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Matthew J. Armstrong & Molly
E. Cummings**

Animal Cognition

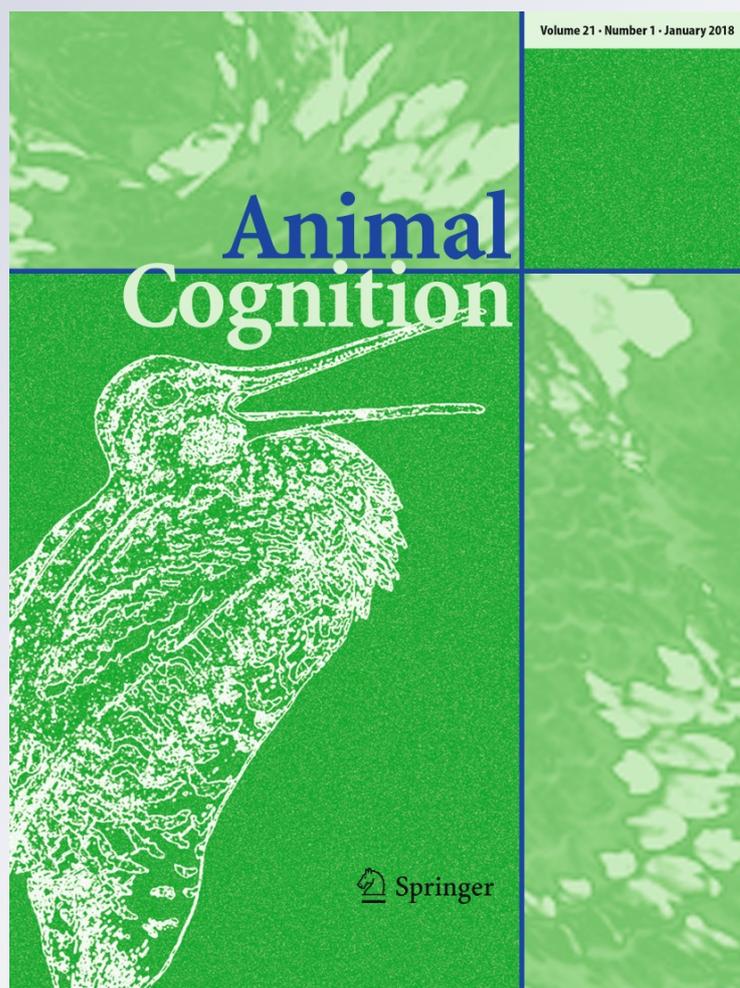
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Sex-specific cognitive–behavioural profiles emerging from individual variation in numerosity discrimination in *Gambusia affinis*

R. Ian Etheredge^{1,2} · Capucine Avenas³ · Matthew J. Armstrong¹ · Molly E. Cummings¹

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Abstract The relationship between an individual's cognitive abilities and other behavioural attributes is complex, yet critical to understanding how individual differences in cognition arise. Here we use western mosquitofish, *Gambusia affinis*, to investigate the relationship between individual associative learning performance in numerical discrimination tests and independent measures of activity, exploration, anxiety and sociability. We found extensive and highly repeatable inter-individual variation in learning performance ($r = 0.89$; ICC = 0.89). Males and females exhibited similar learning performance, yet differed in sociability, activity and their relationship between learning and anxiety/exploration tendencies. Sex-specific multivariate behaviour scores successfully predicted variation in individual learning performance, whereas combined sex analyses did not. Female multivariate behaviour scores significantly predict learning performance across females ($\rho = 0.80$, $p = 0.005$) with high-performing female learners differentiated from female non-learners and low-performing learners by significant

contributions of activity and sociability measures. Meanwhile, males of different learning performance levels (high-, low- and non-learners) were distinguished from each other by unique behavioural loadings of sociability, activity and anxiety/exploration scores, respectively. Our data suggest that despite convergence on learning performance, the sexes diverge in cognitive–behavioural relationships that are likely products of different sexual selection pressures.

Keywords Sexual dimorphic behaviour · Learning · Sociability · Anxiety

Introduction

From humans to bumblebees, there is an extensive intraspecific variation in cognitive abilities (Thornton and Lukas 2012; Dukas 2008; Lucon-Xiccato and Bisazza 2017a); yet the source of this variation and how it is maintained remains elusive. While genes (Goldberg and Weinberger 2004; Morley and Montgomery 2001) and experience (Spence et al. 2011) are likely contributors, additional influences may be even more complex. Recent theories (Carere and Locurto 2011; Sih and Giudice 2012; Griffin et al. 2015) propose a relationship between variation in cognitive performance and behavioural tendencies—suites of behaviours that covary within individuals across contexts (Sih et al. 2004; Dickman 1990; Réale et al. 2007). If cognitive performance is linked to behavioural phenotypes, then selection on behaviour may indirectly drive the evolution of cognitive processes. This reveals the intriguing possibility that variation in cognition may be a product of selection on other behaviours and compels us to quantify the relationship between cognition and behavioural tendencies (cognitive–behavioural profiles) to better understand sources of cognitive variation.

R. Ian Etheredge and Capucine Avenas have contributed equally, deserving co-first author status.

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✉ Molly E. Cummings
mcummings@austin.utexas.edu

¹ Department of Integrative Biology, University of Texas, Austin, TX 78712, USA

² Max Planck Institute for Ornithology and Collective Behaviour, University of Konstanz, Konstanz, Germany

³ Neuroscience and Signaling Department, Paris-Sud University, Orsay, France

To investigate the complex relationship between individual variation in learning capabilities and variation in behavioural tendencies, we can ask whether different behavioural types are associated with different learning performances. Conversely, we can also ask how different behavioural types produce similar learning performances. This latter question, though less intuitive, may be a common phenomenon in nature where selection pressures for cognitive capabilities are shared across individuals; yet selection imposes divergent behavioural strategies between groups of individuals. Sexual selection is an obvious force that diversifies behavioural traits in predictable directions between the sexes. With near ubiquity, sexual selection has led to sexual dimorphism in both reproductive and non-reproductive behaviours (sociability, activity, anti-predator behaviour, boldness, and spatial awareness; Magurran and Garcia 2000; Jones et al. 2003). Yet, despite sexual dimorphism in behaviours and behavioural syndromes (Dingemanse et al. 2004; Piyapong et al. 2010; Schuett and Dall 2009; Pruitt and Riechert 2009), in some cases there appears to be strong convergence for learning capacity between the sexes. While there are numerous examples of sex differences in cognitive performance linked to sex-specific tasks [e.g. spatial memory (Gaulin and Fitzgerald 1986; Lucon-Xiccato and Bisazza 2017b) or motor task (Range et al. 2006) enhanced in males in some taxa; and females in others (Guigueno et al. 2014; Liu and Burmeister 2017)], as the cognitive task becomes further removed from reproductive function, the performance difference between the sexes declines. Conspecific males and females across vertebrates [rats (Rodriguez et al. 2010), fish (Lucon-Xiccato and Bisazza 2014, 2016; Lucon-Xiccato and Dadda 2016; Mamuneas et al. 2015), birds (Titulaer et al. 2012)] show equivalent performances in associative learning trials despite differences in their approach to the cognitive task (“cognitive style”, e.g. fast vs. slow). Hence, sexual selection provides a fruitful avenue to probe for divergent behavioural types leading to variation in cognitive-behavioural profiles (or CBPs, Crespi and Hurd 2014).

If sexual selection has produced divergent behavioural phenotypes between the sexes with similar learning capacity, do different cognitive-behavioural profiles emerge? To answer this question, we conduct cognitive-behavioural profiling in the western mosquitofish, *Gambusia affinis*, a member of the Poeciliidae fish family which are characterized by internal fertilization and multiple sexually dimorphic behaviours (Magurran and Garcia 2000). *G. affinis* has a mating system involving both male coercion (a chase and gonopodial thrust mating strategy) and gonopodial displays (Bisazza 1993; Langerhans et al. 2005), while females engage in avoiding male harassment and maximizing fecundity through foraging (Pilastro et al. 2003). Across many poeciliid species, there are well-documented sex-specific differences in behaviour and cognitive style. For instance,

female *Poecilia reticulata* exhibit greater sociability (Griffiths and Magurran 1998), feeding innovation (Laland and Reader 1999) and predatory inspection behaviours (Magurran and Nowak 1991), whereas males are bolder (Magurran and Seghers 1994; Harris et al. 2010), make faster decisions (Lucon-Xiccato and Bisazza 2016) and are less flexible (Lucon-Xiccato and Bisazza 2014, 2017b). Furthermore, *Gambusia* species have well-documented correlated relationships across behavioural traits (e.g. behavioural syndromes, Cote et al. 2010) that respond to differential selection regimes (e.g. predation; Heinen-Kay et al. 2016) as well as exhibit sex-specific responses for brain size when subject to artificial selection for sexual conflict (e.g. gonopodia length, Buechel et al. 2016).

By examining cognitive-behavioural profiles in species with known sex-specific behavioural differences and behavioural syndromes, we can begin to identify unique relationships between learning and behaviour. Here we focus on learning performance (the accuracy of decisions in learning trials), rather than cognitive style per se, to determine whether there are combinations of behavioural traits that favour learning and whether they are the same or different between sexes. As our goal is to identify sexually unique relationships between learning and behavioural tendencies, and not to identify sex differences in cognitive performance, we employ a learning assay with minimal inherent sexual bias. Previous research with *P. reticulata* has shown that male and female guppies exhibit equivalent learning performances across three different visual discrimination learning tasks (shape, orientation and colour discrimination tasks; Lucon-Xiccato and Bisazza 2016). Here we employ a visual discrimination task of numerosity discrimination of geometric shapes (a common discriminant learning procedure in teleosts, Agrillo et al. 2017) as our learning assay. For our cognitive-behavioural profiling, we couple this visual discrimination learning assay (numerosity discrimination) with a battery of independent behavioural assays conducted outside the learning environment that are likely to capture sex-specific differences (sociability, activity, mate choice) as well as behaviours with common inter-relatedness (anxiety, exploration).

To identify how the variation in learning performance covaries with variation in behavioural tendencies, we needed to adopt a learning task and training regime that would capture variation in learning performance. Our selection of numerosity discrimination as our learning task favours this aim as it has been demonstrated to elicit high levels of individual variation in learning performance in poeciliid fishes (Bisazza et al. 2014; Lucon-Xiccato and Bisazza 2017a, b). To achieve our aim of capturing variation, we further employ a relatively short training regime (4 days, 16 training trials) before evaluating individual learning performance (using performance criteria during non-reinforced

test trials). Typical associative learning trails with fish can range between 10 and 30 training trials (Agrillo et al. 2012a), and with extended training, poeciliid fish have been able to achieve similar ranges of numerosity discrimination performances as mammals and birds (Bisazza et al. 2014). While our approach deviates from more traditional methodology that extends learning trials until individuals meet learning criteria, it enables us to identify a range of performance under standardized conditions. With this range of learning performance, we imposed a learning criterion cut-off to differentiate individuals that did not yet learn at the end of the training period (non-learners), and another criterion to differentiate individuals that marginally met the learning criterion (low-performance learners) from those that significantly exceeded it (high-performance learners). It is this range of learning performance that we further probe to identify behavioural predictors of this variation. We take a multivariate approach to identifying the relationships that underpin cognitive–behavioural profiles as males and females often vary in more than one behaviour and in the interaction between behaviours. We predict to find (1) no sex differences in learning performance in numerosity discrimination, (2) sex differences in other behaviours, (3) a quantitative relationship between behavioural tendencies and learning capabilities (CBPs) and (4) different cognitive–behavioural profiles between the sexes in *G. affinis*.

Methods

We subjected male and female *G. affinis* to an array of different behavioural assays including: (1) associative learning (numerosity discrimination), (2) anxiety/exploration (scototaxis), (3) sociability and (4) dichotomous mate choice (females only). All fish were collected at the same time from a single wild population in the San Marcos River, Texas, and housed in mixed-sex community tanks for circa 8 months prior to testing (e.g. two 20 gallon tanks with even sex ratios), thus minimizing differences in pre-treatment experiences. Fish were socially isolated for a minimum of 8 days prior to the onset of testing to standardize their social encounters prior to testing. Experimental animals ($N = 24$, 12 females, 12 males of which two males subsequently died) were housed and experiments conducted at the University of Texas at Austin under Institutional Animal Care and Use Committee Protocol No. AUP-2013-00156. Male subjects ranged in size from 27 to 35 mm in standard length (SL) with an average of 32.8 mm SL, while female subjects (with indeterminate growth) ranged from 25 to 53 mm SL with an average of 34.9 mm SL. Because we are interested in individual effects, we used a fixed order experimental design following Bell (2012) which standardizes any carry-over effects between assays across individuals. All experimental

fish began with numerosity (a 9-day procedure) and were subsequently tested for scototaxis (day 10), sociability (day 11), and mate choice (females only, day 12), with all fish experiencing the same order of behavioural assays. Mate choice followed sociability trials to avoid confounding a desire to be social (after several days of testing in isolation) with mate preference.

Associative learning assay (numerosity discrimination)

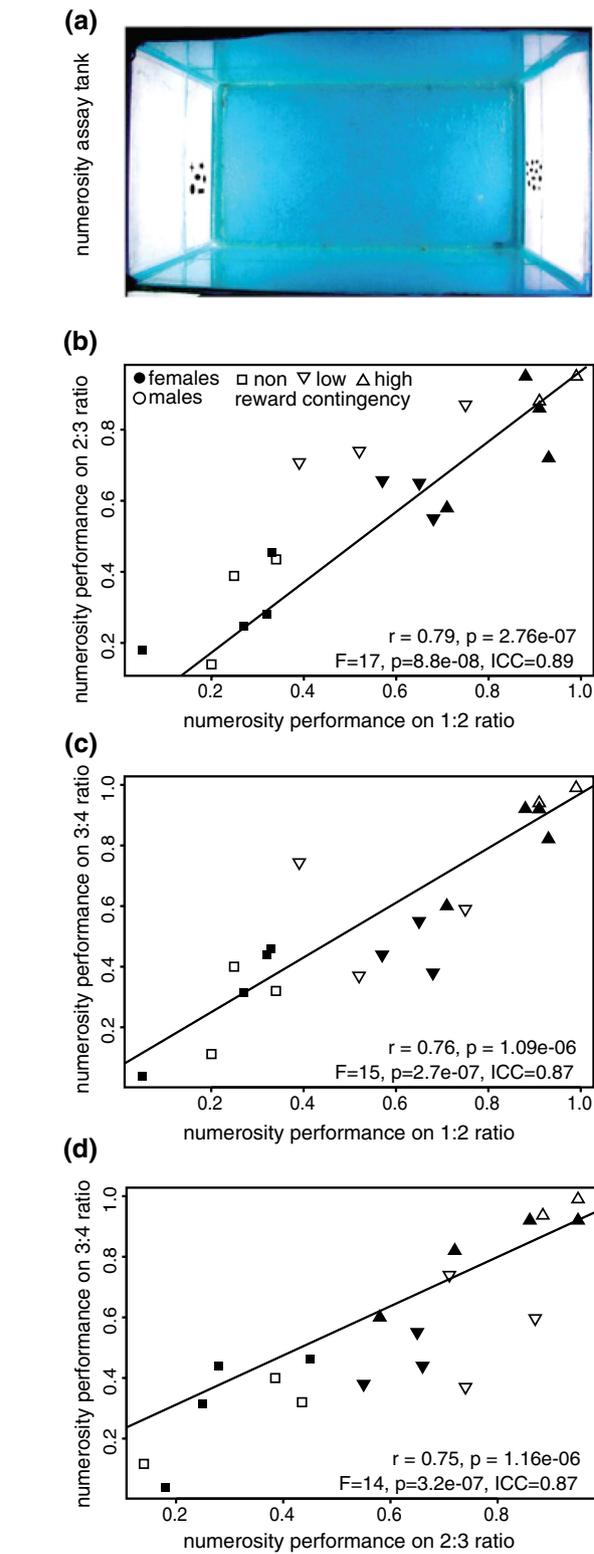
We used a numerical discrimination assay (numerosity discrimination) modified from Agrillo et al. (2008, 2012b) that included LCD screens (1024×768 px, 48 cm, XGA-LCD) positioned flush against the exterior aquaria end walls for stimuli presentation (Fig. 1a) and bloodworms as a food reward. Two experimental tanks ($25 \text{ cm} \times 50 \text{ cm} \times 25 \text{ cm}$) were lined with light blue felt (to provide visual contrast for tracking) and filled with 15 cm of $27 \text{ }^\circ\text{C}$ water for each trial that was changed daily. Video cameras were placed above each tank to record subject positions during trials. All fish were trained on a 1:2 ratio regime of numerical contrasts of 5 versus 10 and 6 versus 12 geometric shapes (see Fig. 1a). To control for continuous quantities that also scale with variation in numerical quantities (e.g. cumulative surface area and the convex hull or the overall space that the objects occupy; Stevens et al. 2007; Agrillo et al. 2017), we varied the sizes of the geometric shapes between the numerical contrasts. For example, the rectangle shape was 15.3 mm surface area on the 5 side of the 5 versus 10 numerical contrast compared to 12.25 mm on the 10 shapes side. Shapes were positioned and selected for each stimulus pair to (a) create the same convex hull ($6 \times 6 \text{ cm}$) and (b) minimize the difference in cumulative surface area (all pairs $<10\%$ difference). Each stimulus was $6 \times 6 \text{ cm}$ centred on the LCD screens, in the middle of the water column (between 4.5 and 10.5 cm depth). To eliminate, internal reflections and the highly angular-dependent refractions that can occur when LCD images travel across media with diverse refractive indices (e.g. travelling from solid through air then through glass and into water), each of the experimental tanks and LCD screen clusters were placed within a larger $45 \times 120 \times 50 \text{ cm}$ mineral oil tank. This eliminates the large difference in refractive indices in the screen-air-glass-water interface and allows for reliable presentation of stimuli that is unaffected by the orientation of the subject.

All fish experienced habituation (days 1–2), training (3–6), numerosity testing (days 7–9) and behavioural assays (days 10–12). During habituation days, fish were familiarized with both experimental tanks, the acclimation tube, LCD screens and our feeding procedure. On the first day of habituation, groups of 4 same-sex experimental subjects were introduced into one of the two experimental tanks where they remained for 3.5 h and then moved to the other

Fig. 1 Consistent individual performance in associative learning across different numerical discrimination contexts. All data points represent median performance across 3 testing trials for each numerosity discrimination context, including: 1:2 (7 vs. 14 shapes), 2:3 (8 vs. 12 shapes), 3:4 (9 vs. 12 shapes). **a** Experimental numerosity tank (from above). We found highly significant correlations of individual learning performance across different testing trials, **b** 1:2–2:3 ratios, **c** 1:2–3:4 ratios, and **d** 2:3–3:4 ratios. Data points are labelled providing information as to the sex [open symbols denoting males and closed symbols denoting females]; and training contingency [squares denote individuals trained to respond to low side of the 1:2 training regime, and circles were trained to high]

experimental tank for an additional 3.5 h. During habituation, LCD screens were on and showed a blank screen (white background without any geometric shapes), and food (bloodworms) were delivered sub-surface with a pipette at both ends of the tanks near the LCD screens and replenished 4 times throughout the day. After each of the acclimation sessions, fish were returned to their home aquaria. As our testing procedure involved four rounds of testing per individual per day, we introduced this regime to each fish during day 2 of habituation. On the second day of habituation, individual fish were introduced to the experimental tank inside an opaque acclimation tube for 5 min followed by a 10-min free-swimming trial with food reward placed near the blank LCD screens at either end. Individual fish experienced 4 acclimation periods (two in each of the two experimental tanks) throughout the day with a 2-h interval between each acclimation session, and were returned to their home aquaria between each acclimation session. Video recordings from above the tank allowed us to evaluate thigmotaxis (tendency to stay near walls, avoidance of open areas) during the first training trial to add an additional context for evaluating anxiety/exploration behavioural tendencies. Our thigmotaxis score measured the amount of time an individual spent within one body length (~ 2 cm) of any side of the experimental tank (excluding the area directly in front of the LCD screens).

Following the acclimation period, each fish was randomly assigned to either the low (rewarded at the lower numerical value, e.g. food presented at the side of tank with the 5 geometric shapes in a 5 vs. 10 training trial) or high (rewarded at the higher numerical value) group with an equal sex ratio in each group. Fish proceeded to partake in numerosity training involving 4 training trials per day (with minimum 2-h interval between trials) across four consecutive days (days 3–6). Training consisted of an initial 5-min acclimation period within an opaque tube followed by a 4-min free-swimming trial. In all trials, the bloodworm food reward was delivered via a pipette just below the water surface in front of the LCD with the assigned reward contingency before individual fish were released from the acclimation tube; and a control pipette (water only) was presented in front of the other LCD screen simultaneously to control for surface disturbances



on both sides of the experimental chamber. Both pipettes were removed before the fish was released from the acclimation tube. Following each trial, all uneaten food was removed and discarded. Fish were otherwise food limited.

The experimental tank each subject trained in, the numerical stimulus pairs (5 vs. 10; 6 vs. 12) used for each training trial, and the side of tank receiving reinforcement (stimulus + food reward) were randomized across training trials.

For three consecutive days following training (days 7–9), individuals experienced testing trials (no food reinforcement) three times a day with a 2-h interval between tests and ended each day with a reinforcement trial (ad libitum food reward during 5 vs. 10 or 6 vs. 12). Individuals were tested (5-min acclimation followed by 4-min free-swim trial) on three novel numerical discrimination stimulus pairs at 1:2 ratio (7 vs. 14), 2:3 ratio (8 vs. 12), and 3:4 ratio (9 vs. 12) once a day each for three successive days. A reinforcement trial with excessive food reward was performed at the end of each testing day to minimize extinction of the learning rule throughout the testing period.

Test presentation order within a testing day was randomized across individuals. The position of test subjects within the tank was recorded with a video recorder and subsequently scored to evaluate the time spent within 16 cm (approximately 1/3 of the inner tank arena) of the LCD exhibiting the reward contingency (either the high or low numerical contrast) that the individual fish was trained upon. Video recordings of test trials were blind-labelled and hand-scored by reviewers (C.A. and M.A., and two additional undergraduate students naïve to the experimental objectives).

Learning performance categorization

We evaluated individual learning performance by examining the time spent near the target numerosity in each individual's nine test trials (non-reinforced presentations of numerosity discrimination contrasts including three replicates of three different numerical contrasts: 7 vs. 14, 8 vs. 12 and 9 vs. 12). The target numerosity was the side of the numerical contrast (high or low side of the ratio) that the individual was previously reinforced during training trials. We employed a standard learning criterion (> 60% time spent near the target numerosity stimulus, Agrillo et al. 2012a, b) to differentiate the non-learning individuals from those that learned to associate the stimulus with food reward. We used the median of an individual's three replicates of a given test ratio as a measure of their learning performance for each specific contrast. Any individual fish that did not achieve at least one median time score of 60% near the numerosity stimuli (within 16 cm of screen) across any of the three test ratios were categorized as “non-learners”. Individuals that met this learning criterion were further differentiated into “high-performance learners” and “low-performance learners” based on their responses across all testing trials. We tested whether an individual's time spent near the target numerosity was significantly different than 50% across all nine testing

trials. Individuals with proportion time spent near the target numerosity that was significantly greater than 50% across all nine test trials were categorized as “high-performance learners”. Individuals that met the learning criterion (achieving a median performance > 60% in at least one of the three test ratios), but failed to show significantly greater than 50% time spent near the trained numerosity across all nine test trials, were categorized as “low-performance learners”.

Anxiety and Exploration:

Differentiating between anxiety, fearfulness, boldness, exploratory tendencies, neophobia and stress has been a procedural challenge for researchers working on mammals to fishes (Walsh and Cummins 1976; Maximino et al. 2010a; Carter et al. 2013; Greggor et al. 2015; Perals et al. 2017). Since any single experimental procedure to evaluate anxiety often elicits different combinations of the aforementioned emotional and physiological responses, it is considered advisable to measure as many possible behavioural responses during any given experimental procedure as well as measure behaviour across contexts to increase validity (Carter et al. 2013; Perals et al. 2017). A particularly agnostic approach that is recommended when testing animals in procedures without pharmacological validity, is to use multi-dimensional statistics to reduce the multifarious behavioural measures into composite scores that encompass the diverse responses (Perals et al. 2017). To this end, we scored multiple behaviours within an anxiogenic procedure (a scototaxis trial) and combined those behaviours with risk-assessment behaviour in another context (thigmotaxis in numerosity training) into a principle components analysis to begin to characterize some of these complicated and inter-dependent traits.

Scototaxis (“shadow favouring”) is an anxiogenic behavioural assay that is analogous to the light–dark box in rodents and an alternative to novel tank diving and open-field tests in teleost (Maximino et al. 2010a). In this procedure, animals are placed into an environmental tank that is 50% white and 50% dark and researchers record the behavioural responses in the threatening (white zone) and non-threatening (dark zone) areas. Scototaxis has been pharmacologically validated as an anxiogenic assay in a number of small prey fish (Maximino et al. 2010b) including poeciliids (Ramsey et al. 2014). The scototaxis tank was 24 × 50 × 30 cm and split into two halves along the long axis and each side covered with either white or black felt. The tank was filled with 15 cm of water and held at 27 °C throughout the experiment. We performed a complete water change between each round of trials. Light blue filters (Lee 063) were wrapped around each 18-in. fluorescent full spectrum (15 W) light bulbs and placed at the top of each tank wall (and offset from the edge by 15 cm) to ensure even lighting within the tank. The total

intensity differed between the two sides by nearly 50% (average intensity measurements with sensor facing upwards at the base of black side: 57.3 lx; white side: 105 lx). At the start of each scototaxis trial, fish were placed in an opaque acclimation tube in the centre of the tank spanning a portion of both the black and white side for 5 min, and then released for a 10 min trial which was recorded on a video camera placed above the scototaxis tank. Video recordings of subject fish were collected and analysed for spatial positioning (XY coordinates) using the tracking platform, idTracker 2.1 (Pérez-Escudero et al. 2014), with slight modification to enable batch processing of videos in MATLAB R2014b. From the tracked data set, we calculated the following behaviours: (1) proportion of time spent in white side of tank, (2) latency to enter white side, (3) number of entries into white side, (4) thigmotaxis (time spent ~ 2 cm from wall) in white side and (5) thigmotaxis in dark side. Each fish was tested only once in a scototaxis trial. The five scototaxis behavioural measures, along with their respective thigmotaxis score during their numerosity training trial (see above), served as input into a principle component analysis (PCA) to reduce the set of six related behaviours down to two (the primary and secondary PCA axes scores).

Sociality, activity and mate choice

We used the same experimental tank (30 × 122 × 50 cm filled with 15 cm of water and held at a 27 °C) to test sociability (shoaling preference to be near conspecifics), mate choice (in females only) and activity. For both sociability and mate choice trials, the two 24-cm ends of the tank were partitioned from the centre region with plexiglass dividers. As many poeciliid species are found in dynamic, mixed-sex shoal groups in the wild (Auge et al. 2016), for our sociability trials we employed a mixed-sex social group (4 female and 2 male conspecifics) placed at one end of the tank behind a plexiglass divider while keeping the other end (partitioned off with a plexiglass divider) empty. Individuals were first acclimated for 5 min in an opaque tube followed by a 10-min trial. The focal fish was then returned to the acclimation tube for 1 min, while the social group was moved to the opposite side before a second 10-min trial was performed. Video recordings of subject fish during sociability trials were collected and analysed using the same customized version of the idTracker program mentioned above to calculate the amount of time each individual fish spent within 24 cm of the plexiglass divider near the social group (approximately 1/3 of the inner tank arena). To measure activity in a non-threatening environment, we collected activity scores (calculated as the total distance travelled within a trial) during sociability trials (using the automated custom tracking program). While our sociability trials are not likely to be completely “non-threatening” as the solitary

test fish is always physically partitioned from the stimulus shoal group, it was our least threatening assay in our series of tests and for that reason was selected as the best candidate to evaluate natural activity levels.

For female mate choice trials, presentation stimuli included one large (2.95 cm standard length) and one small (1.62 cm standard length) conspecific male placed at either end of the tank behind plexiglass dividers as in Wang et al. (2014) and Lynch et al. (2012). Female *G. affinis* have demonstrated a preference to associate with larger males (Lynch et al. 2012) which has been hypothesized to serve a protection function as smaller males are more effective at sneak copulating than larger males (Bisazza and Marin 1995). Females were placed in an opaque tube in the centre of the tank for 5-min acclimation followed by 10-min observation period where female preference (relative time spent in association zone or 24 cm in area adjacent to the stimulus dividers) for each male was recorded using customized idTracker software (see above). The side of the tank that the larger and smaller male were presented on was randomized between females.

Behavioural scores and analyses

All trials were recorded using a web camera placed above each experimental tank via the cross platform video utility FFMPEG (v2.8.5), and scored with in house computer tracking algorithms (scototaxis, sociability, activity and mate choice; see above) or hand-scored (day 1 numerosity training trials and all numerosity testing trials). Numerosity trials were not analysed with our customized tracking program due to the low contrast of the video recordings.

We used Spearman, Pearson and intra-class correlations (ICC) where appropriate and Mann–Whitney *U* tests to compare between groups (sexes, reward contingency training group, and learner groups). To investigate the interaction of learning performance and behavioural scores in sex-dependent ways, we used the “cor” package for univariate correlations (in R v3.2.2) and compared the slopes and standard errors of linear models obtained using the R package lm() by calculating their Z scores and two-tailed p values via the pnorm R function.

For multivariate analyses, input behavioural loadings were first transformed (arcsine transform for sociability; log transform for activity) when required by the analysis (e.g. principal component analysis—PCA, generalized canonical discriminant function analysis—gcDFA). To understand how variation in individual traits varies across learning groups, we utilized generalized canonical discriminant function analysis (gcDFA) using the candisc R package (Friendly and Fox 2016). In contrast to traditional canonical discriminant function analysis or canonical correlation, which is restricted to a one-way MANOVA design, gcDFA examines all factors

in a multivariate linear model. We performed gcDFA on the combined data set as well as for each sex separately. All analyses were conducted in R v3.2.2 using the “prcomp” package for PCA and the “candisc” package for gcDFA.

Results

All twelve females and ten surviving males completed the numerosity discrimination assay. However, three of these individuals (one female and two males) had incomplete behavioural assays (e.g. missing scototaxis or sociability trials due to technical or human error) and were therefore not included in any subsequent analyses. Hence, our cognitive-behavioural profiling was conducted on nineteen *G. affinis* (11 females, 8 males) that completed all numerosity training and test trials and subsequent behavioural assays of scototaxis, sociability and mate choice (for females only).

Numerosity discrimination performance evaluation

We evaluated individual learning performance on the nineteen *G. affinis* with complete numerosity discrimination and behavioural data sets. We evaluated learning performance with each individual's numerosity test trials ($n = 9$, including three each at 7 versus 14; 8 versus 12; and 9 versus 12 numerical contrasts, referred to as 1:2, 2:3, and 3:4 test ratios, respectively). We used an individual's median numerosity accuracy (proportion time spent on target side—the side of the numerical contrast that received reinforcement during training trials) to evaluate learning performance within each numerical contrast task. *G. affinis* showed highly consistent inter-individual learning performances across these different numerical contrasts: 7:14 versus 8:12 (Fig. 1b), 7:14 versus 9:12 (Fig. 1c) and 8:12 versus 9:12 (Fig. 1d) with Pearson and intra-class correlations (r [ICC]) of 0.79 [0.89], 0.76 [0.87] and 0.75 [0.87], respectively, with $p \ll 0.001$ for all statistical analyses (Fig. 1).

We differentiated learners from non-learners with a standard learning criterion ($> 60\%$ median time spent near target numerosity in any one of the three test ratios; Agrillo et al. 2012a, b). We identified 7 individuals that failed to meet the learning criterion (hereafter referred to as “non-learners” with all median test scores $< 60\%$; see Table 1). We subsequently categorized the variation in individuals that met our learning criterion ($n = 12$) into either “high-performance” or “low-performance” learners based on time spent near target across all test trials. We identified 6 individuals that spent significantly greater than 50% of their time near the target numerosity across all test trials and hereafter refer to these individuals as “high-performance” learners (see Table 1). We identified 6 individuals with test scores that did not differ significantly from 50% across all nine

test trials, yet exceeded our learning criterion during at least one of the three test ratios ($> 60\%$ median time spent near target numerosity). We classified these latter six individuals as “low-performance” learners (see Table 1). Of note, three of the individuals that did not meet our learning criterion showed significant deviation from 50% time spent near the target numerosity (see Table 1). While this suggests that these three individuals are reacting to the stimuli in a non-random manner, we retained these individuals in the “non-learner” group. (However, we discuss the learning implications of this unique subset in the “Discussion”.)

It is important to note that in controlling for some non-numerical continuous variables in the design of our stimuli sets (e.g. total surface area, and convex hull), we also introduced other variables that may differ between our numerical contrasts (such as density, and size of the individual shapes; see Milletto Petrazzini et al. (2017) for an alternative methodology to address these confounds). While differences in these non-numerical variables were largest in the 2:1 stimuli (20–29% in shape size), they were substantially reduced in the 3:4 ratio test (e.g. 3% difference in rectangle size; 9% difference in ellipse size). If individuals were using a rule such as visit the side with the smaller objects over the larger objects, then we might have observed a precipitous decline in performance when differences in object size was minimal (9 vs. 12 shapes). However, we find no such trend in our data set, as “high-performance” learners exhibited higher than chance performance across all of our ratio tests (see Table 1). While we view our results as support for putative numerical discrimination capacity in some individuals of *G. affinis*, it should be noted that further testing is required to further validate these tentative conclusions.

We used an individual's average numerosity performance (the mean of their three test ratio median scores) as an overall “learning score” (see Table 1). We found no differences in learning scores between individuals trained to different reward contingencies (average (\pm SE) learning score for “high” end rewarded = 0.56 ± 0.051 , average learning score for “low” end rewarded = 0.57 ± 0.047 , $U = 45$, $p = 1.0$). We also found no differences in learning scores between the sexes (average (\pm SE) male learning score = 0.55 ± 0.079 , average female learning scores = 0.58 ± 0.10 , $U = 42$, $p = 0.90$). Individuals meeting the high-performance learning criterion, exhibited significantly higher learning scores than individuals in the low-performance category (average (\pm SE) high-performance learning score = 0.86 ± 0.05 , low-performance learning score = 0.60 ± 0.03 , Mann–Whitney $U = 1$, $Z = 2.64$, $p = 0.0083$).

Sexually dimorphic behaviour

We found differences in activity and sociability tendencies between the sexes. Females tended to be more active (female

Table 1 Individual learning performances in the numerosity discrimination assay

Fish ID	Sex	1:2 test Median score	2:3 test Median score	3:4 test Median score	Average performance	Learning category
MH1	M	0.99	0.95	0.99	0.977	High*
FH3	F	0.88	0.95	0.92	0.917	High*
ML5	M	0.91	0.88	0.94	0.910	High**
FL5	F	0.91	0.86	0.92	0.897	High*
FL6	F	0.93	0.72	0.82	0.823	High**
FH4	F	0.71	0.58	0.6	0.630	High*
MH3	M	0.75	0.87	0.59	0.737	Low
FL4	F	0.65	0.65	0.55	0.617	Low
ML6	M	0.39	0.71	0.74	0.613	Low
FH2	F	0.57	0.66	0.44	0.557	Low
ML4	M	0.52	0.74	0.37	0.543	Low
FL3	F	0.68	0.55	0.38	0.537	Low
FH5	F	0.33	0.45	0.46	0.413	Non-learner
ML2	M	0.34	0.435	0.32	0.365	Non-learner
FL1	F	0.32	0.28	0.44	0.347	Non-learner
MH5	M	0.25	0.385	0.4	0.345	Non-learner**
FH6	F	0.27	0.25	0.31	0.277	Non-learner**
MH6	M	0.2	0.14	0.11	0.150	Non-learner**
FL2	F	0.05	0.18	0.04	0.090	Non-learner

All fish were trained for 4 days and received the same number of training trials ($n = 16$) on a 1:2 ratio task (either 5 vs. 10 or 6 vs. 12 shapes with food reward). Individuals that were trained with food reward on the higher side of the numerosity contrast have a FishID with “H” and individuals trained with food reward on the lower numerosity contrast have an “L” in their FishID. For each of 3 days following training, fish experienced three test trails (stimuli presentation without reinforcement) followed by a reinforcement trial (1:2 ratio with extra food reward) at the end of each testing day. Fish experienced nine test trials in total, including three testing trials per discrimination task: a novel 1:2 ratio (7 vs. 14 shapes), 2:3 ratio (8 vs. 12) and 3:4 ratio (9 vs. 12). Scores are reported as the median proportion time spent with trained stimulus across three test trials for each test ratio. Overall learning performance (learning score) was evaluated as the mean of the three test median scores (average performance). Individuals were categorized as non-learners if they failed to meet the learning criterion (> 0.60 median time spent with target numerosity in at least 1 test ratio). Individuals who met the learning criterion were further categorized into high-performing versus low-performing learners based on non-random time spent on the reinforced side across all test trails. Individuals that met the learning criterion were classified as high-performance learners if their time spent on the reinforced side was significantly greater than 50% across all nine test trials, and low performance if their scores were not significantly greater than 50% using either a t test or a Wilcoxon nonparametric test following a Shapiro–Wilks test for normality

Individuals with proportion time spent with target numerosity in test trials that significantly differed from 0.50 are indicated by * $p < 0.05$; ** $p < 0.01$

average (\pm SE) activity score = 4.47 ± 0.21 (units = average pixel distance travelled), male average = 3.92 ± 0.71 , $U = 68$, $p = 0.050$) and social (female average (\pm SE) sociability score = 0.86 ± 0.031 , male average = 0.73 ± 0.064 , $U = 67$, $p = 0.063$) than males. Sexually dimorphic trends were found despite the absence of significant repeatability at the individual level (Figure S1: sociability (ICC = 0.20, $p = 0.22$), activity (ICC = 0.37, $p = 0.27$).

Our individual measures of anxiety and exploration showed low repeatability values (see supplementary Figure S1) and revealed no significant differences between the sexes: proportion of time spent in white side of scototaxis tank ($U = 45$, $p = 0.93$), latency to enter white side of scototaxis tank ($U = 43$, $p = 1.0$), number of entries into white

side of scototaxis tank ($U = 39$, $p = 0.74$), thigmotaxis in white side of scototaxis tank ($U = 47$, $p = 0.80$), thigmotaxis in dark side of scototaxis tank ($U = 39$, $p = 0.71$) and thigmotaxis during day 1 of numerosity training trials ($U = 60$, $p = 0.19$).

To reduce the dimensionality of the several anxiety and exploration measures that are potentially inter-related, we performed a principle component analysis (PCA) on the five behavioural measures collected during the scototaxis trials (proportion time on white, latency to enter white, entries into white side, thigmotaxis on white side, thigmotaxis on black side) along with the thigmotaxis responses from the initial day of numerosity training (Fig. 2). The first major axis of the anxiety/exploration PCA analysis explained 28.5% of the

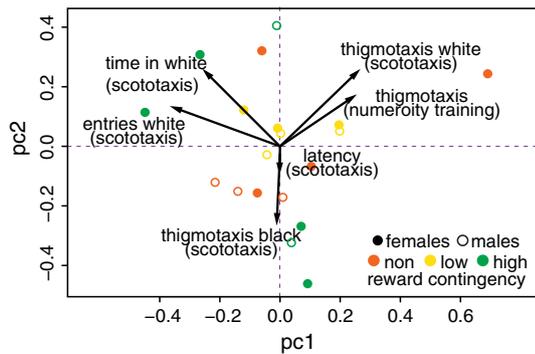


Fig. 2 First two major axes of a principal component analysis of anxiety and exploration behavioural scores. Five behavioural measures collected during scototaxis trials (time in white, entries into white, latency to enter white, thigmotaxis in white, and thigmotaxis in black zone) were combined with thigmotaxis scores during the first day of numerosity training as inputs to the PCA. Data points are labelled as in Fig. 1 (open symbols denote males, closed denote females) (color figure online)

variance across individuals (PC1, Fig. 2) and appears to capture differences in exploratory tendencies. Individuals with positive PC1 scores exhibited higher thigmotaxis in both numerosity training trials and in the white portion of the scototaxis trials, whereas negative PC1 scores were represented by individuals who spent greater proportion of time in the white areas (more threatening zone) during scototaxis assays (scototaxis score) and greater total entries into the white side of the scototaxis tank. The second major axis (PC2, Fig. 2) explains (20.8%) of the behavioural variance and appears to capture more of the anxiety-related behaviours as this axis is characterized by latency to enter the white zone (the more threatening area) of the scototaxis assay, along with thigmotaxis in the black portion of the tank (the less threatening area). We retained the first 2 major axes from this PCA that explained ~ 50% of the behavioural variance as our exploration/anxiety scores. We observed no sex differences in either of the two major axes of the anxiety/exploration PCA [male average (\pm SE) PC1 score = 0.18 ± 0.24 , female average (\pm SE) PC1 score = -0.13 ± 0.48 , $U = 38$, $p = 0.65$; male average (\pm SE) PC2 score = 0.22 ± 0.40 , female average (\pm SE) PC2 score = -0.16 ± 0.35 ; $U = 35$, $p = 0.49$].

Learning performance and behaviour: cognitive-behavioural profiling

Learning scores did not covary with individual behavioural scores or size across *G. affinis* (all $p > 0.10$; Table 2). Furthermore, neither of the two anxiety/exploration axis scores (Anx/Exp PC1, Anx/Exp PC2) revealed a pattern of covariance with learning performance across *G. affinis* (Anx/Exp PC1: $r = 0.10$, $p = 0.69$; Anx/Exp PC2: $r = 0.098$, $p = 0.69$; Table 2).

Table 2 Relationship between discrimination learning scores and individual behavioural traits and size (standard length, SL)

	Combined sexes	Males	Females
Size (SL)	0.25 (0.29)	-0.047 (0.93)	0.49 (0.12)
Sociality	0.25 (0.28)	0.61 (0.11)	0.20 (0.53)
Activity	0.090 (0.71)	0.071 (0.88)	0.39 (0.23)
Anx/Exp PC1	0.10 (0.69)	-0.59 (0.12)	0.32 (0.32)
Anx/Exp PC2	0.098 (0.69)	-0.45 (0.25)	0.51 (0.11)
Thigmotaxis—training	0.13 (0.57)	0.52 (0.19)	-0.37 (0.25)
Thigmotaxis—white	-0.11 (0.63)	0.47 (0.24)	-0.43 (0.18)
Thigmotaxis—black	0.34 (0.15)	-0.024 (0.95)	0.39 (0.23)
Scototaxis time in white	0.074 (0.76)	0.42 (0.29)	0.0091 (0.97)
Entries into white	0.13 (0.58)	-0.048 (0.90)	0.25 (0.45)
Latency to enter white	0.26 (0.27)	0.50 (0.21)	-0.39 (0.22)

Pearson correlation coefficients and associated (p values) are reported for both sexes combined ($n = 19$, overall), males only ($n = 8$), and females only ($n = 11$). See Fig. 3 for a comparison of slopes between the sexes

However, a few of the anxiety/exploration-like behaviours revealed a sex-specific relationship with learning performance (Fig. 3). Specifically, thigmotaxis in the white portion of scototaxis trials (Fig. 3b), proportion of time spent on the white side during scototaxis trials (Fig. 3e) and thigmotaxis during initial training trials (Fig. 3f) showed significant contrasting patterns with learning score between the sexes. In all three cases, the male relationship between anxiety/exploration behaviours and learning score trended positive, whereas females trended negative or neutral.

To identify cognitive-behavioural profiles, we performed generalized canonical discriminant function analysis (gcDFA) to identify multivariate predictors of the different learning groups (non-, low- and high-performance learners). We conducted three analyses including: (1) using all 8 individual behaviours from Fig. 3 as input loadings (Figure S2), (2) using 4 independent behavioural inputs (sociability, activity, exploration/anxiety PC1, anxiety/exploration PC2) with the sexes combined (Fig. 4), (3) using the 4 independent behavioural inputs in a sex-specific manner (males only, Fig. 5a; and females only, Fig. 5b with the addition of mate choice).

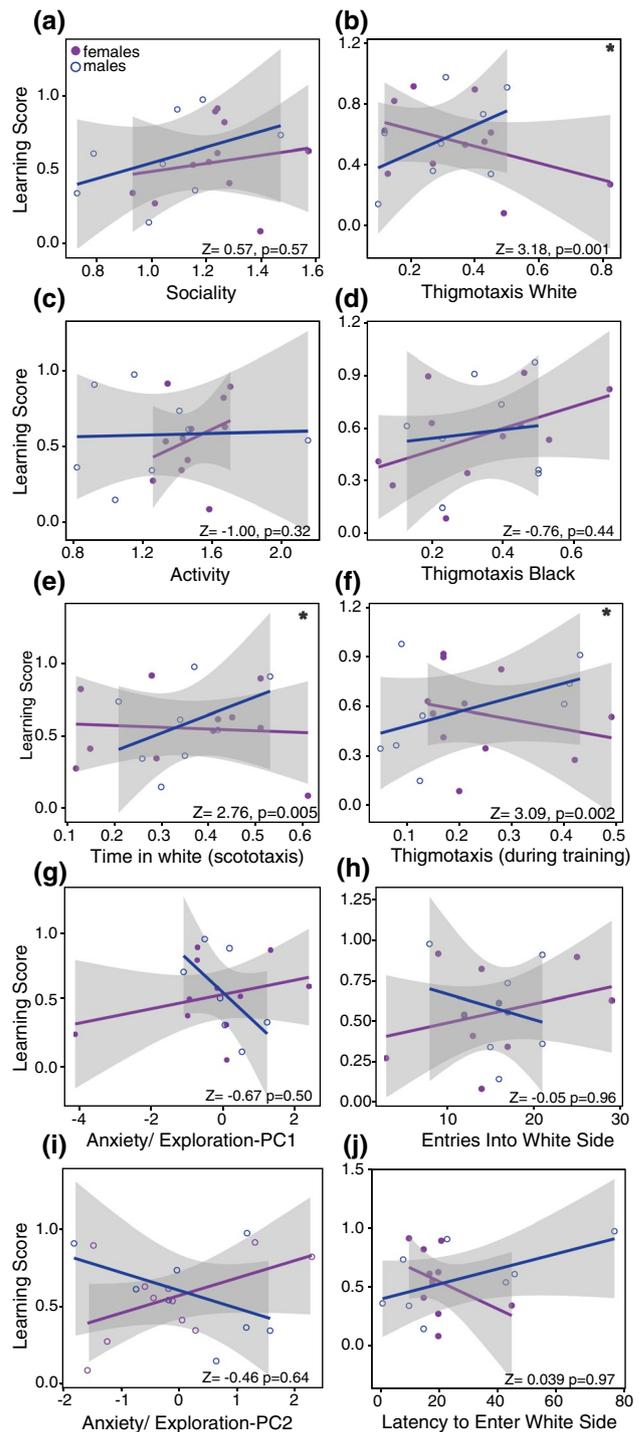
Generalized canonical discriminant analyses (gcDFA) showed learner group membership was predicted from variation in multiple behavioural traits. Across all gcDFA analyses, the most significant behavioural loadings differentiating the three learning phenotypes were sociability and activity scores (Figure S2, Figs. 4, 5). The gcDFA with the full 8 behavioural inputs (Figure S2) significantly differentiated

Fig. 3 The relationship between learning performance and **a** sociality, **b** thigmotaxis in white zone (during scototaxis), **c** activity levels (measured during sociability trials), **d** thigmotaxis in black zone (during scototaxis), **e** time in white (during scototaxis), **f** thigmotaxis during numerosity training day 1, **g** anxiety/exploratory PC1 (see Fig. 2), **h** entries into white side during scototaxis, **i** anxiety/exploratory PC2 (see Fig. 2), and **j** latency to enter white side during scototaxis. We compared the slope between male (blue, unfilled) and females (purple, filled) by calculating the normal Z computed as the difference between slopes divided by the difference in slope standard errors ($Z = (b_1 - b_2) / \sqrt{SE_1^2 + SE_2^2}$). We found significantly different slopes (* denotes $p < 0.05$) between males and females for learning performance and thigmotaxis during training (f), thigmotaxis on the white side of the tank during scototaxis trials (b), and time spent on white side during scototaxis trials (e) (color figure online)

the low-, mid- and high-level learning phenotypes by a primary axis defined largely by sociability ($p = 0.057$) and activity ($p = 0.069$) loadings; and the individual DFA1 scores were significantly correlated with individual learning scores ($r = 0.57, p = 0.009$). To reduce the interdependence of the inputs, we next applied a more conservative gcDFA approach (Fig. 4) that incorporated only 4 behavioural inputs (sociability, activity, anxiety/exploration-PC1, anxiety-exploration PC-2). The combined sex analysis with 4 behavioural inputs gcDFA failed to differentiate the three learning groups (Fig. 4) with significant overlap of the normalized 95% confidence ellipses and the DFA axis 1 scores did not correlate with individual learning scores ($p = 0.28$).

Using the 4 behavioural inputs with sex-specific gcDFAs showed strong differentiation of the learning groups (Fig. 5a, c), and the resulting multivariate behaviour axis scores significantly predicted learning performance variation across individuals (Fig. 5d, females only). Male-specific gcDFA revealed clear differentiation among the three learning phenotypes with a primary axis (male DFA1) accounting for 93.6% of the variation and discriminating between the non-learning males from the two learning male groups, and a secondary axis (male DFA2) accounting for 6.4% of the variation and discriminating the high-performance group from the others by distinct behavioural loadings (Fig. 5a). Activity was the only significant loading ($\rho = 0.76, p = 0.036$) for the primary axis (male DFA1) and differentiated the low-learning males from all others. Neither of the male-specific DFA axis behavioural scores (DFA1, DFA2) correlated with individual male learning scores (DFA1: $\rho = 0.45, p = 0.27$, DFA2, $\rho = 0.71, p = 0.06$).

Female-specific gcDFA revealed strong multivariate differentiation between high-learning phenotypes and all others (Fig. 5c) with a primary axis (female DFA1) explaining 91.9% of the behavioural variation across individuals with significant loadings of activity ($\rho = 0.66, p = 0.030$), near-significant sociability ($\rho = 0.58, p = 0.059$), and non-significant associations of Anx/



Exp PC1 ($\rho = 0.41, p = 0.20$), Anx/EXP PC2 ($\rho = 0.28, p = 0.40$), and mate choice ($\rho = 0.23, p = 0.50$) scores. There were no significant behavioural loadings onto the secondary axis (female DFA2) that explained only 8.1% of the variation. Female DFA1 multivariate behavioural scores strongly predicted the variation in individual female learning scores ($\rho = 0.80, p = 0.005$, Fig. 5d).

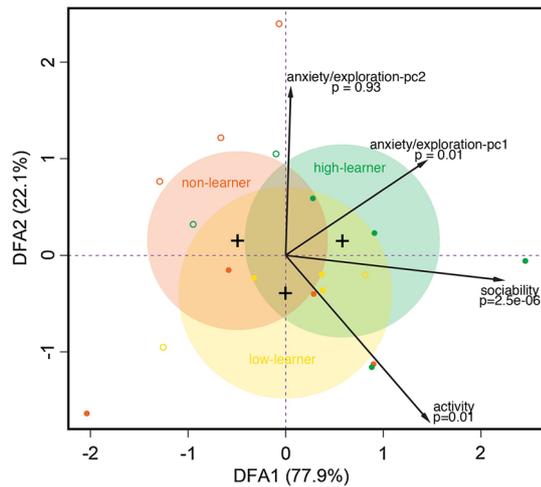


Fig. 4 Generalized canonical discriminant analysis plotting sociability, activity, and the first two major axis of the anxiety/exploration PCA (from Fig. 2) for mixed-sex analysis ($n = 19$) with 95% normalized confidence ellipses associated with each learning category (non-, low- and high-learning performance) outlined by the colours orange, yellow and green, respectively. Centroids for each category are shown as +, and male and female individuals are denoted by unfilled and filled symbols, respectively (color figure online)

Discussion

Across different numerosity discrimination contrasts, individual *G. affinis* exhibited highly consistent learning performances (Fig. 1). This highly repeatable, inter-individual pattern of associative learning suggests that a consistent range of learning phenotypes exist within this species. The nearly continuous range of performance included individuals that spent significantly higher proportions of time on the side of the tank with the trained ratio contingency across all three novel numerical contrasts (“high-” performance learners), as well as individuals who passed the learning criterion for some of these ratios but not all (“low-” performance learners), and individuals that did not bias their time near the trained numerosity in any of the test ratios (“non-learners”, Table 1). This variation in learning performance may be due to individual differences in acquiring the association between numerical contrasts and food reward, differences in extinction rates (as individuals experienced three non-reinforced trials before a reinforcement trial on testing days), or differences in motivation across individuals. While further investigation will be required to dissect the source of variation among learning phenotypes, the robust consistency in individual performances suggests these phenotypes are distinct.

While subjecting individuals to multiple tests has its drawbacks (e.g. increases extinction), it is a recommended means to identify variation in learning performance (Thornton and Lukas 2012). Individuals exhibiting a significantly

greater than 50% time across all nine test trials suggest that these high-performance learners adopted the basic contingency rule provided during numerosity training (visit the high or the low side of the 1:2 numerical presentation) and generalized this rule across novel test ratios (2:3 and 3:4). Meanwhile, individuals that met the learning criterion (achieving a median performance > 60% in at least one of the three test ratios), but failed to show significantly greater than 50% time spent near the target numerosity across all nine test trials, may represent partial or more limited learning capacity in this numerosity discrimination test. Alternatively, these low-performance learners may also represent individuals with an ability to utilize the contingency rule but with less of an ability to generalize across contexts, or individuals with lower motivation or higher extinction tendencies than individuals in the high-performance category.

An examination of individual variation in learning performance can often benefit from examining the individuals that fail to perform the task as much as we can learn from individuals that succeed at the task (Thornton and Lukas 2012; Seed et al. 2012). Within our “non-learners” group, we observed individuals that failed to show a significant bias towards the training numerosity as well as individuals that revealed a significant bias “away” from the target numerosity (Table 1). While the former may represent a group that may have less of a capacity to acquire the association between a numerical representation and food reward, they may also represent a group that were less motivated than those that passed the learning criterion. Additionally, they may also represent individuals that did not focus on the relevant cues provided during numerosity training. Meanwhile, the latter individuals may represent individuals that learned to avoid the numerosity stimuli associated with food for unknown reasons, or individuals with rapid learning extinction rates or greater willingness to innovate under non-rewarding contexts. For our purposes, we do not differentiate the non-random from the random “non-learners”, yet this is likely to provide a very interesting future direction of study.

Sexually dimorphic behaviours and relationships to learning

We observed great variation in learning performances across individuals, yet we observed no sex differences in learning scores. While it should be noted that we have relatively small sample sizes to identify sex differences in learning performance, we did observe near-significant differences in other behaviours between the sexes (activity and sociability). Female *G. affinis* trended to be more active and social than their male counterparts; yet we observed no significant difference between the sexes in any of the six individual measures of anxiety or exploration (nor the anxiety/exploration PC axes). Combining the sexes, or investigating each sex

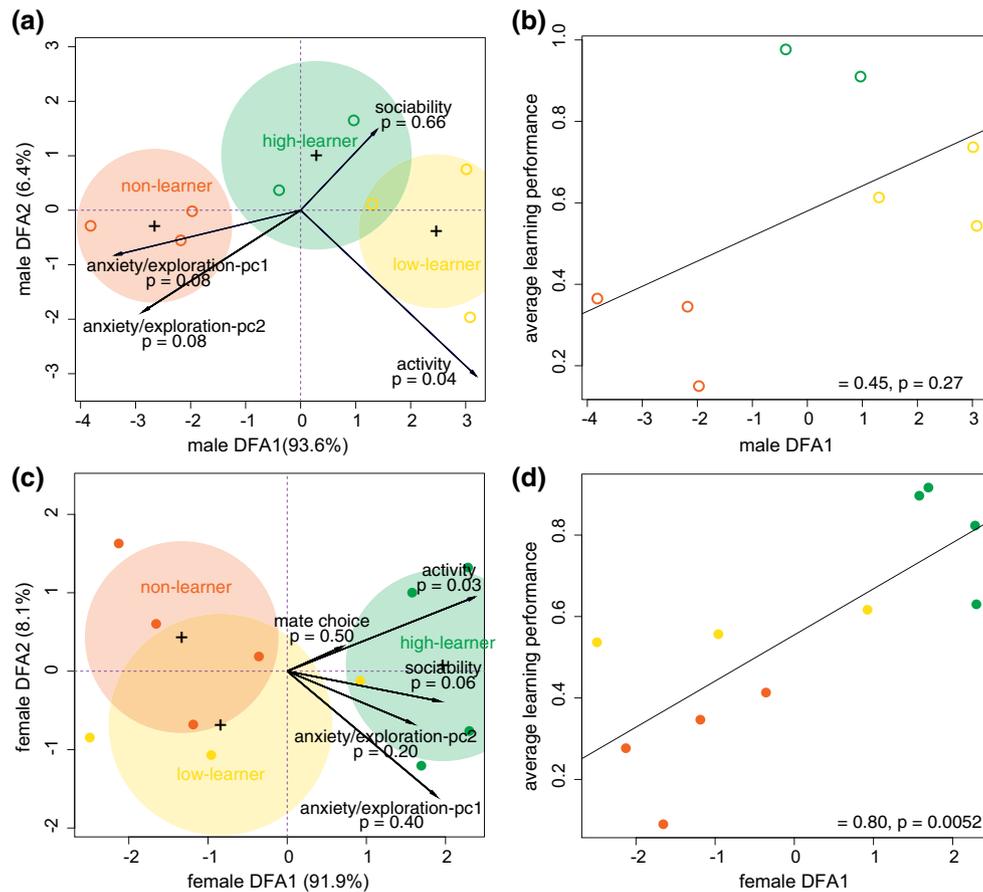


Fig. 5 Generalized canonical discriminant analysis plotting sociability, activity, mate choice (females only) and the first two axis of the anxiety/exploration PCA (from Fig. 2) for males (a) and females (c). Coloured circles represent the normalized 95% confidence ellipses associated with each learning category: non-learners (orange), low-performance learners (yellow) and high-performance learners (green). For males, sociability showed a significant correlation with the primary canonical axis (male DFA1), while activity showed a signifi-

cant correlation with the secondary axis along with near-significant behavioural loadings of the two anxiety/exploration PCA scores. For females, activity showed a significant correlation and sociability a near-significant correlation with the primary canonical axis (female DFA1). Learning scores (mean of each individual's median performances in all 3 tests shown in Fig. 1) were significantly correlated with individual primary canonical axis scores (DFA1) for females (d) but not for males (b) (color figure online)

separately, we observed no significant correlation between each individual behaviour and learning scores (see Table 2). However, we identified sexually dimorphic relationships between some anxiety and exploration measures with learning scores (Table 2, Fig. 3).

Anxiety, exploration and activity (Guillette et al. 2009; Brust et al. 2013) have each been individually correlated with learning performances in different taxa, and sometimes in sex-dependent ways (Titulaer et al. 2012; Mamuneas et al. 2015). In this study, male and female *G. affinis* exhibited significantly different slopes of individual learning scores with some exploratory or risk-assessment measures in our scototaxis trials (proportion of time and thigmotaxis on the white side, Fig. 3b, e). Males trended towards a positive relationship between these behavioural variables and learning performance, whereas females trended towards a negative or neutral relationship. The same contrasting pattern

was also observed when examining the relationship between learning performance and thigmotaxis tendencies of males and females during their initial numerosity training sessions (Fig. 3f). This contrasting relationship between the sexes is similar to that found in the great tits (*Parus major*) where males were found to have a positive relationship and females a negative relationship between exploration and reversal learning success (Titulaer et al. 2012).

Within poeciliid fishes it is still too early to determine whether this sex-specific difference is common across species. The positive relationship between learning performance in appetitive associative learning assays (e.g. food reward) and exploration in more threatening environments (thigmotaxis in novel environment during training; white side of scototaxis trials) in males is consistent with previous research in male guppies (*P. reticulata*). Dugatkin and Alfieri (2003) found a positive relationship between predator inspection

tendencies and associative learning task with food reward in male guppies. Yet, our trends for female *G. affinis* differ from those found in poeciliid Panamanian bishop fish, *Brachyrhaphis episcopi*. Female *B. episcopi* from a high predation population revealed a positive relationship between exploratory tendencies in an open-field test and learning rate in an appetitive associative learning task (DePasquale et al. 2014). While both of these studies only examined a single sex, more recent studies are directly comparing cognitive differences between the sexes. A robust body of work comparing cognitive performance and cognitive styles between male and female guppies has found they differ in decision speed for some tasks (Lucon-Xiccato and Bisazza 2016), cognitive performance in others (spatial abilities and cognitive flexibility; Lucon-Xiccato and Bisazza 2014; Lucon-Xiccato and Bisazza 2017a, b) and comparable cognitive performances on a number of different cognitive tasks (visual discrimination: Lucon-Xiccato and Bisazza 2016; novel object recognition: Lucon-Xiccato and Dadda 2016). Interestingly, despite showing similar performances, males and females guppies did differ in their exploratory styles within the novel object recognition task with males being more exploratory at the beginning of trials and females more so at the end (Lucon-Xiccato and Dadda 2016).

Sexually dimorphic cognitive–behavioural profiles

Given the sex-specific relationships between exploratory tendencies and learning performances, it should be unsurprising that these differences contribute to sex differences in cognitive–behavioural profiles. Male and female *G. affinis* have different behaviours associated with each learning performance category (non-, low- and high-performing learners, Fig. 5a, c). Both of the anxiety/exploration combined measures axes (Anx/Exp PC1, Anx/Exp PC2) are associated with male non-learners, while more closely associated with high-performance learning females. In addition, activity levels are significantly associated with female high-performers while associated with low-performance learning in males. Meanwhile, sociability was associated with high-performing learners from both sexes. While our small sample sizes warrant caution in interpretations of these patterns, it is noteworthy to point out that each of the sex-specific multidimensional analyses more clearly differentiate learning phenotypes than the combined sex analyses with double the sample size (Figs. 4 vs. 5). Furthermore, the multivariate primary axes of the female-specific DFA significantly predicted individual variation in learning scores (females, $p = 0.005$, Fig. 5d), whereas the mixed-sex multivariate DFA primary axis did not ($p = 0.28$, Figure S2).

By evaluating multiple behavioural attributes (anxiety, activity, exploration and sociability) of individuals in different contexts, we are able to predict the performance

variation of these individuals in numerosity discrimination tests (Fig. 5b, d). These multivariate behavioural scores were only successful when sex-specific, suggesting that in western mosquitofish each sex has a unique suite of behavioural traits associated with associative learning performance. These unique cognitive–behavioural profiles for each sex may be results of divergent sexual selection or ecological selection. In some poeciliids, males and females occupy distinct ecological habitats leading to one sex experiencing higher predation threats than the other (e.g. guppies, Croft et al. 2006), which may differentially impact cognitive–behavioural profiles between the sexes. Across poeciliids, females are subject to selection pressures to increase reproductive success through increased fecundity (through increased foraging efficiency) and reduction of male harassment (Bisazza 1993). Social tendencies have known fitness benefits among poeciliid females including more efficient foraging in *G. holbrooki* (Pilastro et al. 2003) and reduction in sexual harassment (Pilastro et al. 2003, Agrillo et al. 2006). Meanwhile, activity levels are also likely to influence a female's ability to escape male harassment. Since this combination of behaviours is likely to impact a female's reproductive strategy, this may underpin the very strong variance capture ($> 91\%$ variance) and predictive power ($\rho = 0.80$, $p = 0.005$) of the primary axis of the female CBP. Whether the sex-specific CBPs are driven by sexually dimorphic reproductive strategies will be an interesting avenue of research in the future.

Linking variation in learning performance to variation in social tendencies

In all of our multidimensional analyses (Figure S2, Figs. 4, 5a, c), social tendencies were the most salient behavioural driver associated with high-performance learning phenotypes. While not a direct aim of the current study, sociability has been previously identified as a repeatable behavioural trait with great individual variation in this species (Cote et al. 2010, 2011, 2012). Previous work has highlighted social tendencies in *Gambusia* species that are highly stable over time and linked to dispersal tendencies (*G. affinis*, Cote et al. 2010), and that are strongly responsiveness to predation regimes (*G. hubbsii*, Heinen-Kay et al. 2016). Here we find that though the sexes generally tend to differ in social tendencies, male and female western mosquitofish that exhibited the highest numerosity discrimination performances were also individuals with higher sociability scores. Recognizing that associative learning performance and sociability may be part of a more general cognitive–behavioural profile should be an important aspect to future studies.

Why might a strong association between social behaviour and learning performance exist? This question is commonly posed in taxa with highly complex social interactions (e.g. social hierarchies, Seyfarth and Cheney 2003), with a

common focus on the role of social learning (information received through others). Researchers have proposed that demands on cognitive hardware (Dunbar 1998; Dunbar and Shultz 2007) and processing (Fernald 2017) increase with increased complexity of social interactions. Even in pociliid taxa with minimal social hierarchies, researchers have theorized (Laland and Williams 1997) and found (Chapman et al. 2008) a relationship between social tendencies and social learning. However, there has been little theory regarding how social tendencies might influence individual learning ability. One possible mechanism for the link between social tendencies and individual learning capabilities is that social types are accustomed to greater information processing on a daily basis than asocial types. Individuals who spend more time with conspecifics may be subject to processing greater amounts of information, and/or face a greater number of decisions, than asocial individuals who predominantly process environmental information only.

Social individuals may be simultaneously processing environmental stimuli as well as processing conspecific interactions and making discriminating decisions about other individuals, therefore having more total information to process at any given time. Recent research investigating decision-making differences between asocial and social behavioural types in *G. affinis* is consistent with this proposition. Cote et al. (2012) found that *G. affinis* social types are not only making the decision to shoal with conspecifics over being alone, but they are also making discriminating decisions of the type of individuals (social or asocial) with whom they choose to shoal. Furthermore, research with guppies has found that individuals who can discriminate among shoal groups based on individual composition of personality types (e.g. bold vs. shy) can increase their foraging success (Dyer et al. 2009), suggesting that social types are facing a myriad of discrimination decisions that have direct impacts on their fitness.

The link between social tendencies and individual variation in *G. affinis* learning capabilities is present for both sexes, but the mechanism governing this association remains unknown. Heritable associations (pleiotropic gene effects or linkage) between social tendencies and associative learning performance may be responsible. Examination of natural variation in behavioural syndromes in wild Bahamian *G. hudsonii* suggests that some features of behavioural suites may be heritable (e.g. exploratory tendencies, (Heinen-Kay et al. 2016)). It is also possible that these seemingly non-related or independent traits (cognitive performance and social tendencies) may be linked through experience. For instance, social tendencies may enhance associative learning capacity (e.g. past social experiences augment learning processes) through increased exposure to decision-making (see Discussion above). While these findings may highlight a general difference in social tendencies between learning phenotypes,

they may also be a function of the particular discrimination task we employed (numerosity discrimination). Numerical discrimination tasks where individuals differentiate between groups of geometric shapes are similar to the task of differentiating between conspecific shoals of differing sizes (Agrillo et al. 2008). Hence, our results may be driven by more socially oriented individuals learning a group size differentiation task better than less social individuals. However, recent research with *P. reticulata* females suggests that this may not be the case, as more social females performed worse in shoal discrimination tasks (Lucon-Xiccato and Dadda 2017). Further experimentation that exposes individuals to diverse set of associative learning tasks (e.g. colour discrimination; spatial learning tasks) will help to differentiate these implications and provide additional hypotheses for unraveling the relationship between learning performance and sex-specific CBPs.

Summary

Taken as a whole, this study reveals that the cognitive-behavioural profiles in the western mosquitofish, *G. affinis*, differ between the sexes, and that some of the most important behaviours contributing to these differences may be under divergent sexual selection. These results have important implications for how cognitive variation may be maintained. Selection on behaviours with strong links to learning performance might serve to maintain variation in cognitive performance across populations. Variation in sociability, activity and anxiety behaviours may be maintained through spatiotemporal dynamics of food availability, predation intensity and/or social densities. High sociability, activity and even anxiety may be favoured under some conditions, whereas low sociability and activity in others. For instance, if selection for sociability varies by environmental or population density, as it does for the common lizard (Cote et al. 2008); then we might expect to observe spatiotemporal variation in female and male cognitive performance driven by selection for social tendencies.

These results also suggest that we can learn much about the behavioural inputs to cognitive performance by studying divergent behavioural types. While cognitive-behavioural profiling is already common practice with human disease aetiology (Crespi and Hurd 2014), the currently young field of cognitive-behavioural profiling in non-human animals has much to offer in understanding the complex link between behavioural and cognitive phenotypes. By extending our investigation of cognitive-behavioural relationships beyond humans, we can not only identify a broad array of CBPs driven by distinct selection pressures but also employ manipulative studies to better understand the mechanisms and developmental origins

behind these associations and address age-old queries regarding sources of cognitive variation.

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Compliance with ethical standards

This research was conducted without any external financial support.

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval The authors certify that this work followed ethical treatment of animals outlined in their IACUC protocol (AUP-2016-00246).

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