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Supplemental Information

Artificial Selection on Relative

Brain Size in the Guppy Reveals Costs

and Benefits of Evolving a Larger Brain

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Supplemental Inventory

Figure S1: This figure accompanies *Figure 1*. It depicts the selection regime and the raw data of brain size plotted against body size for two generations for the two sexes separately.

Figure S2: This Figure accompanies *Figure 2* and shows the testing apparatus in which guppies were conditioned to learn to discriminate four from two symbols.

Table S1: This table gives the parameters of models used to analyze the impact of various factors on brain size in adult and juvenile guppies selected for large and small relative brain size.

Table S2: This table gives the parameters of models used to analyze the impact of various factors on body size in adult and juvenile guppies.

Table S3: This table gives the parameters of models used to analyze the impact of various factors on gut size in adult guppies.

Table S4: This table gives the parameters of models used to analyze the impact of various factors on aspects of fecundity in female guppies.

Table S5: This table gives the specifics of the model selection process for all models used in this study (apart from the analysis of numerical learning ability).

Supplemental Experimental Procedures and Results

Here we describe in greater detail how:

- we selected for large and small relative brain size
- we quantified body and brain size of juveniles
- we conducted the test of numerical ability
- we tested for a potential sensory bias
- we quantified gut size
- we analyzed our data
- we calculated realized heritabilities of brain size.

We furthermore provide additional results for the numerical learning test and its control.

Supplemental References

Additional references for Supplemental Information.

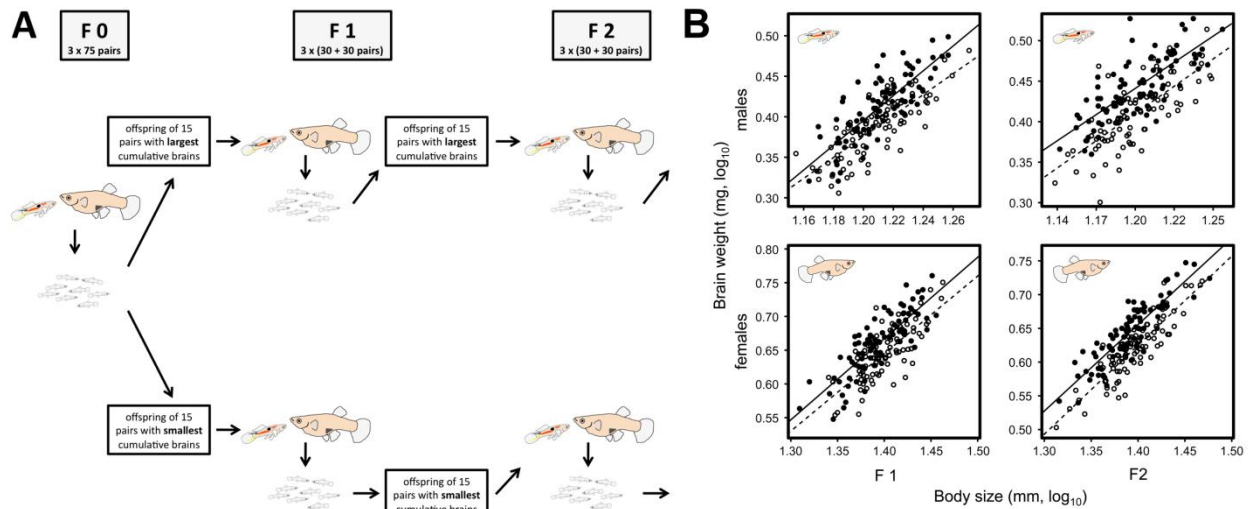


Figure S1, Related to Figure 1.

(A) Artificial selection procedure for large and small relative brain size in guppies. Depicted is the experimental procedure for each of the three replicates. For generation F0, we used guppies (*Poecilia reticulata*) of a large, outbred stock population to set up three replicate populations of 75 breeding pairs. Since it is impossible to determine brain size in live fish, we sacrificed the parents after offspring production. Brains of those parents were removed and weighed. From the 75 pairs of every population we kept the offspring of the 15 pairs with the relatively largest and smallest brains (controlled for body size) to start (F0) the “up” and “down” selected lines in the three replicates. Within each of these six lines we used two males and females of every family to form 30 breeding pairs for the next generation. We randomly assigned partners, but avoided full-sib pairs. For the F2 generation we followed the same procedure.

(B) Relative brain size responded rapidly to divergent selection in guppies. Depicted is the relationship between body size and brain size, where F1 and F2 are the first and second brain size-selected generations, respectively. Top row: males, bottom row: females. Filled circles denote the lines selected for large brain size, empty circles the selection lines for small brains. Replicates are shown together here, but were controlled for in the analysis.

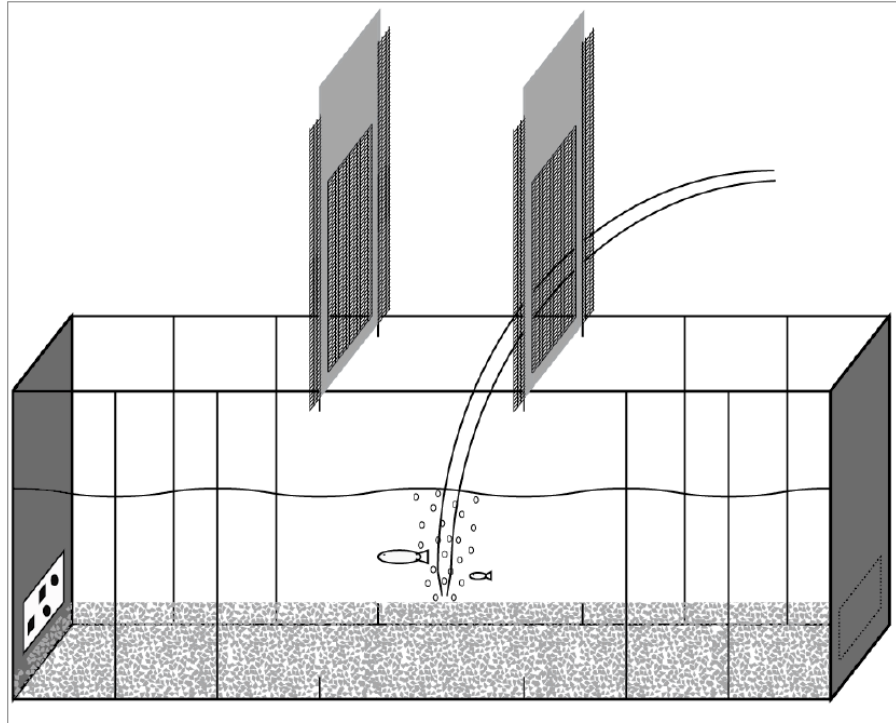


Figure S2, Related to Figure 2.

Test of cognitive performance in guppies (*Poecilia reticulata*) selected for large and small relative brain size. We trained 48 adults to associate a specific number of symbols (two or four) with food. Training occurred twice per day. To test whether they learned to discriminate two symbols from four we presented the stimulus card pairs, without food reward, on opposite sides of their individual holding tanks. Then we lifted see-through dividers, which confined the fish to the center of the tank, and assessed to which side the fish swam. Tests of cognitive ability were performed after each three-day period of training resulting in a total of 48 trainings and eight cognitive tests.

Table S1. The Effect of Body Size, Sex, and Brain Size Selection on Brain Size in Juvenile and Adult Guppies

Adults Generation F1				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	-2.33	-2.74	-1.94	<0.001
Log size	1.18	1.05	1.3	<0.001
Sex	-0.8	-1.41	-0.22	0.009
Selection	0.052	0.04	0.063	<0.001
Log size * Sex	0.26	0.065	0.46	0.013
Random terms	Variance	Lower CI	Upper CI	P
Residual	0.0027	0.0023	0.0031	NA
Replicate line	0.004	2.45×10^{-10}	0.0082	NA
Replicate line * Sex	0.00022	1.2×10^{-11}	0.001	NA
Adults Generation F2				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	-2.81	-2.9	-2.72	<0.001
Log size	1.32	1.29	1.35	<0.001
Selection	0.071	0.06	0.082	<0.001
Random terms	Variance	Lower CI	Upper CI	P
Female Residual	0.0022	0.0018	0.0027	NA
Male Residual	0.0036	0.0029	0.0044	NA
Replicate line	0.0001126	6.08×10^{-15}	0.0002024	NA
Replicate line * Sex	3.56×10^{-5}	1.95×10^{-12}	0.00017	NA
Juveniles Generation F4				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	-0.32	-0.70	0.06	0.10
Log size	0.27	0.08	0.46	0.005
Selection	0.041	0.019	0.061	<0.001
Random terms	Variance	Lower CI	Upper CI	P
Residual	0.0017	0.0011	0.0024	NA
Replicate line	0.00056	1.37×10^{-12}	0.00082	NA

Adults: For each generation, the table reports the result of a GLMM calculated in the R package MCMCglmm. The “Intercept” term is set to the intercept of the regression of log body size on log brain size of females in the brain selection regime for smaller brains. The other estimates represent differences between the intercept (of small brained females) and the specified experimental unit (according to the default contrast matrix in R, “contrast treatments”). Thus, “Selection” is the difference in relative brain size between females in selection for smaller (“down”) and larger (“up”) brains, “Sex” is the difference between females selected for smaller brains and males selected for smaller brains, “Log Size” is the effect of log body size on log brain size of individuals selected for smaller brains, the interaction “Log Size * Sex” indicates the differences in the slopes of the regression of log body size on log brain size between the sexes. “Estimate” is the parameter estimate; “Lower CI” and “Upper CI” denote its associated 95 % credibility intervals. “P” is the probability of falsely rejecting the null hypothesis that the parameter equals 0. For the random effects, P-values are not available, but the variance

component explained by each random effect is accompanied by upper and lower CIs. “Female Residual” is the residual variance between female individuals in brain size, “Male residual” is the male residual variance in brain size, “Replicate line” is the variance explained by replicate line, “Replicate line * Sex” is the variance explained by the interaction between sex and replicate line. **Juveniles:** The effect of log offspring body size and brain size selection on log offspring brain size (optic tectum width) in F4 juveniles. The terms are set analogous to the adult model.

Table S2. Body Size in Juvenile and Adult Guppies Selected for Large and Small Relative Brain Size

Adults				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	24.84	24.30	25.36	<0.001
Selection	-0.06	-0.26	0.16	0.571
Sex	-8.89	-9.48	-8.35	<0.001
Random terms	Variance	Lower CI	Upper CI	P
Female Residual	140.77	129.98	149.83	NA
Male Residual	0.65	0.52	0.80	NA
Replicate line	0.19	7.9×10^{-16}	0.32	NA
Sex * Replicate line	0.09	4.72×10^{-11}	0.34	NA
Juveniles				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	7.46	7.31	7.62	<0.001
Selection	0.057	-0.17	0.27	0.602
Mother size	0.32	0.11	0.54	0.004
# Siblings	0.0038	-0.15	0.17	0.980
Selection * Mother size	-0.32	-0.59	-0.068	0.019
Selection * # Siblings	-0.018	-0.25	0.22	0.875
Mother size * # Siblings	-0.18	-0.39	0.063	0.123
Selection * Mother size * # Siblings	0.26	0.011	0.5	0.037
Random terms	Variance	Lower CI	Upper CI	P
Residual	0.18	0.12	0.25	NA

Adults: The effect of sex and brain size selection on adult body size. The terms are set analogous to the juvenile model. **Juveniles:** The effect of maternal size, number of siblings and brain size selection on juvenile body size. The terms are set analogous to Table S1, but the “Intercept” term here is interpreted as the mean body size of juveniles selected for smaller brains (since all covariates are standardized to a mean of 0). “Mother size” is the effect of maternal size on juvenile body size, “# Siblings” is the effect the number of siblings has on juvenile body size and the interaction “Selection * Mother size” indicates that the slopes of the regression of maternal size on juvenile body size depends on the selection treatment. This suggests that the positive effect of maternal size on offspring size was stronger in the “down” selected guppies. The interaction “Selection * # Siblings” shows that the slopes of the regression of number of siblings on juvenile body size also depends on selection treatment. The interaction “Mother size * # Siblings” denotes an interaction among the two covariates and the three way interaction “Selection * Mother size * # Siblings” indicates that this interaction is different in the two brain selection regimes. This interaction indicates that the negative relationship between maternal size and number of offspring is weaker in the “up” selected guppies. Parts of these relationships may relate to the fact that large-brained guppies had a lower reproductive investment than small-brained individuals.

Table S3. The Effect of Body Size, Sex, and Brain Size Selection on Gut Size

Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	23.30	22.19	24.46	<0.001
Standardized size	4.96	3.94	6.06	<0.001
Sex	-18.34	-19.43	-17.22	0.009
Selection	-0.81	-1.14	-0.49	<0.001
Standardized size * Sex	-4.37	-5.46	-3.31	<0.001
Random terms	Variance	Lower CI	Upper CI	P
Female Residual	31.36	23.06	40.11	NA
Male Residual	1.14	0.91	1.40	NA
Replicate line	0.11	2.86×10^{-12}	0.28	NA
Sex * Replicate line	0.05	1.0×10^{-11}	0.18	NA

Since size and sex are highly correlated in guppies, we standardized size to a mean of 0 and a standard deviation of 1 within each sex in order to avoid problems associated with collinearity. Apart from the different response variable (here gut mass instead of brain mass), the terms are set analogous to Table S1.

Table S4. Reproductive Traits

<i>Offspring number</i>				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	1.93	1.39	2.43	0.009
Age at offspring	0.3	0.23	0.39	<0.001
Selection	-0.19	-0.33	-0.046	0.0105
Random terms	Variance	Lower CI	Upper CI	P
Residual	0.04	0.00027	0.086	NA
Replicate line	0.89	2.7×10^{-8}	0.92	NA
<i>Age at first reproduction</i>				
Fixed terms	Estimate (β)	Lower CI	Upper CI	P
Intercept	140.77	129.98	149.83	<0.001
Selection	0.71	-3.94	5.28	0.757
Random terms	Variance	Lower CI	Upper CI	P
Residual	197.3	151.7	241.7	NA

Offspring number: The effect of age at first parturition and brain size selection on number of offspring. The terms are set analogous to Table S1. **Age at first reproduction:** The effect of brain size selection on the age of first reproduction. The terms are set analogous to Table S1.

Table S5. Model Simplification Procedure

	DIC
Adult brain size – F1	
Random effects	
Null	-998.4
+ Replicate line	-1041.0 *
+ Sex : Replicate line	-1040.4
+ Sex : Residual	-1038.6
Fixed effects	
Full	-1041.0
- Log size * Sex * Selection	-1042.8
- Sex * Selection	-1043.5
- Log size * Selection	-1045.3 *
- Log size * Sex	-1040.3
Adult brain size – F2	
Random effects	
Null	-1029.5
+ Replicate line	-1029.1
+ Sex : Replicate line	-1029.0
+ Sex : Residual	-1036.4 *
Fixed effects	
Full	-1036.4
- Log size * Sex * Selection	-1038.5
- Sex * Selection	-1040.5
- Log size * Selection	-1042.4
- Log size * Sex	-1044.2
- Sex	-1046.1 *
- Selection	-916.3
Juvenile brain size	
Random effects	
Null	-207.8
+ Replicate line	-207.56 **
Fixed effects	
Full	-207.56
- Log size * Selection	-209.2*
- Log size	-203.4
Juvenile body size	
Random effects	
Null	77.0 *
+ Replicate line	77.4
Fixed effects	
- Full	77.0 *
- Selection * Maternal size * # siblings	80.1
Adult body size	
Random effects	
Null	1212.4

+ Replicate line	1212.4
+ Sex : Replicate line	1212.3
+ Sex : Residual	1115.7 *
+ Sex residual – Sex : Replicate line	1118.6
Fixed effects	
Full	1115.7
- Sex * Selection	1114.6 **
- Selection	1112. *
<hr/>	
Gut mass	
<hr/>	
Random effects	
Null	1460.3
+ Replicate line	1460.6
+ Sex : Replicate line	1461.0
+ Sex : Residual	1159.6 *
Fixed effects	
Full	1159.6
- Log size * Sex * Selection	1157.7
- Sex * Selection	1155.7
- Log size * Selection	1153.6 *
- Selection	1175.0
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Number of offspring	
<hr/>	
Random effects	
Null	737.5
+ Replicate line	734.5 *
Fixed effects	
Full	734.5
- Selection * Age at first reproduction	733.3 *
- Selection	737.6
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Age at first reproduction	
<hr/>	
Random effects	
Null	1204.5
+ Replicate line	1198.5 *
Fixed effects	
Full	1198.5 *
- Selection	1196.6 **
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The models used to analyze body size, offspring body size, brain size, age at first offspring and fecundity were built based on the best (lowest) Deviance Information Criterion (DIC), starting with 1) a model with a full fixed effect formula (all fixed main effects and their interactions), to which the random effects were added sequentially according to the simplification tables. When the random effect formula that had the lowest DIC value had been chosen, we continued with simplifying the fixed effect formula. The fixed effects were removed sequentially starting with the highest order interactions and then continuing until the model only contained statistically significant parameters. The removal procedure of fixed effects that did not improve the model was based on obtaining the lowest DIC value. Thus, in the tables below, the random effect formula “Null” denotes that no random effects are added to the model and a “+” denotes the inclusion of a random effect. For the fixed effect formula, “Full” denotes a full fixed effect

formula including all higher order interactions, while a “-“ denotes the removal of a fixed effect. The model with the lowest DIC value (denoted with a “**”) is presented in the supplementary tables (S1-S4). However, sometimes the purpose of a specific model was to demonstrate non-existent influence of a specific variable (for example, the effect of brain selection on body size). In this case we instead present statistics from a model that have a higher DIC value (i.e. since selection had no effect on body size and would thus have been excluded from the analysis under a strict simplification scheme) these models are denoted with “***”.

Supplemental Experimental Procedures and Results

Artificial Selection on Relative Brain Size

We used laboratory-descendants of wild guppies (*Poecilia reticulata*), whose founders (> 500 individuals) were imported from Trinidad in 1998 and since then kept in large populations (> 500 individuals at any time) where they were allowed to reproduce freely. From these, four stock populations were formed using 100 l aquaria each stocked with 100 individuals of equal sex ratio. The parental population (F0) was formed as follows: we scanned the population tanks daily and removed newborn offspring. These juveniles were then reared in groups of three to five individuals in 4 l tanks with a 2 cm layer of gravel and constant aeration, on a 12:12 l:d lighting schedule. Temperature was held at 26-27°C and fish were fed flake food and live brine shrimp six days per week. Java moss (*Taxiphyllum sp.*) provided spatial structuring and hiding opportunities while 5-10 snails (*Planorbis sp.*) removed food remains. We separated males from females at the first signs of maturation (gonopodium growth in males) and reared them separately until maturity (when males showed fully developed color pattern). Since it is not possible to determine brain weight in live fish to select breeder individuals, we used a selection design in which parents were paired at random and sacrificed for brain size quantification *after* offspring production. The offspring of parents with the largest and smallest brains were then used to form breeder pairs for the next generation (see below). At 82.4 ± 0.3 days of age (mean \pm SE) we used 450 of the F0 fish to set up three experimental replicate populations of 75 breeding pairs each (225 breeding pairs in total), in similar tanks as described above. Guppies are live-bearing and we divided the breeding tanks with a net divider (3 mm mesh size) to create a zone for new-born fish. We checked for offspring daily and moved juveniles to separate tanks in groups of up to six individuals. After offspring production (Mean age: 126 ± 0.8 days) we euthanized the parents with an overdose of benzocaine and measured their standard length (from the tip of the snout to the end of the caudal peduncle) to the nearest 0.01 mm using digital calipers and placed them in 5 % buffered formalin. Brains were removed under a stereomicroscope and weighed to the nearest 0.001 mg. To select the offspring that would form breeder pairs for the subsequent generation (F1) we extracted the residuals from sex-specific regressions of (log-transformed) brain weight on (log transformed) body size, and standardized these, within sexes, to a mean of zero and a standard deviation of unity. We then added the standardized residuals of the male and female in each breeding pair and ranked them according to their sum. Each replicate population and selection regime combination was handled independently. From the 75 pairs of every replicate population we kept the offspring of the 15 highest- and lowest-ranking pairs to start the “up” and “down” selected lines respectively (see Figure S1 for a scheme of the selection procedure). We thus attained six replicate populations of juveniles (i.e. three replicates of up- and down-selected lines respectively), which we reared in sibling groups consisting of up to 6 offspring. We separated the sexes at first signs of maturation in males and kept them separated till all fish reached maturity. Within each of the six lines we used two males and two females each of the selected 15 families to form 30 breeding pairs for the next generation (180 pairs in total; we randomly assigned partners, but avoided full-sib pairs; F1 age at pairing 80.3 ± 0.6 days; age at sacrifice: 136 ± 0.8 days). For the next generation (F2) we followed the same procedure as described above, again using offspring of the top and bottom 15 breeding pairs for the “up” and “down” selected lines respectively (F2 age at pairing

102.9 ± 0.6 days, age at sacrifice: 161.7 ± 1 days). Since brain size is a plastic morphological trait [1], we did all comparisons across the selection regimes within generations, where the three replicate populations experienced identical conditions.

Quantification of Neonate Body and Brain Size

To determine offspring body and brain size at birth we used one offspring each of 67 F3 clutches from all lines. We placed the newborn fish in small Petri dishes and took dorsal pictures through a dissecting microscope using a digital camera (QImaging, Go-3). We used ImageJ (1.43u NIH) to determine standard length (from the tip of the snout to the end of the caudal peduncle) and width of the optic tectum which is the largest separate brain structure and also clearly visible through the semi-transparent skull-plate of newborn fish.

The Numerical Learning Test

To investigate cognitive ability in the different lines, we first trained fish to associate a visual numerical cue with a food reward and then we determined the number of correct decisions made when individuals were presented with the visual cue but without the reward. The experimental fish were 48 mature offspring of F2 (mean age 150 ± 7 days) balanced from all replicates from large- and small-brained lines and of both sexes. Each experimental fish was individually kept in 15 x 40 x 15 cm Plexiglas tanks with a 2 cm layer of coarse sand and constant aeration. To minimize isolation stress, a smaller non-mature “friend”, who was changed three times during the experiment, accompanied each fish. We blocked visual contact between tanks with cardboard dividers. For the association learning phase, we presented stimuli randomly on opposite sides of the holding tanks. Stimuli consisted of white 6 x 3 cm cardboard cards with two or four black objects. To avoid potential shape-bias, half of the objects were circles, the other half squares. Since cumulative surface area is important for quantity discrimination in fish [2], the cumulative surface area on both type of cards was held constant at 1 cm² (4 objects: 0.25 cm² each, 2 objects: 0.5 cm² each). The separate objects on the stimuli cards were randomly placed in eight different positions to exclude a potential location bias. Forty-eight stimulus card pairs were randomly chosen for every trial. Two see-through dividers were used to confine the fish and the friend in the center, prior to each training (Figure S2). During a training session, we placed the respective stimulus card on each side of the tank and placed flake-food on the tank bottom on the side with four objects. To avoid potential side-biases, the stimulus cards with different number of objects were randomly placed on either the left or right side for each training session. Fish were kept in the center for 5 minutes, thereafter the dividers were lifted and the fish were allowed to feed in front of the four objects for 90 minutes. All food remains and the stimulus cards were removed after each training session. Fish were trained twice per day on three consecutive days prior to each numerical learning ability trial. The observer was unaware of the identity of the experimental fish both during the training phase and during the numerical learning trial.

To test for numerical learning we performed tests every fourth day as described above and placed the stimuli on both sides but this time without adding food. To ensure that fish were choosing according to object number, the trial objects were all of the same size (0.375 cm²). To exclude the possibility of pattern preference they consisted randomly of either squares or circles, but were not mixed within trials. The stimuli used during the test phase was slightly

different than during training to ensure stimuli number was the only aspect that could be remembered by focal individuals between training and trial. The test procedure followed those during the training phase. When entering the correct side the fish was rewarded with food on the correct side. When swimming to the wrong side of the tank no food was given. When the fish did not enter either choice area within the first 5 minutes or showed signs of severe stress when the dividers were lifted (characterized by a “dart-and-freeze” behavior, where the fish shoots to any position in the tank and stays there motionless), we added food and the trial was scored as “no choice”. On the fifth day we started a new round of training as described above. We repeated this training/trial routine eight times so that every fish was trained and trialed 48 and 8 times, respectively.

Control for Preexisting Bias for Specific Number of Objects.

Since we trained all focal individuals to associate the higher number of objects (i.e. four objects) with food, we used 48 additional individuals, naïve to the experiment, to determine whether untrained individuals showed a preference for either four or two objects. This was done to assure that our results are due to the previous training, thereby excluding the possibility that any differences found in the test may be driven by an inherent preference to feed in front of either four or two objects. We used 4 vs. 2 objects of the intermediate size and randomly chose either squares or circles. Following the protocol for the numerical learning assay, we confined the focal individual to the center of the tank and placed the stimulus cards with two and four objects on either side of the tank. However, this time we dripped flake food mixed with water simultaneously on both sides of the tanks and noted to which side the focal individual would swim. We tested every individual once per day on three consecutive days.

Quantification of Gut Size

To explore the predicted trade-off between brain size and gut size, we used 360 randomly chosen fully grown and mature F3 (age: 159 ± 1.4 days) balanced for lines, replicate and sex. F3 were raised identically to previous generations. To ensure guts were empty we food-fasted them for 24 hours [3] and then euthanized them with an overdose of benzocaine. We measured the standard length (from the tip of the snout to the end of the caudal peduncle) to the nearest 0.01 mm using digital calipers and placed them in 5 % buffered formalin. After fixation (90.1 ± 0.1 days in formalin) we removed the gut under a stereomicroscope and weighed it to the nearest 0.001 mg. Gut mass may be influenced by fixation time but since all samples were fixed for the same duration before processing we are confident that this does not influence our results. To ensure that only individuals with fully evacuated guts were included in the analysis, we checked for food remains in the gut and excluded all individuals whose guts were not completely empty from the analysis (♀♀: 37 small-brained, 28 large-brained; ♂♂: 3 large-brained, 3 small-brained). We note that the results were qualitatively identical also when all individuals were included.

Statistical Analysis

The models for the variables response to selection, body size, inter-brood interval, gut mass and fecundity were fit using a Bayesian approach implemented in the R package MCMCglmm [4, 5]. Flat priors were used for the fixed effects and locally uninformative priors were used for the

random effects, both representing little prior knowledge. Initially, the models were fit with all possible interactions across the fixed effects. However, after evaluation of DIC values, several parameters were removed, and the final model contained the parameters presented in the tables (see Table S5 for model simplification procedure). After a burnin of $8 * 10^5$, a sample of the posterior distribution of $3.2 * 10^6$ was made with a thinning interval of 800, yielding a total posterior sample of 4000. All autocorrelations across successive posterior samples were in the interval < 0.1 and > -0.1 . In addition to the Bayesian model, we also analyzed the data using Restricted Maximum Likelihood and found these models to yield results that were highly congruent with the Bayesian models. Specific details of the models are described below. To analyze the response to artificial selection on relative brain size as well as its interactions with sex, we fit linear mixed effect models with the fixed effects selection regime (“down”, “up”), sex (“female”, “male”) and the covariate size (standard length in mm). The models were fit for generation F1 and F2 separately. We fit the logarithm of the response (brain weight) against the logarithm of the co-variate (size) in order to avoid problems associated with allometry. Initially, the model was fit with all possible interactions across the fixed effects. However, after evaluation of DIC values, several parameters were removed, and the final model contained the parameters presented in Table S1. Replicate line (3 levels) was added to the model as a random effect together with the interaction variance of the replicate*sex interaction.

Divergences in body size were modeled with sex and selection regime as fixed effects (“down” and “up”) and replicate line, the interaction of replicate line and sex, as well as sex specific residual variances as random effects.

Fecundity (estimated by number of offspring in the first brood) was assessed under the F2 generation and analyzed using a female specific model where number of offspring was modeled dependent on the age at first offspring, selection (“down” and “up”) as well as their interaction. Since the interaction was non-significant (based on DIC values) it was discarded from the analysis. Number of offspring was modeled using a Poisson distribution. Body size is known to affect number of offspring in fishes [6]. Since body size was measured on average 36 days after the first brood, we used age at first reproduction as a proxy for female body size at first reproduction. Equal growth rates between the two selection regimes were assumed because all fish were fed similar *ad libitum* rations and because there were no significant size differences across the selective regimes both at birth and when sacrificed (Table S2). Age at first reproduction should therefore present an accurate proxy of size at first reproduction. Replicate line was added to the model as a random effect. Divergence in age at first reproduction was modeled with selection regime as fixed effect (“down” and “up”) and replicate line as random effect.

To analyze how the relative gut size changed over the brain selection lines, we fit a linear mixed effect model with the fixed effects selection regime (“down”, “up”), sex (“female”, “male”) and the covariate size (length in mm standardized to a mean of 0 and a standard deviation of 1 within each sex in order to avoid collinearity between sex and size). Length rather than mass was used as co-variate for these analyzes since some of the females were pregnant, and mass would thus have been a biased measure. These analyzes were run for generation F3. Initially, the model was fit with all possible interactions across the fixed effects. Replicate line (3 levels) was added to the model as a random effect. Since the sexes differed greatly in their residual variance, we modeled different residual variances for the sexes. Divergence in juvenile

body size was modeled as dependent on the clutch size, maternal size, with selection regime as fixed effect (“down” and “up”) and replicate line as a random effect. To analyze the size and brain size of newborn fish we fit a linear mixed model with the fixed effects selection (“down” and “up”), body size as a co-variate and replicate line as a random effect.

To analyze cognitive performance after the first training session, we first assessed the number of correct choices on the first day of testing, using a probit-link generalized linear mixed model (GzLMM) with correct choice as dependent variable. We included selective regime and sex as fixed factors and replicate line as random factor. We then analyzed the number of correct choices of all eight numerical learning ability trials. Since not all fish participated in every trial we used binary probit-link generalized linear mixed models (GzLMM) to analyze the cognitive ability with the total number of correct choices as dependent variable and the number of times the fish participated as independent variable [7, 8]. We included selective regime and sex as fixed factors and replicate line as random factor. These analyzes were done with SPSS 19.0, SPSS Inc., Chicago.

All experiments were done in accordance with the ethical regulations for research involving animal subjects in Uppsala, Sweden.

Calculation of Realized Heritabilities

We used line means to estimate realized heritability. We followed Walsh and Lynch [9] and estimated realized heritabilities as the ratio between the cumulative selection response R_C and the cumulative selection differential S_C , which are defined by

$$R_C = \bar{z}_{u,t} - \bar{z}_{d,t} \quad \text{and}$$

$$S_C = \sum \left(\bar{z}_{u,t-1}^{selected} - \bar{z}_{u,t-1} \right) - \left(\bar{z}_{d,t-1}^{selected} - \bar{z}_{d,t-1} \right)$$

Here, z represents the means of the specified groupings (the overall mean in the “up” and “down” selection lines at generation t , or the means of the artificially selected individuals included in the analysis). In the following description “*selection*” refers to the selection regimes, i.e. the populations that are under selection for either larger or smaller brains. “*Selected*” refers to the group of guppies *within each selection regime* that were chosen to establish the following generation. We estimated the realized heritabilities for males and females separately. Brain size means exhibited plasticity across generations and we detected generation-dependent sex differences in brain-body size allometry (table S1). It was thus problematic to extract the means of interest from a single model, and we therefore ran separate models for each generation and sex combination for the models estimating the response to *selection* (calculated as the difference between the “up” and “down” populations). For the models estimating the means of the *selected* individuals and the population mean (the mean of the guppies in the selection regimes for either smaller or larger brains), we ran models specific to each generation, sex and *selection* combination.

The models estimating the response to *selection* contained the explanatory variables selection (“up” and “down”), log body size, and replicate line as a random effect. The models estimating the means of the *selected* individuals contained the explanatory variables selected

("yes", "no"), log body size as a covariate and replicate line as random effect. To evaluate main effects independently of the covariate, log body size was standardized to a mean of 0 and a standard deviation of 1. All models were fit using MCMCglmm as previously described.

Results from Numerical Learning Test

Already on the first day after the training, we found a significant interaction between sex and selection (GzLMM, $n = 39$, selection: $\chi^2 = 0.16$, $df = 1$, $P = 0.695$, sex: $\chi^2 = 0.02$, $df = 1$, $P = 0.889$, selection*sex: $\chi^2 = 7.05$, $df = 1$, $P = 0.012$). Large-brained females outperformed small-brained females in the learning assay (GzLMM_{females}, $n = 23$, selection: $\chi^2 = 5.36$, $df = 1$, $P = 0.031$), while no difference was found between males of different brain sizes (GzLMM_{males}, $n = 16$, selection: $\chi^2 = 2.26$, $df = 1$, $P = 0.155$). The combined results for all trials are given in the main article.

Results from Control for Preexisting Bias for Specific Number of Objects

Analogously to the numerical learning ability trial, we first tested the bias on the first day and then analyzed the data for all three days combined. All but one individual participated readily in all trials. We therefore excluded this individual and, analogously to the numerical learning assay analysis, used a binary probit-link generalized linear mixed model (GzLMM) to test for preexisting bias towards two or four objects on the first day of testing. To then test all three days, we used a general linear mixed model (GLMM). These analyzes were done with SPSS 19.0, SPSS Inc., Chicago. On the first day of the control test we found no preexisting bias towards four or two objects (GzLMM, $n = 47$, selection: $\chi^2 = 0.01$, $df = 1$, $P = 0.913$, sex: $\chi^2 = 1.10$, $df = 1$, $P = 0.300$, selection*sex: $\chi^2 = 0.012$, $df = 1$, $P = 0.913$). This did not change after three days of testing (GLMM, $n = 47$, selection: $\chi^2 = 1.76$, $df = 1$, $P = 0.192$, sex: $\chi^2 = 0.01$, $df = 1$, $P = 0.911$, selection*sex: $\chi^2 = 0.25$, $df = 1$, $P = 0.623$). We therefore conclude that the significant results shown and discussed in the main article are not due to a preexisting bias for either two or four objects, but rather due to an increased learning performance in the large-brained females.

Supplemental References

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