visual tests were removed and a white plastic T maze (Figure S2C) was inserted in the housing apparatus. The maze consisted of a 9×6.5 cm start box, a 12×3 cm corridor, and a choice sector leading to two identical arms (9.5×4 cm). One of the two arms led to a passage closed by means of a grid net (unrewarded arm). The other arm had a grid net that could be opened as a curtain if the subject pushed it, therefore allowing the subject to return to the housing apparatus (rewarded arm). The side of the correct arm was randomised and counterbalanced across subjects and sexes and did not affect performance (Wilcoxon rank sum test: $W = 293.000$, $p = 0.337$). Both arms were blocked outside the trials to prevent the subject from accidentally entering the maze. The water level inside the maze was kept at 1.5 cm to motivate the subject to return the housing apparatus.

The testing procedure involved a series of trials administered across consecutive days. The length of this period varied across subjects based on the time needed to reach the learning criterion (described below). In each day, the experimenter administered 12 trials separated in two sessions (morning and afternoon; 3 hours interval). Trials within sessions were separated by a 10-minute interval. In each trial, the experimenter collected the subject with a net and moved it into the start box of the T maze. The subject was let undisturbed until it exited the maze, while the experimenter recorded the first arm entered (i.e., rewarded or unrewarded arm). The learning criteria was as in the previous test: 20 correct choices out of 24 trials in two consecutive days.

Cognitive flexibility in spatial discrimination

After a subject reached the learning criterion in the spatial task, we administered a spatial reversal learning task to assess cognitive flexibility.⁵⁷ The procedure consisted in switching the correct and the incorrect arm of the maze and training the subject to the same criterion described for the learning phase.

QUANTIFICATION AND STATISTICAL ANALYSIS

For data analysis, we used RStudio (version 2023.12.1 + 402). The sample size was decided *a priori* based on a previous study on cognitive sex differences in guppy.⁵⁸ Using G Power 3.1.9.7,¹²⁰ the required sample size for detecting a large effect size (estimated Cohen's $d = 0.98$) with a power of 80% and α error probability of 0.05 was 23 subjects per group. Statistical tests were two-tailed and the threshold for the statistical significance was set at $p = 0.05$. 41 subjects completed all the tests, while for the other 7 subjects we

have missing data for the visual learning ($n = 2$), visual cognitive flexibility ($n = 3$), spatial tasks ($n = 3$) and physiology measures ($n = 4$). Descriptive statistics are reported in the main text as mean \pm standard deviation. We initially applied adjusted generalised linear mixed-effects models¹²¹ ("*rpt*" R function, $nboot = 1000$, $nperm = 1000$, $datatype = \text{Gaussian}$; *gname* argument = individual identification name) and two-sample t-tests ("*t.test*" R function) to assess repeatability by restricted maximum likelihood and average differences in performance across trials and sexes, respectively. Asterisks reported in the figures indicated statistical significance (" ** ", $p < 0.05$). Temporal variation of each behavioural trait was analysed via linear mixed-effects models ("*lmer*" R function from "*lmerTest*" package, significance of the models' parameters obtained with the Satterthwaite's degrees of freedom method) fitted with the continuous variable "time blocks" (i.e., 20 temporal blocks of 1 minute) as covariate, "trial" as fixed factor, and subject ID as random factor. In the permutation analysis, repeatability of traits was assessed by setting sex and trials as fixed factors. One-sample t-tests ("*t.test*" T function) were used to compare the population score against the one attended by chance for the thigmotaxis (expected value: 0.5), for the scototaxis (expected value = 0.5), and for the relative lateralization index (expected value = 0). We performed the PCA on the behavioural traits with the "*prcomp*" function ("*stats*" R package).

To ensure that the scores observed in the visual and the spatial learning tasks were not due to chance, we conducted a simulation analysis following De Russi et al.⁵⁷ We calculated the number of days necessary to reach the learning criterion in a simulated set of subjects that chose randomly between the stimuli. This was done simulating 12 trials using a random binomial distribution (50% probability of success) per day of the simulated experiment. The number of days in the simulation was set to the maximum number of days necessary to reach the criterion in the real subjects. The number of subjects was equal to the number of tested guppies. The simulation process was repeated for multiple interactions ($n = 10000$) to compute the percentage of simulated subjects that reached the learning criterion. Then, we calculated the probability of obtaining the observed number of successful guppies from the simulated population.

To investigate sex differences in the associations between traits, we estimated the structure of the covariation using a network analysis. In the network analysis, each trait represented a node and the associations with other nodes (i.e., edge) were controlled for the influence of all the other nodes in the network. This approach allowed us to mitigate the problem of spurious correlation of the common correlation approaches, i.e., when two variables are not directly associated but are connected through a third one,^{89,90} and does not require assumption on latent variables like for instance the factor analysis.

The input for the network analyses was the correlation matrix between pairs of traits (12 traits) calculated using Spearman rank tests ("*rcorr*" R function, library "*Hmisc*"). For traits measured twice, we used the trials' means as the variable. In case of non-repeatable traits, the average score was expected to provide an estimation of individual differences that was more accurate compared to the score of a single trial. Based on the results of the PCA, we fit the originally measured behavioural traits, albeit we excluded swimming distance and area covered due to strong covariance with activity and thigmotaxis. We obtained a correlation matrix including all the traits per each sex. Missing data represented 2.43 % (7 out of 288 observations from 2 subjects) and 3.82 % (11 out of 288 observations from 5 subjects) for the male and female datasets, respectively. To avoid discarding subjects, we imputed the missing data using the "*mice*" package.¹²² Data imputation via multiple imputations reduces sampling discarding by creating several datasets based on the initial distribution of available data without specifying predictor variables.^{123,124} We performed repeated multiple imputations (argument $m = 5$) for each 50 iteration (argument $maxit = 50$). Then, we used the univariate imputation method of predictive mean matching (argument $meth = \text{"pmm"}$, $seed = 500$) to impute missing data.

We then estimated correlation network structures of measured traits independently for each sex via the "*estimateNetwork*" function from the bootnet package.⁹⁰ Preliminary results, indicating a high number of correlated traits, discouraged the use of regularised techniques for estimating network structure, which can poorly fit dense networks.^{125,126} Since we hypothesised that all observed traits were biological correlated in such a manner that defined the individual, we computed a non-regularised estimation approach to estimate the network via "*cor*" function from psych package (argument $default = \text{cor}$; argument $corMethod = \text{"cor"}$; argument $corArgs = \text{"spearman"}$). The stability of the significant relationships in the weighted network was assessed using a bootstrap method⁹⁰ ("*bootnet*" package; $samples = 1000$). Resulting 95% Confidence Interval of simulated nodes' relationships that did not contain zero, indicated statistical significance (Figure S3). Estimated networks were graphically represented using the plot function from *qgraph* package.¹²⁷ In the network graphs, nodes' colour represented the assigned domain, i.e., physiology (blue), behaviour (light grey), and cognition (green). The width of the edges represented the strength of the association between nodes. The colour of the edges indicated positive (blue) and negative (red) relationships. We compared the number of connections per trait between the two networks via two-sample t-tests.

From the estimated weighted networks, we extracted all weights, i.e., numeric value identifying the strength of association between two nodes, to compare the average strength of traits' relationship between sexes ("*cor.test*" function, method Pearson). We also analysed the effect of sex and domain on the traits' relationship by using a linear mixed-effects model ("*lmer*" function from the *lmerTest* package) fitted with sex and domain as fixed effect, and trait pair as random effect. For the latter analysis, we removed the association between the two physiological traits from the datasets. Then, we compared the two estimated networks using the package *NetworkComparisonTest*¹²⁸ ("*NCT*" function, independent data, number of iterations = 10000). Structural differences were analysed using the network structure invariance test and global strength invariance test; if significant, the first metric would indicate a difference in the covariance structure across groups, while the second metric would indicate a difference in the strength of covariations. Moreover, structural differences were further compared between sexes considering centrality indices that described the importance of each individual node and edge in the network.¹²⁷ The centrality indices considered were: the

proximity of each node to the other nodes in the graph (closeness) calculated as the sum of the length of shorter paths (edges) between them; the absolute importance of a certain node (strength) calculated as the sum of the weights (absolute value) of a target node; and the relative importance of a certain node (expected influence) calculated as the sum of the relative weights of a target node. Since we estimated the network structure based on traits' covariances, nodes with a high number of correlations showed high values of closeness, while strength and expected influence defined the importance of a node with respect to the strength of its correlations with all other nodes. We graphically represented the differences in centrality indices between sexes using the *centralityPlot* function from *qgraph* package.¹²⁷