Hybridization may promote variation in cognitive phenotypes in experimental guppy hybrids

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Abstract

Hybridization is an important mechanism of evolution. While hybrids often express inferior traits and are selected against, hybridization can promote phenotypic variation and produce trait combinations distinct from the parentals, generating novel adaptive potential. Among other traits, hybridization can impact behaviour and cognition and may reinforce species boundaries when hybrids show decreased cognitive abilities. However, the hypothesized role of hybridization in the diversification of cognitive phenotypes remains enigmatic. To test this idea, we compare the performance of female guppies (*Poecilia reticulata*), Endler's guppies (*Poecilia wingei*), and their experimental hybrids in colour association and reversal learning. In addition, we introduce a new approach to compare multidimensional cognitive phenotypes. We found that hybrids showed intermediate learning abilities in both tasks compared to the parentals. Moreover, hybrids had slightly higher phenotypic dispersion, new trait combinations occurred in some hybrid individuals, and the mean phenotype of one hybrid group deviated away from the axis of variation of the parentals. Our method should hence be useful in further exploring how hybridization, and other evolutionary processes, impact behavioural and cognitive traits. Our results suggest that hybridization may promote cognitive variation and generate new trait combinations, even when learning performance at the group level is intermediate between parentals.

Keywords

Transgressive segregation; phenotypic novelty; kernel density estimation; associative learning; cognitive flexibility

1. Introduction

Interspecific hybridization often leads to hybrids that are less fit than the two parental species (Cooper et al. 2018; Coughlan and Matute 2020). Such fitness costs come from hybrid inviability or infertility (Ellison and Burton 2008; Stelkens et al. 2015; Walter et al. 2020), but also from physiological or behavioural phenotypes that are impaired or ecologically mismatched. For example, hybrids of *Drosophila* have decreased foraging efficiency (Turissini et al. 2017) and hybrids of migratory fish that express intermediate migratory strategies have higher predation rates (Pärssinen et al. 2020). In these cases, intrinsic (genetic) and extrinsic (ecological) selection against hybrids can become a postzygotic isolating barrier that reinforces species boundaries (Coughlan and Matute 2020). On the other hand, there is now widespread evidence that hybridization can also be a catalyst of phenotypic variation, leading to ecological and evolutionary innovations (Mallet 2007; Rieseberg et al. 2003a; Seehausen 2004). Through recombination of parental alleles, hybridization can generate high levels of heritable variation in hybrids, including individuals that express new trait combinations that exceed the range of both parental species, referred to as transgressive (Dittrich-Reed and Fitzpatrick 2013; Lewontin and Birch 1966). Under certain ecological conditions, such variation may provide hybrids with opportunity for the evolution of novel adaptations or to explore underused niches (Mallet 2007; Seehausen 2013). For example, transgression in bill morphology allowed the hybrids of two Galapagos finches to forage on novel food items and led to the formation of a new species (Lamichhaney et al. 2018). Despite an apparent contrast, the ideas of hybridization resulting in unfit hybrids that are selected against or in transgressive hybrids with evolutionary novelty potential are not mutually exclusive. Even if most of the variation produced is deleterious, a few rare transgressive individuals can become established if intrinsic selection is not strong and extrinsic selection is favourable (Dittrich-Reed and Fitzpatrick 2013; Pereira et al. 2014). For instance, early hybrids of the *Tigriopus* copepod show reduced survival during development, but a few generations of recombination are enough to recover fitness to the levels of the parentals (Pereira et al. 2014). At the same time, some of these hybrid lineages can thrive in extreme temperatures that are lethal to the parental species, and thus may become established in these unused niches (Pereira et al. 2014).

Transgressive phenotypes are common in both plants and animals, and different mechanisms have been proposed to explain how recombination of parental genomes can result in new traits (Bell and Travis 2005; Rieseberg et al. 2003b; Stelkens et al. 2009; Thompson et al. 2021). When alleles have complementary additive effects, first-generation (F1) hybrids can only express intermediate phenotypes, but effects may sum in the F2 and higher generations and lead to transgression (Rieseberg et al. 2003b). By contrast, extreme hybrid phenotypes can arise in F1 hybrids through dominance of some loci contributing to variation in the same trait or epistatic interactions of alleles at different loci, among other mechanisms (Mérot et al. 2020; Stelkens et al. 2009; Thompson et al. 2021). More inclusive definitions of transgression may also encompass ecological or functional transgression, not classically considered a transgressive effect from a genetic point of view (Dittrich-Reed and Fitzpatrick 2013; Holzman and Hulsey 2017). In these cases, novel hybrid variation may arise from new combinations of traits that fall between disjunct parental ranges, which can perform new functions or facilitate expansion into new areas of functional space.

One way in which hybridization can influence the trajectories of species is through effects on behavioural phenotypes. When hybrids show potentially maladaptive behaviours, such as impaired foraging, reproductive, or migratory behaviours, then behavioural traits can contribute to selection against hybrids and play a role as reproductive isolation barriers (Bridle et al. 2006; Linn et al. 2004; Pärssinen et al. 2020; Turissini et al. 2017). However, hybridization can also lead to behavioural novelty. Even if hybrid individuals show intermediate or poorer behaviours in the parental species niches, for example lower feeding efficiency on parental food types, they may have higher efficiency on novel food types and therefore be able to exploit resources outside the parental species niche, as observed in hybrids of African cichlids (Selz and Seehausen 2019). In addition, behavioural novelty can indirectly arise from transgression in other traits, such as novel combinations of morphological characters that may allow the expression of new behaviours (Feller et al. 2020).

Similar to behavioural phenotypes, recent studies have proposed a role for cognitive abilities such as learning and memory in influencing the outcome of hybridization events (Rice 2020; Rice and McQuillan 2018). On average, hybrids of black-capped (*Poecile atricapillus*) and Carolina (*P. carolinensis*) chickadees performed worse in a spatial task mimicking seed caching and were less likely to solve a novel problem than their parental species (McQuillan et al. 2018). Since these birds rely on scatter-hoarding to survive harsh winters, hybrids showing poor learning and memory may suffer a fitness disadvantage in the wild (McQuillan et al. 2018). In other cases, better learning and memory scores have been reported in F1 hybrids of domesticated mice and of horses and donkeys (mules); such cases are likely a result of heterosis, i.e., enhanced heterozygosity from outbreeding domesticated species (Osthaus et al. 2013; Proops et al. 2009; Võikar et al. 2001). If heterosis underlies enhanced cognitive abilities, this should be a transient effect restricted to F1 hybrids (Lippman and Zamir 2007). Surprisingly, there is a complementary outcome of hybridization on cognition that may also influence ecological selection on hybrids, and which remains to be tested. Hybridization may increase cognitive phenotypic variation in hybrids and promote transgression in cognitive performance. Here we test this hypothesis.

By measuring the learning performance of hybrids between guppies (*Poecilia reticulata*) and Endler's guppies (*Poecilia wingei*) and the parental species in two cognitive tasks, we examine how the individual learning abilities of hybrids compare to the parentals and test whether hybrids display larger variation and transgression in learning performance. *P. wingei* evolved from *P. reticulata* (Meredith et al. 2010) and occurs in a small area in northern Venezuela, largely separate from the ubiquitous *P. reticulata*. As the two species seem to have a very similar ecology (M. Kempkes pers. comm.) their cognitive abilities can be adequately compared using the same set up (Cauchoix et al.

2018). Males differ in colour pattern and courtship behaviour (Alexander and Breden 2004; Poeser et al. 2005), and since those differences are most pronounced in areas where they co-occur (Poeser et al. 2005) it is assumed that the species are separated by divergent sexual selection (Alexander and Breden 2004). While field data on hybridization is lacking, hybridization events seem likely as *P. wingei* and *P. reticulata* are genetically compatible (Alexander and Breden 2004) and readily hybridize in the laboratory (Ramsay 2014). Of potential importance is the field observation that male *P. wingei* and *P. reticulata* differ in home range size. While *P. reticulata* are known to vagrantly cover large areas (Croft et al. 2003), *P. wingei* seem to stay within areas of 50-70 cm diameter (M. Kempkens, pers. comm.). It is unknown whether females show similar differences. As home range size is often related to cognition (Sherry et al. 1992), we may speculate that *P. wingei* and P. *reticulata* evolved cognitive differences. Hence, their hybridizability, similar ecology, yet potentially divergent cognitive ability makes them a suitable species pair to test for the cognitive consequences of hybridization. Specifically, we assess individual performance on two different cognitive tests – a colour association task testing simple learning abilities and a reversal learning task testing cognitive flexibility. In addition to differing in complexity, the two cognitive traits assayed by these tasks are encoded by different brain regions and may represent different trade-offs between costs and benefits of investment in each cognitive trait (Chaves and Hodos 1997; López et al. 2000; Rice 2020; Vila Pouca et al. 2021). To characterise phenotypic dispersion and assess the occurrence of transgression, we use a novel approach that combines the learning scores of the two tasks into clusters of cognitive phenotypes. Borrowing from methods used in movement ecology and ecological niche analyses (Mammola and Cardoso 2020; Worton 1989), together with a geometric approach to quantify patterns of hybrid phenotypes (Mérot et al. 2020; Thompson et al. 2021), we quantitatively compare phenotypic dispersion between hybrids and parentals and quantify the extent to which hybrids are transgressive and/or deviate from parental mean phenotypes. If hybrids perform worse in these cognitive tests compared to one or both parental species, then these traits may represent a postzygotic isolating barrier against hybridization (Rice and McQuillan 2018). However, if hybrid phenotypes are over-dispersed relative to parents or deviate from parental mean phenotypes, then those hybrids may have cognitive innovation potential (Seehausen 2013; Thompson et al. 2021).

2. Methods

2.1. Parental species and F1 hybrid breeding

Parental fish used for breeding derived from laboratory populations that were kept in identical conditions at Wageningen University & Research. Common guppy (*Poecilia reticulata*) populations descended from wild individuals from the upper Aripo river, Trinidad, and Endler's guppy (*P. wingei*) populations descended from wild individuals from Cumaná, Venezuela in 2006. Experimental fish were produced according to standard hybridization methods (Stelkens et al. 2009) and tested for cognitive ability in two blocks. In each block, reciprocal first-generation (F1) hybrids (i.e., *P. reticulata* females crossed with *P. wingei* males, and *P. wingei* females crossed with *P. reticulata* males) and non-hybrid (parental) crosses were bred and raised simultaneously. We will refer to the parental species as R for *P. **r**eticulata* and W for *P. **w**ingei* and use these abbreviations for the hybrid crosses as mother species × father species (i.e., R×W and W×R). All aquaria were part of a recirculation system, in which water chemistry, temperature, light conditions, and feeding regime were kept constant. All fish were bred and raised in identical conditions in a common garden environment. In the first block, F1 hybrids and parental offspring were obtained each from 12 families. In the second block, F1 hybrids were obtained each from 16 families and parental offspring each from 12 families. For further details on breeding and rearing, see supplementary material.

2.2. Learning assays

Individuals used in the learning tasks were adult females, at an approximate age of six months. Only females were tested as males are often difficult to motivate with a food reward (Fuss and Witte 2019). The fish were tested in two blocks in 60 experimental tanks, where they were individually housed for the duration of the learning tasks. In each block, females from the two F1 hybrid crosses and the two parental crosses were assigned a unique identifier and randomly distributed across the 60 tanks, and the experiments were run blind to the group of each animal. The experimental tanks included a home compartment (45×20 cm) and an experimental compartment (15×20 cm) at the front of the tank, only accessible during training sessions through a guillotine door (Fig. 1; see supplementary material for additional details). Fish were confined to the home compartment outside of training sessions. In the first block of experiments, the home compartment was further divided into a small compartment (15×20 cm) at the back of the tank that housed two juvenile females as companions, separated from the focal fish by a transparent and perforated divider, to avoid keeping fish in isolation. Due to logistical constraints, focal fish in the second block only had the neighbouring fish as social companions. The experimental compartment was visually isolated to avoid social learning effects.

To test for associative and reversal learning, we used a well-established colour discrimination assay for fish, using red and yellow as stimulus colours (Buechel et al. 2018; Fuss and Witte 2019). Before the start of the experiment, fish were haphazardly assigned to either red or yellow as the correct stimulus, balanced across the four groups. The experimental compartment contained a white plate with 20 identical circular holes (5 mm deep, 10 mm diameter).

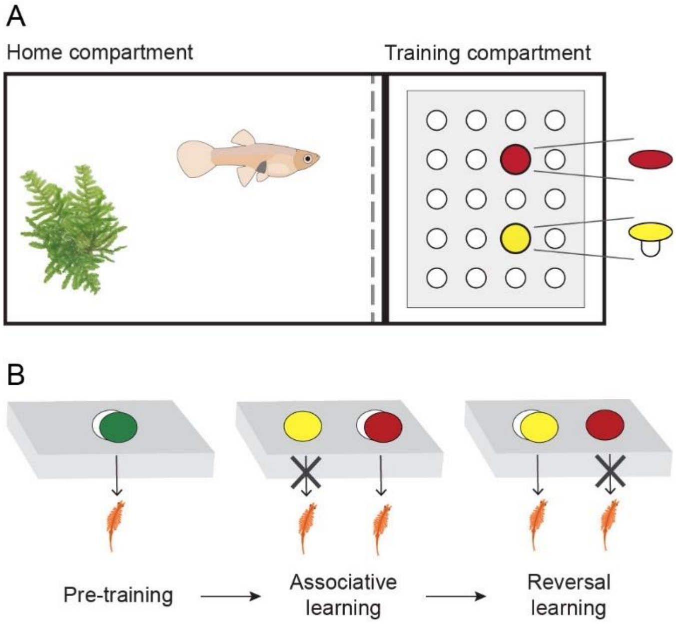

Figure 1. Schematic representation of the experimental tank and associative and reversal learning tasks. (a) The experimental tank consisted of a home compartment and a training compartment. (b) In pre-training, fish were taught to dislodge a green disc to access a food reward (one brine shrimp) in a hole underneath. During the learning tasks, fish were given a choice between two coloured discs, both concealing a food reward. The negative stimulus was fixed in the hole with a plastic knob and could not be moved by the fish to uncover the food item beneath it.

2.2.1. Pre-training

During pre-training trials, fish learned to dislodge a green disc to access a food reward (one frozen *Artemia*) hidden in one of the holes. The trial started with the opening of the opaque door; 5 s later, we opened the transparent door. The fish could then voluntarily enter the training compartment and find the food reward. During the first trials, the disc only partially covered the hole, leaving the reward exposed. We then trained the fish to dislodge the green disc by successively moving the disc from partially to fully covering the hole. Six females (R, n=2; W, n=1; R×W, n=2; W×R, n=1) did not feed during early pre-training stages and were replaced by new fish. A further seven females (R, n=3; R×W, n=3; W×R, n=1) did not dislodge the disc during late pre-training stages and were excluded from the experiment. In total, fish ran between 30 and 34 pre-training trials, with the exception of a single W×R individual who ran 24 trials (R, n=33 ± 0.7; W, n=33 ± 0.2; R×W, n=32.9 ± 0.6; W×R, n=32.7 ± 1.7). A total of 113 females (R, n=27; W, n = 30; R×W, n=27; W×R, n=29) succeeded to dislodge the disc in all trials of the last two days of pre-training and continued the experiment.

2.2.2. Associative learning

In the associative learning task, fish were given a choice between a red and a yellow disc, both concealing a food reward (to ensure fish could not be learning through olfactory cues). Only one of the discs could be dislodged by the fish to reveal a reward (positive stimulus), as the negative stimulus was kept in the hole with a plastic knob and could not be moved to uncover the food item beneath it. For each trial, we randomised the position (left/right) of the correct colour, with the constraint of no more than two consecutive trials in the same position, to avoid side biases. Choice was recorded as the first disc the fish touched. The fish was given 1 min to dislodge the correct coloured disc and eat the reward. For incorrect trials, correction was allowed within 3 min. If the fish failed to correct its choice within 3 min, or when fish failed to make any choice within 1min, we moved the rewarded disc 5 mm to the side to allow easy access to the food. This ensured that all fish experienced the same number of reinforced trials throughout the experiment, regardless of their initial choice. In the first block of testing, fish ran one or two daily sessions of three trials over seven or eight days. In the second block, fish ran one daily session of four trials over five days. The learning criterion consisted of 7 correct choices consecutively (significant according to a binomial probability). As soon as a subject reached the learning criterion, with the constraint that the fish ran a minimum of 12 trials, the next phase commenced. If a subject did not reach the learning criterion within 40 trials, it was excluded from further training (R, n=7; R×W, n=1; W×R, n=1).

2.2.3. Reversal learning

After passing the learning criterion of the associative learning task, the reversal learning task started. The procedure was the same as the during the associative learning phase except the reward contingency was reversed: fish previously trained on yellow were trained on red and vice-versa (Fig. 1). Each subject ran a minimum of 24 training trials and continued the reversal task until it reached the ‘7 out of 7’ learning criterion, up to a maximum of 72 trials.

2.3. Analysis of learning performance

Out of 113 females that completed the cognitive assays, three females (R, n=2; R×W, n=1) were excluded from the analysis due to repeated refusal to dislodge the discs (< 40% recorded choices during training trials). Statistical analyses were performed in R v.3.6.3 (R Core Team 2020) using ‘*lme4’* (Bates et al. 2015). For both associative and reversal learning tasks, we compared the performance of the parental species and F1 hybrids in (i) number of trials to reach learning criterion using a generalised linear model (Poisson distribution) with Crossing Group (R, W, R×W, W×R), Colour (red, yellow), and Testing Block (block 1, block 2) as potential predictor variables; and (ii) learning rate, i.e., probability of success per trial (correct = 1; incorrect = 0) using a generalised linear mixed-effect model (binomial distribution) with Trial Number, Crossing Group, Colour, Testing Block, and the interaction of Trial Number × Crossing Group and Trial Number × Colour as predictor variables, as well as a random intercept and slope for Fish Identity, which accounts for the repeated observations of individual fish. If Crossing Group was a significant predictor in the model, we assessed Tukey corrected multiple comparisons between Crossing levels using the ‘*glht’* function in the *‘multcomp’* package (Hothorn et al. 2008). We tested the significance of the random effects in (ii) models with likelihood ratio tests, by comparing models which culled the intercept or slope term to our final model. To examine if performance in the associative learning task had carryover effects on performance in the reversal task, we tested the inclusion of the predictor ‘Trials to learning criterion in the associative learning task’ and its interaction with Crossing Group in models (i, ii) for the reversal task. For model (ii) in the reversal task, Trial Number was log-transformed to meet the assumption of linearity on the logit-scale.

2.4. Analysis of transgressive trait variation in hybrids

2.4.1. Quantifying phenotypic dispersion

To quantify phenotypic dispersion, we used Kernel Density Estimation (KDE), an established method for probability density estimation that is commonly used in ecology (e.g. home range, morphological, or ecological niche analyses; Mammola and Cardoso 2020; Worton 1989). With this method, trait space can be approximated using the raw distribution of individuals within the multidimensional space, without the need of categorizing data. Furthermore, KDE allows the assumption that there might be empty space within extreme values of traits, contrary to other methods such as convex hull polygons or simply considering the range of values observed in the parentals (Feller et al. 2020; Mammola and Cardoso 2020). From individual scores in the associative and reversal learning tasks (number of trials to reach learning criterion), KDE allowed us to estimate the clusters of ‘cognitive space’ occupied by each group across the two dimensions of learning performance. KDE clusters were computed with the *‘kde’* function in the *ks* package (Duong 2021), with the bandwidth estimated using a grid-search estimation. The number of trials to reach criterion was log-transformed to approximate a continuous variable with Gaussian distribution. Phenotypic dispersion was estimated as the hypervolume of the KDE cluster that contains 95% of the individuals in the group with the ‘*contourSizes’* function in the *ks* package (Mérot et al. 2020).

2.4.2. Identifying transgressive hybrids

We considered hybrid phenotypes to be transgressive if their trait values fell outside the range of both parental species (Dittrich-Reed and Fitzpatrick 2013; Stelkens et al. 2009), i.e., outside the 95% KDE of both parents. For each hybrid group, we quantified the frequency and proportion of transgressive individuals.

2.4.3. Comparing mean hybrid phenotype to simulated hybrid populations

Under an expectation of complete additive inheritance, F1 hybrids should be a linear combination of the parental phenotypes (Thompson et al. 2021). To test whether the mean phenotypes of R×W and W×R hybrids deviate from the expected intermediate trait value, we first simulated hybrid phenotypes as a linear combination of our parental individuals. Simulated hybrids were generated by randomly sampling a pair of individuals from each parental species and calculating the mean trait values of the parental pair. To approximate the sample size of our experimental groups, we used 30 randomly chosen parents from each species to create 30 simulated hybrids and repeated this process 100 times. We then compared the observed trait values of R×W and W×R hybrids to the simulated intermediate hybrid populations with regards to two metrics: 'parental bias', which captures deviation of hybrid phenotypes from the arithmetic mean of the parental phenotypes in the direction of either parent (Mérot et al. 2020; Thompson et al. 2021); and 'phenotypic mismatch', which captures the deviation of the hybrid mean phenotype away from the line connecting parental mean phenotypes (Mérot et al. 2020; Thompson et al. 2021). The two metrics were estimated by considering the geometric position of the mean phenotypes of the two parents and the hybrid. 'Parental bias' is given by the position of the hybrid projection on the line connecting parental mean phenotypes (where a value of zero corresponds to a hybrid phenotype that is exactly intermediate), while 'parental mismatch' corresponds to the Euclidian distance of the average hybrid phenotype to the line drawn between the two parental means (where a minimum value of zero corresponds to a hybrid phenotype that falls on the line connecting the parents).

3. Results

3.1. Do parental species and hybrids differ in learning performance?

Pre-training success was similar across the four groups, with 113 females (R, n=27; W, n = 30; R×W, n=27; W×R, n=29) succeeding in dislodging a green disc and progressing in the experiment. In the associative learning task, common guppies (R) had the lowest success rate, with 20/27 (74%) R females reaching the learning criterion. The two F1 hybrid groups had high success rates similar to Endler's guppies (W): 26/27 (96%) R×W females, 28/29 (97%) W×R females, and 30/30 (100%) W females reached learning criterion. When comparing the number of trials needed to learn the colour association, Endler's guppies succeeded in the task faster than common guppies and their hybrids (Fig. 2a; Tukey post-hoc tests, all $P < 0.001$; Table S1a; Table S2). All four groups improved their success rate as the task progressed, indicating they succeeded in learning the task (Est. = 0.332, SE = 0.050, $z = 6.665$, $P < 0.001$). Endler's guppies and W×R hybrids showed a steeper learning curve compared to the other two groups (Fig. 2c; W: Est. = 0.192, SE = 0.057, $z = 3.348$, $P = 0.001$; W×R: Est. = 0.095, SE = 0.045, $z = 2.091$, $P = 0.037$; Table S1b). The number of trials to learn differed between testing blocks (Est. = 0.118, SE = 0.051, $z = 2.324$, $P = 0.02$; Table S1a) and so did success rate (Est. = -0.192, SE = 0.255, $z = -3.583$, $P < 0.001$; Table S1b). Individual fish differed in their naïve probability of choosing the rewarded colour in the first trial (random intercept for Fish Identity, $\chi^2 = 28.117$, df =2, $P < 0.001$; Table S1b) and in their speed of acquisition over trials (random slope for Fish Identity, $\chi^2 = 32.019$, df =2, $P < 0.001$; Table S1b).

In the reversal learning task, success rates were also very high with 19/19 (100%) R females, 24/26 (92%) R×W females, 28/28 (100%) W×R females, and 30/30 (100%) W females reaching learning criterion. Female Endler's guppies and R×W hybrids needed fewer trials to learn the colour reversal compared to common guppies and W×R hybrids (Fig.2b; Tukey post-hoc tests, all P<0.02; Table S1c; Table S3). All four groups improved their success rate as the task progressed (Est. = 1.138, SE = 0.215, z = 5.284, P < 0.001), with female Endler's guppies showing a steeper learning curve compared to the other groups (Fig.2d; Est. = 0.673, SE = 0.259, z = 2.598, P = 0.009; Table S1d). The effect of the associative learning task in learning the reversal was unclear. On one hand, we found that fish in the W×R group alone showed a positive correlation between trials needed to learn the association and trials needed to learn the reversal task (Fig. 3; Est. = 0.023, SE = 0.007, z = 3.156, P = 0.002; Table S1c). On the other hand, performance in the associative task had a marginal negative effect on success rate in the reversal task, i.e., fish that were faster in learning the initial association tendentially made more mistakes in the reversal (Est. = -0.023, SE = 0.012, z = -1.964, P = 0.05; Table S1d), with no interaction effects between groups (all P > 0.3). The number of trials to learn the reversal task, but not the success rate, differed between testing blocks (trials to learn: Est. = -0.136, SE = 0.043, z = -3.195, P = 0.001; success: Est. = -0.023, SE = 0.012, z = -1.964, P = 0.05; Table S1c,d). Individual females differed in their initial persistence to the previously learnt response (random intercept for Fish Identity, χ^2 = 9.875, df =2, P = 0.007; Table S1d) and in their speed of acquisition over trials (random slope for Fish Identity, χ^2 =42.134, df =2, P < 0.001; Table S1d).

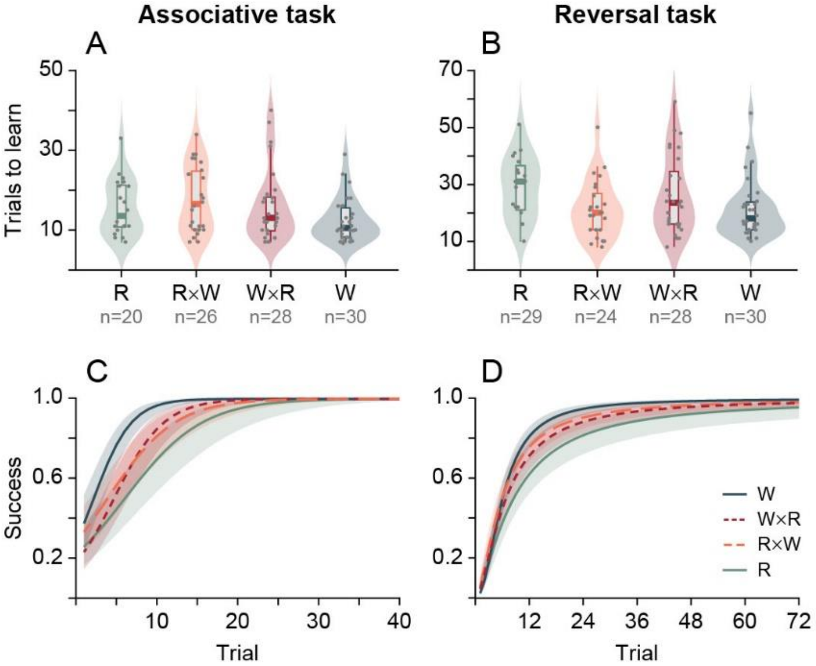

Figure 2. Performance of female fish from the two parental species (R; W) and the two F1 hybrid crossings (R×W; W×R) in the associative learning (left panels) and reversal learning (right panels) tasks. (a, b) Number of trials taken to learn each task. Different letters indicate significant differences

between groups (Tukey post-hoc tests). (c, d) Probability of correct choice over trials (lines show predicted model outputs and shaded areas indicate 95% confidence intervals). Points in (a, b) represent individual fish.

Females in this study showed a naïve preference for the red discs; fish that were assigned red as a positive stimulus during the initial discrimination were faster to reach criterion and had a steeper learning rate (both $P < 0.001$; Table S1a,b). Similarly, fish initially trained to yellow and with red as the rewarded stimulus in the reversal task took less trials to succeed in the reversal and had a steeper learning rate (both $P \leq 0.003$; Table S1c,d).

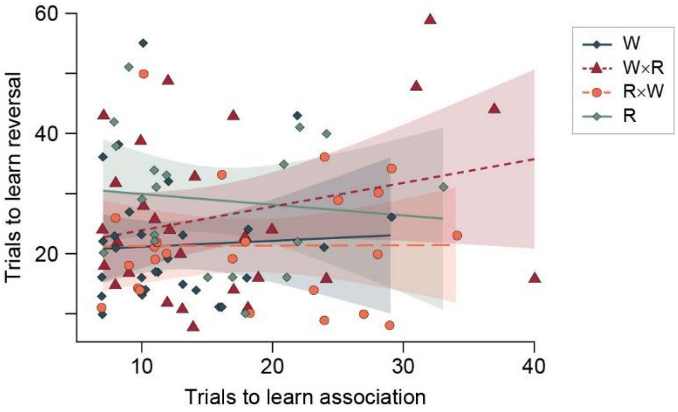

Figure 3. Relation between individual performance in the associative learning and the reversal learning task, showing a positive correlation for fish in the W×R group alone (triangles, short-dash line). Each point represents one fish.

3.2. Are hybrid phenotypes over-dispersed compared to parentals?

The hypervolume of 'cognitive space' describing phenotypic dispersion was similar between the four groups, with W×R hybrids showing a slightly larger dispersion value and common guppies (R) showing the least dispersion (Fig. 4a,b; Table S4). The simulated hybrid populations had consistently lower dispersion, with 95% KDEs having half the hypervolume of our four experimental groups (Fig. 4d; Table S4).

3.3. Are hybrid phenotypes transgressive?

The frequency of hybrid individuals found outside parental ranges, considered as transgressive, was of 17% for R×W and 14% for W×R individuals, corresponding to a total of eight transgressive hybrids (four from each group; Fig. 4a,b; Table S4).

3.4. Is transgression linked to a deviation of the mean hybrid phenotype?

We found that ‘parental bias’ was low since the projection of the mean phenotypes of R×W and W×R was positioned between the two parentals and overlapped with the simulated hybrid populations (Fig. 4c,e; Table S4). However, R×W hybrids showed high phenotypic mismatch with parentals, since the mean phenotype of R×W deviated away from the line connecting parental mean phenotypes (Fig. 4c), with a value higher than any hybrid population simulated as a linear combination of parental phenotypes (Fig. 4f; Table S4). The mismatch of R×W was observed towards poor performance in associative learning but slightly better performance in reversal learning (Fig. 4a,c).

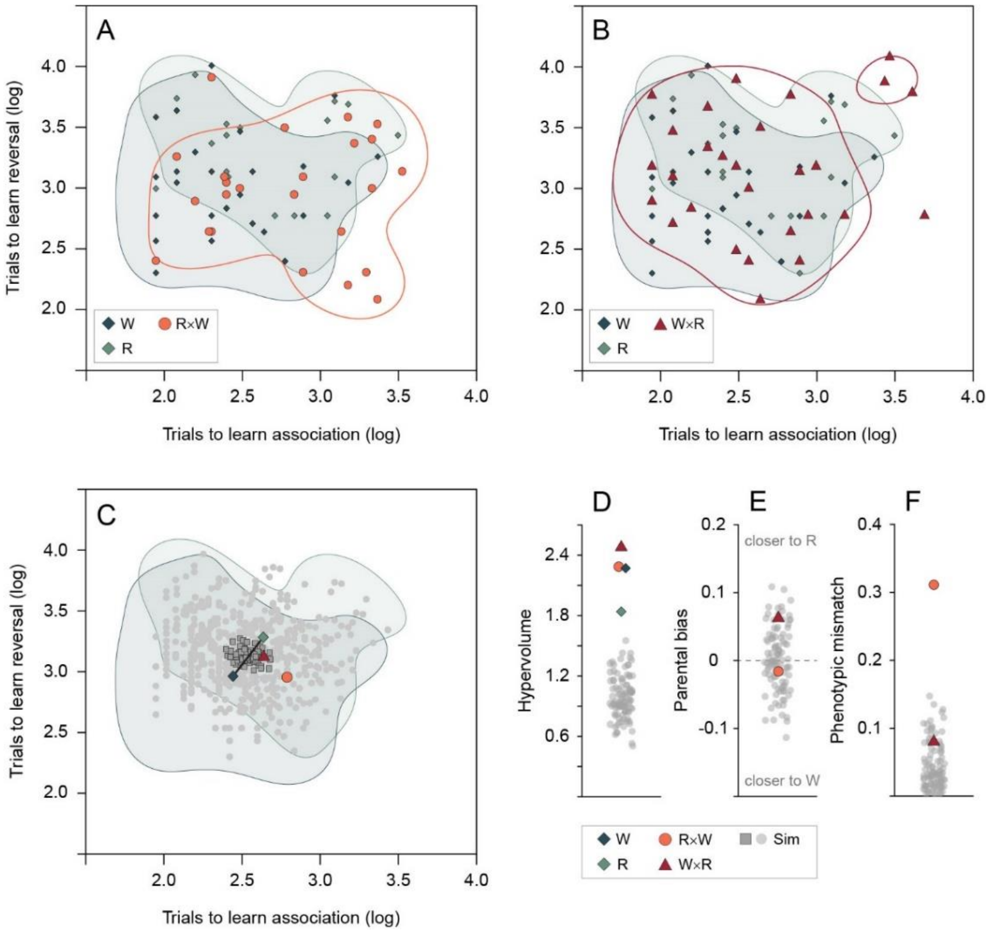

Figure 4. Distribution of individual fish in a two-dimensional ‘cognitive space’; comparison of parentals (R, n=19; W, n = 30; diamonds, shaded 95% KDEs) and **(A)** R×W hybrids (n=24, circles), showing four transgressive R×W fish; and **(B)** W×R hybrids (n=28, triangles), showing four transgressive W×R fish. **(C)** Position of the mean phenotype of the four groups and of simulated hybrid populations (grey), in relation to the parental 95% KDEs. The black line connects parental mean phenotypes. **(D)** Hypervolume of observed and simulated 95% KDEs (phenotypic dispersion). **(E)** Distance of observed and simulated hybrid phenotypes from the midpoint between the parentals (parental bias). **(F)** Deviation of observed and simulated hybrid phenotypes from the line connecting parental mean phenotypes (phenotypic mismatch).

4. Discussion

We experimentally investigated how hybridization impacts cognitive abilities in first-generation reciprocal crosses between two closely related Poeciliid species. We found that both hybrid crosses showed mean learning scores that were intermediate between the parentals. However, hybrid phenotypes had slightly higher dispersion relative to parents, some hybrid individuals were transgressive, i.e., showed trait combinations outside the parental ranges, and the mean phenotype of one hybrid group deviated away from the axis of variation of the parental species.

Interspecific hybridization can be an important source of phenotypic and genetic variation (Dittrich-Reed and Fitzpatrick 2013; Mallet 2007; Seehausen 2004). Such variation may enlarge the working surface for natural selection and promote the evolutionary potential of hybrid populations (Rieseberg et al. 1999; Seehausen 2013). For example, some hybrids of African cichlids or Galapagos finches show greater variation in morphological and/or behavioural phenotypes and express transgressive traits that allow them to explore ecological opportunities unavailable to the parentals (Feller et al. 2020; Lamichhaney et al. 2018; Selz and Seehausen 2019). Here, we found that cognitive phenotypes of F1 hybrids were slightly over-dispersed compared to parentals and that some individuals among the hybrids had transgressive combinations of learning scores. In addition, R×W hybrids showed a significant deviation away from the axis of variation of the parentals. We therefore suggest that hybridization may generate variation and transgression in cognitive abilities. These experimental results, even if just on first-generation hybrids, provide an important first test of whether hybridization can promote cognitive variation, a prerequisite for hybrids to have cognitive innovation potential (Seehausen 2013; Selz and Seehausen 2019).

We found that only R×W hybrids showed a phenotypic mismatch with the parentals, while the reciprocal crossing (W×R hybrids) did not deviate from a linear combination of parental phenotypes. Several studies have documented asymmetries between F1 hybrid lineages in plants and animals, particularly in the degree of viability or sterility (Bolnick et al. 2008; Tiffin et al. 2001; Turelli and Moyle 2007). Hybrid asymmetries typically arise from incompatibilities involving uniparentally inherited genetic factors (from mitochondria, chloroplasts, maternal transcripts, or sex chromosomes) and tend to be more pronounced in the heterogametic sex (Bolnick et al. 2008; Turelli and Moyle 2007). In *Drosophila*, hybrid males (the heterogametic sex) show greater impairment of foraging behaviour (Turissini et al. 2017), and in wild *Poecile* chickadees the females (heterogametic sex in birds) show poorer cognitive skills (McQuillan et al. 2018). The asymmetry we found between R×W and W×R hybrids hints at two general features of the genetic architecture of the traits we measured. First, the R×W mean phenotype significantly deviated from simulated linear combinations of the parental phenotypes, implying that additive inheritance, one of the primary causes of transgression (Mérot et al. 2020; Rieseberg et al. 2003b; Stelkens et al. 2009; Thompson et al. 2021), is unlikely to regulate expression of the traits we measured. Second, the observed asymmetry between reciprocal crossings suggests the contribution of uniparentally inherited genetic factors. However, the genetic mechanism underlying extreme trait expression cannot be conclusively determined from phenotypic distribution alone and so we refrain from speculating further. Despite examining reciprocal hybrids, our study tested only females which are the homogametic sex in *Poecilia* guppies. Future work should compare cognitive performance of male and female reciprocal hybrids to test if greater reciprocal asymmetries are observed in males compared to females. Additionally, it remains unclear what would be the functional consequences of a phenotypic mismatch with the parentals, such as that observed here in R×W hybrids. The phenotype of an individual is often an integrated suite of traits that covary and function together and that can ultimately influence fitness (Sih et al. 2004). Some argue that a phenotypic mismatch might break up suites of integrated traits and thus mismatched hybrids might be poorly suited to the parental environments (Thompson et al. 2021), but further empirical work is required to test this hypothesis.

When comparing the learning performance of hybrids and parentals in associative and reversal learning independently, the mean phenotype of both hybrid groups was intermediate between the parentals. Such intermediate phenotypes at the group level might indicate sub-optimal performance and decreased chances that hybrids escape competition from the parentals (Donovan et al. 2010; McQuillan et al. 2018; Montgomery et al. 2021; Pärssinen et al. 2020; Pereira et al. 2014). However, it is possible that intermediate or poor hybrid phenotypes appear in early generations, but performance is rescued in later generations (Pereira et al. 2014). Further studies should compare the performance of F1 hybrids with that of F2 and later generations, to test how the expression of these traits varies after a few generations of recombination. Additionally, comparing F1 to later hybrid generations would indicate if transgression of F1 hybrids is due to heterosis and therefore transient, or if hybrid phenotypic variation in cognitive abilities may be heritable (Lippman and Zamir 2007; Stelkens et al. 2009). Using experimental hybrids reared in similar conditions in the lab and later tested as adults, as in our study, can help to disentangle genetic effects from selection or plasticity effects, as opposed to using wild hybrids (McQuillan et al. 2018).

The phenotypic variation generated by hybridization can potentially enlarge the working surface for selection. However, the functional relevance and potential benefits of the transgressive trait values detected in this analysis are mostly unknown. Some studies indicate that higher learning scores, including associative and reversal learning, are linked with survival and other fitness benefits (Dayananda and Webb 2017; Dukas and Bernays 2000; Kotrschal et al. 2013; Roth et al. 2010), but a few studies find the opposite pattern. For example, pheasants *Phasianus colchicus* that were slower to reverse a learnt association were more likely to survive when released into the wild (Madden et al. 2018). In addition, cognitive investment may be linked to other costly functions or traits, and therefore better or poorer learning abilities may not linearly map into fitness benefits or costs (Kotrschal et al. 2013; Mery and Kawecki 2003; Rice 2020). A promising direction of research is to assay the same hybrid individuals in a battery of tasks testing different cognitive traits and functional behaviours, integrated with additional fitness-related traits such as viability and survival, growth, and reproduction.

Surprisingly, common guppies– frequently used as a test species in cognitive research (Buechel et al. 2018; Fuss and Witte 2019; Lucon-Xiccato and Bisazza 2014; Vila Pouca et al. 2021) – showed worse performance in both tasks compared to Endler’s guppies and their hybrids. It is possible that differences in the ability to perceive and discriminate yellow from red between the four groups may have contributed to the observed cognitive differences between groups. Perception is an important cognitive process, together with learning and memory (Shettleworth 2010), that is relevant to the learning tasks we used. Colour vision is achieved by comparing signals from cone cells with differences in wavelength sensitivity, which is largely determined by opsin proteins (Kelber et al. 2003). Common and Endler’s guppies both have three cone cells sensitive in the long-wavelength range (i.e., the green, yellow, and red portion of the visible light spectrum); the two species also have similar genetic architecture of long-wavelength sensitive opsin genes (Sandkam et al. 2017), indicating they have similar ‘machinery’ for colour vision in the yellow and red spectrum. However, as the two species may have differential expression of opsin genes, they may show differences in the ability to discriminate yellow and red (Sakai et al. 2016). To disentangle to what extent the cognitive differences we found are due to differences in perception or learning and memory, other colour combinations and tests of behavioural sensitivity to those (Sakai et al. 2016) would be necessary.

The differences in cognitive performance between common and Endler’s guppies could also be linked to species differences in ecological traits. Despite generally having a similar ecology (Alexander and Breden 2004; Poeser et al. 2005), the two species differ in a few traits, including male home range size. While *P. reticulata* males vagrantly cover large areas (Croft et al. 2003), *P. wingei* seem to stay within ‘territories’ of 50-70 cm diameter (M. Kempkens, pers. comm.). As home range size is often positively correlated with cognitive performance (Sherry et al. 1992), learning differences between common and Endler’s guppies may be expected. However, the results we found in this study using females as experimental subjects are in the opposite direction as predicted by home range; common guppies (with a larger home range, at least in males) show poorer learning performance compared to Endler’s guppies. It is unknown, however, if females show similar home range differences and whether adaptive cognitive differences linked to home range are expected in both sexes. Additionally, our fish have been in captive conditions in the laboratory for several generations. They were always kept in several large aquaria to keep as much genetic variation in the populations, yet domestication effects cannot be excluded. While any such captive selection pressures were similar for the two species, care must be taken when interpreting their behaviour in light of natural ecological conditions. Further work using male fish as experimental subjects should prove important in testing if male’s performance parallels that of females, and a comparison of learning performance of wild-caught and laboratory individuals from both species could ultimately indicate if home range differences could contribute to some of the cognitive differences found between them.

Inbreeding depression in our laboratory population of guppies could also be a possible explanation for the poorer performance of the common guppy. However, we think this is unlikely as guppies seem resilient to inbreeding depression (Deacon et al. 2014) and the performance of the animals in this study falls within the learning rates of other studies using similar tasks and training procedures on different lab populations (Buechel et al. 2018; Fuss and Witte 2019; Lucon-Xiccato and Bisazza 2014; Vila Pouca et al. 2021). It is also unlikely that our results were driven by other mechanisms rather than learning, such as differences in foraging motivation or physical constraints, since we initially pre-trained all fish to dislodge a single disc and found no differences between groups in pre-training success. Furthermore, the pre-training task ensured that fish were highly motivated to feed, as they had to voluntarily engage in the task, and that all individuals were physically capable of dislodging a plastic disc to access the reward.

We found some differences in associative and reversal learning between the two testing blocks. These block effects are most likely due to slight protocol differences, mainly the presence of two social companions behind a clear divider at the back of the home compartment during the first block but not during the second block; and the different number of trials run per day in the first and second block. However, as the four experimental groups within each testing block were tested in similar conditions, our results in terms of species differences in learning abilities are unlikely confounded by differences between blocks.

Methods to quantify transgression and phenotypic dispersion vary across studies. Transgression is sometimes measured from individual hybrids in comparison to the range of values occupied by the parentals (Feller et al. 2020; Holzman and Hulsey 2017; Stelkens et al. 2009) or defined from the mean phenotype of the hybrid group (Johansen‐Morris and Latta 2006; Pereira et al. 2014). Our methodology allows for a combination of the two measures, resulting in a more comprehensive test of functional transgression applied to multidimensional cognitive phenotypes. Importantly, this method can be easily extended to other studies in ecology and evolution that aim to compare multivariate phenotypes between species or populations.

In conclusion, the results we present here indicate that hybridization may promote phenotypic variation and transgression in cognitive abilities, even when mean hybrid phenotypes are intermediate between the parentals. These results are an important step towards understanding the potential role of hybridization in promoting cognitive novelty and fuelling cognitive evolution.

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7. References

Alexander, H. J., and F. Breden. 2004. Sexual isolation and extreme morphological divergence in the Cumaná guppy: a possible case of incipient speciation. Journal of Evolutionary Biology 17:1238-1254.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 1.

Bell, M. A., and M. P. Travis. 2005. Hybridization, transgressive segregation, genetic covariation, and adaptive radiation. Trends in ecology & evolution 20:358-361.

Bolnick, D. I., M. Turelli, H. n. López-Fernández, P. C. Wainwright, and T. J. Near. 2008. Accelerated Mitochondrial Evolution and "Darwin's Corollary": Asymmetric Viability of Reciprocal F1 Hybrids in Centrarchid Fishes. Genetics 178:1037-1048.

Bridle, J., C. Saldamando, W. Koning, and R. Butlin. 2006. Assortative preferences and discrimination by females against hybrid male song in the grasshoppers *Chorthippus brunneus* and *Chorthippus jacobsi* (Orthoptera: Acrididae). Journal of Evolutionary Biology 19:1248-1256.

Buechel, S. D., A. Boussard, A. Kotrschal, W. van der Bijl, and N. Kolm. 2018. Brain size affects performance in a reversal-learning test. Proceedings of the Royal Society B: Biological Sciences 285:20172031.

Cauchoix, M., P. Chow, J. Van Horik, C. Atance, E. Barbeau, G. Barragan-Jason, P. Bize et al. 2018. The repeatability of cognitive performance: a meta-analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 373:20170281.

Chaves, L. M., and W. Hodos. 1997. Hyperstriatum ventrale in pigeons: Effects of lesions on color-discrimination and color-reversal learning. Visual Neuroscience 14:1029-1041.

Cooper, B. S., A. Sedghifar, W. T. Nash, A. A. Comeault, and D. R. Matute. 2018. A maladaptive combination of traits contributes to the maintenance of a *Drosophila* hybrid zone. Current Biology 28:2940-2947.e2946.

Coughlan, J. M., and D. R. Matute. 2020. The importance of intrinsic postzygotic barriers throughout the speciation process. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 375:20190533.

Croft, D. P., B. Albanese, B. J. Arrowsmith, M. Botham, M. Webster, and J. Krause. 2003. Sex-biased movement in the guppy (*Poecilia reticulata*). Oecologia 137:62-68.

Dayananda, B., and J. K. Webb. 2017. Incubation under climate warming affects learning ability and survival in hatchling lizards. Biology letters 13:20170002.

Deacon, A. E., M. Barbosa, and A. E. Magurran. 2014. Forced monogamy in a multiply mating species does not impede colonisation success. BMC Ecology 14:18.

Dittrich-Reed, D. R., and B. M. Fitzpatrick. 2013. Transgressive hybrids as hopeful monsters. Evolutionary biology 40:310-315.

Donovan, L., D. Rosenthal, M. Sanchez ‐ Velenosi, L. Rieseberg, and F. Ludwig. 2010. Are hybrid species more fit than ancestral parent species in the current hybrid species habitats? Journal of Evolutionary Biology 23:805-816.

Dukas, R., and E. A. Bernays. 2000. Learning improves growth rate in grasshoppers. Proceedings of the National Academy of Sciences 97:2637-2640.

Duong, T. 2021.ks: Kernel Smoothing, version R package version 1.13.2.

Ellison, C. K., and R. S. Burton. 2008. Interpopulation hybrid breakdown maps to the mitochondrial genome. Evolution 62:631-638.

Feller, A. F., O. M. Selz, M. D. McGee, J. I. Meier, S. Mwaiko, and O. Seehausen. 2020. Rapid generation of ecologically relevant behavioral novelty in experimental cichlid hybrids. Ecology and evolution 10:7445-7462.

Fuss, T., and K. Witte. 2019. Sex differences in color discrimination and serial reversal learning in mollies and guppies. Current Zoology 65:323-332.

Holzman, R., and C. D. Hulsey. 2017. Mechanical transgressive segregation and the rapid origin of trophic novelty. Scientific Reports 7:40306.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal: Journal of Mathematical Methods in Biosciences 50:346-363.

Johansen ‐ Morris, A., and R. G. Latta. 2006. Fitness consequences of hybridization between ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor, and transgressive segregation. Evolution 60:1585-1595.

Kelber, A., M. Vorobyev, and D. Osorio. 2003. Animal colour vision–behavioural tests and physiological concepts. Biological Reviews 78:81-118.

Kotrschal, A., B. Rogell, A. Bundsen, B. Svensson, S. Zajitschek, I. Brännström, S. Immler et al. 2013. Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. Current Biology 23:168-171.

Lamichhaney, S., F. Han, M. T. Webster, L. Andersson, B. R. Grant, and P. R. Grant. 2018. Rapid hybrid speciation in Darwin’s finches. Science 359:224-228.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a source of variation for adaptation to new environments. Evolution 20:315-336.

Linn, C. E., H. R. Dambroski, J. L. Feder, S. H. Berlocher, S. Nojima, and W. L. Roelofs. 2004. Postzygotic isolating factor in sympatric speciation in Rhagoletis flies: reduced response of hybrids to parental host-fruit odors. Proceedings of the National Academy of Sciences 101:17753-17758.

Lippman, Z. B., and D. Zamir. 2007. Heterosis: revisiting the magic. Trends in Genetics 23:60-66.

López, J. C., C. Broglio, F. Rodríguez, C. Thinus-Blanc, and C. Salas. 2000. Reversal learning deficit in a spatial task but not in a cued one after telencephalic ablation in goldfish. Behavioural brain research 109:91-98.

Lucon-Xiccato, T., and A. Bisazza. 2014. Discrimination reversal learning reveals greater female behavioural flexibility in guppies. Biology Letters 10:20140206.

Madden, J. R., E. J. Langley, M. A. Whiteside, C. E. Beardsworth, and J. O. van Horik. 2018. The quick are the dead: pheasants that are slow to reverse a learned association survive for longer in the wild. Philosophical Transactions of the Royal Society B: Biological Sciences 373:20170297.

Mallet, J. 2007. Hybrid speciation. Nature 446:279-283.

Mammola, S., and P. Cardoso. 2020. Functional diversity metrics using kernel density n-dimensional hypervolumes. Methods in Ecology and Evolution 11:986-995.

McQuillan, M. A., T. C. Roth II, A. V. Huynh, and A. M. Rice. 2018. Hybrid chickadees are deficient in learning and memory. Evolution 72:1155-1164.

Meredith, R. W., M. N. Pires, D. N. Reznick, and M. S. Springer. 2010. Molecular phylogenetic relationships and the evolution of the placenta in *Poecilia* (*Micropoecilia*)(Poeciliidae: Cyprinodontiformes). Molecular Phylogenetics and Evolution 55:631-639.

Mérot, C., V. Debat, Y. Le Poul, R. M. Merrill, R. E. Naisbit, A. Tholance, C. D. Jiggins et al. 2020. Hybridization and transgressive exploration of colour pattern and wing morphology in *Heliconius* butterflies. Journal of evolutionary biology 33:942-956.

Mery, F., and T. J. Kawecki. 2003. A fitness cost of learning ability in *Drosophila melanogaster*. Proceedings of the Royal Society of London. Series B: Biological Sciences 270:2465-2469.

Montgomery, S. H., M. Rossi, W. O. McMillan, and R. M. Merrill. 2021. Neural divergence and hybrid disruption between ecologically isolated *Heliconius* butterflies. Proceedings of the National Academy of Sciences 118.

Osthaus, B., L. Proops, I. Hocking, and F. Burden. 2013. Spatial cognition and perseveration by horses, donkeys and mules in a simple A-not-B detour task. Animal cognition 16:301-305.

Pärssinen, V., K. Hulthén, C. Brönmark, C. Skov, J. Brodersen, H. Baktoft, B. B. Chapman et al. 2020. Maladaptive migration behaviour in hybrids links to predator‐mediated ecological selection. Journal of Animal Ecology 89:2596-2604.

Pereira, R. J., F. S. Barreto, and R. S. Burton. 2014. Ecological novelty by hybridization: experimental evidence for increased thermal tolerance by transgressive segregation in Tigriopus californicus. Evolution 68:204-215.

Poeser, F. N., M. Kempkes, and I. J. Isbrücker. 2005. Description of *Poecilia* (*Acanthophacelus*) *wingei* n. sp. from the Paría Peninsula, Venezuela, including notes on *Acanthophacelus* Eigenmann, 1907 and other subgenera of *Poecilia* Bloch and Schneider, 1801 (Teleostei, Cyprinodontiformes, Poeciliidae). Contributions to Zoology 74:97-115.

Proops, L., F. Burden, and B. Osthaus. 2009. Mule cognition: a case of hybrid vigour? Animal cognition 12:75-84.

R Core Team. 2020.R: A language and environment for statistical computing.R Foundation for Statistical Computing, Vienna, Austria.

Ramsay, C. 2014. Mate Choice and Hybridization: Comparing the Endler's Guppy (*Poecilia wingei*) and the Common Guppy (*Poecilia reticulata*), University of Colorado Boulder, USA.

Rice, A. M. 2020. The Overlooked Influence of Hybridization on Cognition. Frontiers in Ecology and Evolution 8:39.

Rice, A. M., and M. A. McQuillan. 2018. Maladaptive learning and memory in hybrids as a reproductive isolating barrier. Proceedings of the Royal Society B: Biological Sciences 285:20180542.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy et al. 2003a. Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211-1216.

Rieseberg, L. H., A. Widmer, A. M. Arntz, and B. Burke. 2003b. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 358:1141-1147.

Roth, T. C., L. D. LaDage, and V. V. Pravosudov. 2010. Learning capabilities enhanced in harsh environments: a common garden approach. Proceedings of the Royal Society B: Biological Sciences 277:3187-3193.

Sakai, Y., H. Ohtsuki, S. Kasagi, S. Kawamura, and M. Kawata. 2016. Effects of light environment during growth on the expression of cone opsin genes and behavioral spectral sensitivities in guppies *(Poecilia reticulata)*. BMC Evolutionary Biology 16:1-10.

Sandkam, B. A., J. B. Joy, C. T. Watson, and F. Breden. 2017. Genomic environment impacts color vision evolution in a family with visually based sexual selection. Genome biology and evolution 9:3100-3107.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in ecology & evolution 19:198-207.

—. 2013. Conditions when hybridization might predispose populations for adaptive radiation. Journal of Evolutionary Biology 26:279-281.

Selz, O. M., and O. Seehausen. 2019. Interspecific hybridization can generate functional novelty in cichlid fish. Proceedings of the Royal Society B: Biological Sciences 286:20191621.

Sherry, D. F., L. F. Jacobs, and S. J. Gaulin. 1992. Spatial memory and adaptive specialization of the hippocampus. Trends in neurosciences 15:298-303.

Shettleworth, S. J. 2010, Cognition, evolution, and behavior. New York, NY, Oxford University Press.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in ecology & evolution 19:372-378.

Stelkens, R. B., C. Schmid, and O. Seehausen. 2015. Hybrid breakdown in cichlid fish. PLOS One 10:e0127207.

Stelkens, R. B., C. Schmid, O. Selz, and O. Seehausen. 2009. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. BMC evolutionary biology 9:283.

Thompson, K. A., M. Urquhart-Cronish, K. D. Whitney, L. H. Rieseberg, and D. Schluter. 2021. Patterns, predictors, and consequences of dominance in hybrids. The American Naturalist 197:E72-E88.

Tiffin, P., S. Olson, and L. C. Moyle. 2001. Asymmetrical crossing barriers in angiosperms. Proceedings of the Royal Society of London. Series B: Biological Sciences 268:861-867.

Turelli, M., and L. C. Moyle. 2007. Asymmetric Postmating Isolation: Darwin's Corollary to Haldane's Rule. Genetics 176:1059-1088.

Turissini, D. A., A. A. Comeault, G. Liu, Y. C. G. Lee, and D. R. Matute. 2017. The ability of *Drosophila* hybrids to locate food declines with parental divergence. Evolution 71:960-973.

Vila Pouca, C., D. J. Mitchell, J. Lefèvre, R. Vega-Trejo, and A. Kotrschal. 2021. Early predation risk shapes adult learning and cognitive flexibility. Oikos.

Võikar, V., S. Kõks, E. Vasar, and H. Rauvala. 2001. Strain and gender differences in the behavior of mouse lines commonly used in transgenic studies. Physiology & behavior 72:271-281.

Walter, G. M., T. J. Richards, M. J. Wilkinson, M. W. Blows, J. D. Aguirre, and D. Ortiz-Barrientos. 2020. Loss of ecologically important genetic variation in late generation hybrids reveals links between adaptation and speciation. Evolution Letters 4:302-316.

Worton, B. J. 1989. Kernel methods for estimating the utilization distribution in home ‐ range studies. Ecology 70:164-168.