Learning is a fundamental source of behavioral individuality

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Learning and memory are thought to be essential for our individual uniqueness and sense of self (*1*). Yet, individuality is rarely studied in the context of behaviors that depend on learning. While it is established that such behaviors can vary across individuals, it remains unknown whether their variation stems from learning or from classical sources of individuality, the developmental stochasticity, environment and genetics (*2–12*). To answer this fundamental question, we measured behavior in thousands of flies in the presence and absence of learning, and compared the extent of their individuality. We discover an excess of non-normally distributed individual behavior only in flies that experienced learning, even though they were genetically identical, raised under the same conditions and exposed to the same environment as the non-learning flies. By tracking each fly's decision-making process, we find that learning does not simply change individual behavior, but it also diversifies it. We could recreate the emergence of this excess of individuality using computer simulations of behaving flies only when we enabled reinforced learning. Although individual experience and genetics shaped behavioral biases, we demonstrated that behavior can diverge due to learning even in individuals that share biases. Our results thus establish learning as the fundamental source of individuality, rather than a phenotypic outcome of genetics, environment, or development.

Behavioral individuality is an inevitable, fundamental aspect of life. Even identical twins who share life history eventually diverge behaviorally into distinct individuals (*12–14*). How behavioral individuality emerges is crucial for understanding the mechanisms underlying behavior. Behavior is initially shaped by deterministic, genetic factors (*15–18*) and stochastic, environmental or molecular events that occur during development and

early life (*6, 9, 18–21*). These classical sources of behavioral individuality have been studied extensively (*22*). Behavior can further change during life due to life experience, either through learning and memory, or learning-independent process (*10,22–27*). While it seems intuitive that learning would play a key role in shaping individuality because it can change behavior, surprisingly, there is no direct experimental evidence that would support it. It is still unclear whether variability in learning-dependent behavior is a phenotypic consequence of genotype and environment shaping learning ability and, through it, behavior, or if the very process of learning directly induces divergence in individual behavior. The challenge in understanding the role of learning in individual behavior lies in its entanglement with classical sources of individuality. (Figure 1A). Specifically, the ability to learn and remember is known to vary across individuals (*1, 22, 26, 28, 29*), genotypes (*30, 31*), environments (*32–34*), and even maternal status (*35–37*). So to study the emergence of individuality in the context of learning, a high level of trans-generational experimental control over individual genetic, environmental, experiential and learning conditions across many genotypes is critical but technically challenging. Previous studies have largely focused on individuality of spontaneous behaviors that are independent of learning (*5, 7, 9, 12, 25, 27, 38–41*) or they could not account for the effects of all sources of individuality due to the lack of experimental throughput and parallelization, the lack of genetic diversity, or unknown life-history of the studied system (*4, 11, 26, 31, 42–46*). Consequently, the role of learning in individuality remains largely unexplored. In this study, we develop a data collection technology that is scaled up 100-fold compared to the current state-of-the-art for studying individual learning behavior. Leveraging the experimental utility of the Drosophila model system, we achieved extreme environmental, genetic and experiential uniformity across thousands of individuals (Table S1). This allowed us to study the emergence of individuality in 90 different genotypes, rather than focusing on one or a few. By analyzing, modeling and computationally recreating the time-resolved individual experiences and almost half a million decisions made by flies in the presence or absence of learning, we partitioned the contributions of all known sources of behavioral individuality, including genetics, development, momentary- and life-experience from that of learning (Figure 1A). We demonstrated experimentally that learning is a fundamental force underlying behavioral diversification.

Behavioral individuality is reduced in learning-deficient individuals

We designed a platform that implements operant conditioning assay parallelized across 64 freely behaving individual *Drosophila melanogaster* (Figure 1B, Video S1, S2). The system takes input from real-time video tracking of individuals (Video S1, S2). This is then used for closed-loop coupling of visual stimuli and delivery of a mild foot shock to individual flies upon an undesired behavior (Figure 1C-D, S1A). Operant conditioning paradigms are based on color and place avoidance learning in a Y-shaped arena ("Y-maze"; see Methods), where the conditioned task was to spend less time in the arm or color that was associated with shock (task performance, $T_{shocked}$, Figure 1C, S1A; see Methods). In the control group, the shock was not applied, allowing the flies to behave spontaneously,

without a need to learn (Figure 1D-E). The difference between mean control and conditioned $T_{shocked}$ of a genotype (*"relative task performance"*) emulated the traditionally used group-average measures of learning and memory task performance in learning- and sensory-deficient flies, and uncovered genetic diversity (Figure 1E-F, Figure S1C) (*16*). Interestingly, it also revealed a reduced inter-individual variability of $T_{shocked}$ among flies that are unable to learn and form memories (*16, 47, 48*) (Figure S1B-E, S2A-B).

Distribution of individual learning-dependent behavior is altered

We measured the genetic effects on individual learning-dependent behavior in 5632 flies derived from 88 genetically distinct yet isogenic wild-type genotypes (*49*) (Figure 1F, 2A, S3A). We ensured strict multi-generational control over their life-history (Table S1) across two independently reared replicates of each genotype (Figure 2B, 2F, S3B-I). Individuals from each replicate were then split into **conditioned** and **control** group (Figure 1A) and we tracked their momentary task-relevant behaviors, including ~half a million individual decisions (i.e., switches from one arm in the Y-maze to another arm; N = 497450; Video S2). Task performance (Figure S4A, S4F, S4K; Supplementary text) and other task-relevant behaviors changed significantly between control and conditioned flies (Figure 2A, 2D, 4A-E), and consistently over time (Figure S4G-J), across conditioning paradigms (Figure S1C, Table S3) and sexes (ANOVA $p = 0.537$, Sum Sq. = 0.004, Table S2). Despite the strict experimental control (Table S1, Figure S3B-I, Table S4), we found non-negligible behavior variability within groups of genetically identical individuals (Figure 2A, S3A). Curiously, not only the means and variances (Figure 2D), but also the shapes of the distributions of individual behaviors varied reproducibly between genotypes, particularly in the conditioned group (Figure 2A-B, S3B). Individual behavior in the control group was spontaneous and normally distributed around chance level ($T_{shocked}$ 1/3; Figure 2A-B, S3A, S5). On the other hand, and despite the shared life experience with the control flies, conditioned flies expressed within-genotype individuality in an apparently non-Gaussian and multi-modal fashion (Figure 2A-B, S3A, S5). We asked whether this altered, intra-genetic individuality in conditioned flies is sourced stochastically.

Learning exposes residual individuality

The observed variable shapes of distribution were reproducible across replicates (Exact general independence test, $p > 0.05$ for 83 out of 88 genotypes; see Methods; Figure 2B, S3B), indicating a deterministic, genetic control over individuality. Genotype, environment, and development give rise to individuality by shaping the mean and the variance of behavior within a group of individuals (*15, 50, 51*). To dissect the genetic contribution to the unusual non-normal display of individuality, classical characterization of individuality using genotype mean and variance was not appropriate. Instead, we used *differential entropy* (H) and Hellinger distance (Methods)

to capture the extent of individuality within group and change in individuality between groups, respectively, without assuming Gaussian probability distributions of individual behavior. We found that the entropy of the $T_{shocked}$ distribution was highly genotype-dependent (Resampling test, parametric $p < 2.2e^{-16}$ resampled $p = 1e^{-06}$, Figure S6A), and it correlated between conditioned and control environments (Pearson's product-moment correlation between $H_{control}$ and $H_{conditioned}$ $r = 0.232$, $p = 0.03$, n= 88 genotypes, Figure S6A). This suggested that most of the individuality is sourced from processes that preceded the behavior assay, such as life history. The difference in expressed individuality between the conditioned and control flies varied substantially across genotypes (Resampling test, parametric $p < 2.2e^{-16}$, resampled $p = 1e^{-06}$, Figure S6B), again pointing to genetic as well as environmental control over individuality. At the same time, the difference in expressed individuality between replicates within genotype was consistently low in both conditioned and control flies (Mean $Hellinger_{control}$ = 0.06, Mean $Hellinger_{conditioned}$ = 0.05, T-test $p = 0.256$, n = 88, Figure S6C), demonstrating tight experimental control over life history. Together, these results indicated that the observed behavioral variability is sourced from mechanisms that are not entirely random or individual, but are systematically shared between all flies of the same genotype. Nevertheless, the difference in shapes of the distributions of individual task performance between genotypes indicated that the individuality expressed within a genotype did not linearly translate from spontaneous to learning behavior (Figure 2A-B, S3A, S6A). This can only be explained by an additional non-random source of individuality that cannot be attributed to genotype, environment or life history differences prior to the task. To capture this additional expression of individuality, we introduce a measure of *"residual individuality"* (H_{resid}), calculated as Shannon entropy of a probability distribution after scaling the distribution within its own range. This measure nullifies the variability induced by life-history factors shared across all groups of flies, such as stochastic processes, genotype, environment, and their interactions (Figure S7, Methods). We use different measures of shape divergence by calculating divergence in residual individuality of two groups (see Methods). *Environmental variability* (*EV*) quantifies the change in expressed residual individuality (H_{resid}) of a genotype due to conditioning. *Measurement variability*, (*MV*) reflects the change in residual individuality caused by systematic experimental noise distributed over individual flies during the behavior assay (Figure 2F). *Genetic variability*, (*GV*) captures the genetic contribution to the expressed residual individuality that takes place during the behavior assay (Figure 2E). *Environmental variability* of $T_{shocked}$ (*EV*) was differed significantly across genotypes (Resampling test, parametric $p < 2.2e^{-16}$, resampled $p = 1e^{-06}$; Figure 2C), and evolved with every made decision (Figure 2G), yet we found no difference in *measurement variability* between conditioned and control groups ($MV_{control}$ = 2.08, $MV_{conditioned}$ = 1.85, T-test $p = 0.206$, n = 88, Figure 2F). This suggested that the circumstance under which the behavior was measured, namely the presence or absence of conditioning, induced the observed change in residual individuality. Genetics is expected to be the only deterministic variable that can shape individuality in a fixed environment that is shared in parallel by individuals. In isogenic populations, the effect of genotype on individuality is assumed

to be absent except for its effect on phenotype variance. Yet, even after discounting mean and variance effects, we found that the shapes of the distributions of individual $T_{shocked}$ were significantly more divergent between genotypes in the conditioned group than in the control group ($GV_{control}$ = 0.70, $GV_{conditioned}$ = 0.83, T-test $p < 2.2e^{-16}$, n = 88 genotypes; Figure 2E). This was found in other behaviors as well, including the learning score, handedness, decision-making, velocity and relative velocity (Figure S3E-I, S8A) and could not be explained by residual heterozygosity (*49*) (Figure S8A,S9A, 2C, 2E), thus supporting our hypothesis that residual individuality cannot be attributed to effects of genotype. Residual individuality diverged reproducibly between genotypes (*GV*) and between environments (*EV*), but when averaged across all genotypes, it was the same in conditioned and control environment (Control $T_{shocked}$ H_{resid} = 2.04, Conditioned $T_{shocked}$ H_{resid} = 2.02, T-test p = 0.321, n = 88 genotypes, Figure S8B). This was the case for all behaviors, except for the proportion of correctly made decisions (*percent correct*), where residual individuality (H_{resid}) was higher in conditioned flies (Figure S8A), and for *velocity* (Figure S8A), where it was lower in the conditioned flies, compared to control. This showed that the observed changes in residual individuality upon conditioning do not emerge from a stochastic source of individuality. How can genetically uniform experimental populations tested under parallel environmental conditions still express varying, non-stochastic and genotype-dependent residual individuality across behavioral paradigms? Our results suggested that the residual individuality is shaped by a process specific to conditioning. Because the divergence in residual individuality between conditioned and control flies evolved and increased over time (Figure 2C, 2G), we hypothesized that the cumulative individual learning experience, and genotype's response to it must be the culprit behind the genotype-specific expression of residual individuality.

Individuality is shaped continuously through learning from experience

The only discriminating state between the conditioned and the control groups was the presence of a shock as a stressful or valent experience (punishment). We ruled out shock response as the cause of observed individuality in task performance (Figure S8C, S9A-B, Supplementary text). On the other hand, *learning score* (LS; Methods) correlated negatively and significantly with individual *relative velocity* and *velocity*, as well as *task performance* across individuals (Pearson's product-moment correlation r = -0.201, $p < 2.2e^{-16}$, n = 2611, Figure S8C) and across genotypes (Pearson's product-moment correlation r = -0.399, $p = 1e^{-04}$, n = 88 genotypes; Figure S9A) in the conditioned group. Hence, learning, must play a key role in shaping individual behavior. However, there was a lack of correlation of residual individuality in task performance with genotypes' mean learning score and with residual individuality in learning score. Thus, genetically encoded learning ability could not explain genotype-specific residual individuality in task performance (Figure S9A). Under the assumption of fixed environment and shared momentary experience across genotypes and individuals, neither stochasticity, genetics, nor variation in individual learning ability could provide a satisfactory explanation for the emergence of residual individuality.

This led us to consider the possibility that we overestimated the uniformity or randomness of individual experience of flies during parallelized behavior tests. Despite the efforts to perfectly control and parallelize experienced environments across every individual, in practice, perfect control over biological, freely behaving agents, is rarely achievable. Momentary experience of freely behaving individuals depends on their previous decision (Figure 1A). This is practically intractable in a natural environment, but the Y-maze arena minimizes the diversity of unique experiences and decisions a fly can make and allows us to track and enumerate them (Figure 3A-C). By aligning decisions across thousands of individuals, we asked how individual decisions and momentary experiences impact the emergence of individual behavior in the presence or absence of learning. We found that the initial strategy of a fly exploring the Y-maze conformed to individual- and genotype-specific color biases, reflected in their positions in the maze at the start of a learning sequence (Figure S10A-B). We had initially overlooked the importance of this experiential variation, as it represented a seemingly negligible difference in the way a fly experiences the first punishment. However, this minor variation in initial learning-dependent decision-making influenced the evolution of future decisions (Figure 3A, 3C, 3E). Task performance of conditioned flies was (unsurprisingly) correlated with color bias (Figure S8C), but the *change* in task performance was more sensitive to starting position compared to the control flies. The *change* in task performance also correlated with the flies' individual learning score (Figure 3D) and not with their color bias (Figure 3A, S10C). In the presence of learning, the convergence of task performance of individuals starting at different positions lagged substantially, even for flies that shared a learning score or color bias (Figure 3A, S10D). Moreover, the divergence in residual individuality between flies starting at different positions was persistently high for good learners, especially those expressing an unfavorable initial color bias. We hypothesized that the effect of the starting position on divergent task performance stems from the clash between genetically or stochastically determined individual color bias (*21*) and individual learning at the very beginning of the learning task, as the association between shock and color is just becoming established (Figure 3E). In support of this, an individual's learning rather than bias was a progressively more salient predictor of task performance over time (Figure S10E) while the task performance of the control group consistently reflected the fly's initial bias (Figure S10F). We could directly observe this clash between learning and bias as it played out in the form of persistent color-switching behavior, amplified in flies with high learning score and unfavorable bias (Figure 3C, 3E). Together, these dynamics illustrate how learning directly affects individuality in behavior. First, the individual and genotype-specific bias creates a genotype-specific divergence in initial distribution of experience across individuals. In the absence of learning, this bias will define the distribution of individual behaviors. However, as they begin to learn, flies independently and individually abandon unfavorable genetic biases or lean into favorable ones through individual decisions. As soon as learning is present, every individual decision, including the first one, changes individual future experience, moving away from genetic bias. As flies advance through decisions, their experience and behavior diverge independently (Figure 1A, 3C, 3E). This dynamic process produces an initially

genetically seeded experience (Figure S10B) that only in the presence of learning instigates additional individual diversification of behavior (Figure 2G, 3A, 3C, 3E).

Learning diversifies individual behaviors

While we do not track individual flies throughout their entire lives, the design of our experiment allows us to dissect the effects of life experience prior to the task from the effects of momentary experience during the task. We asked how extensive is the effect of the entire life's experience on individual momentary behavior, compared to the effect of genetics or learning during the task? To quantify the relative contributions of different sources of individuality, we turned to modeling of observed behaviors. We tested how well our models capture and represent the observed behavior by using them to simulate behaving flies and by comparing their simulated behavior to the behavior of observed, real flies. Simulated flies incorporate a range of behavioral parameters (Table S6). They decide how long they want to stay in the current arm of a simulated Y-maze, and whether they want to turn left or right when leaving the current arm. The decisions are made by sampling the escape duration (time to leave one arm) from a log-normal distribution with a time-dependent mean and stationary variance, and the left-right turns are sampled from a Bernoulli distribution with a time-dependent rate (Methods). The mean of their escape duration distribution depends on the color of the arm and the presence of shock at the time point t. Importantly, the mean escape duration and the rate of turning left or right can change with a reinforcement learning rule, which depends on the presence of shock. To assess the effects of genetics, learning, and individual life experience, we consider four different behavior models. First, all flies of the same genotype have identical parameters, but different genotypes have different parameters (*no individuality* model), thereby omitting the effect of experience or variability obtained prior to expressing a behavior in the task (for example through developmental stochasticity). Second, the i-th parameter of an individual simulated fly is sampled from a normal distribution with genotype-dependent mean ($m_i^{(g)}$) and standard deviation ($s_i^{(g)}$). These simulated flies have individuality in the sense that their initial behavioral policies differ from one another, because no two flies have the exact same parameters (*individuality* model). In other words, simulated flies start the task as if in addition to their genetics and learning, their life experience prior to the task (including stochastic processes during development) had shaped their individual behavior. Third, parameters are sampled as in the *individuality* model, but the learning rate is set to zero, such that the presence of shock or color induce no lasting change on their behavior beyond the immediate effect of changing the escape duration (*no learning* model). Fourth, parameters are sampled per individual, but there is no genotype dependence of the means and standard deviations of the parameter distributions. This way the effect of genetics is omitted, including its effect on developmental variability (*no genetics* model).

Best fit was found for *individuality model*, followed by *no learning* and *no genetics* model. *No individuality model* failed to recapitulate the observed behavioral individuality (Figure 4A-B). Particularly striking was the

mismatch in the distribution of the number of decisions made by flies simulated without individuality compared to the actually measured distributions of the number of decisions made by real flies (Figure S11). Compared to whole-life experience, the effect of momentary learning on individual behavior was smaller, and for some genotypes seemed irrelevant (Figure 4A). However, we found that the model without learning could not capture the temporal dynamics of observed behavior, namely the change in task performance, the overall decrease of the escape duration, and the change in handedness from the first 5 minutes to the last 5 minutes of the experiment (Figure 4C). Interestingly, the excess of observed non-Gaussian distributions of individual behavior in conditioned flies was recreated only if the simulated flies could learn (Figure S5) and despite all parameters of the model being drawn from normal distributions. This reaffirmed that residual individuality emerges from the process of learning. Lastly, repeated simulations revealed that the genotype distribution of simulated task performance matches the observed behaviors better for the model with learning (Figure 4D, S11-12). These results confirm our conclusion that beyond life experience, environment and genetics, learning makes an essential contribution to expressed behavioral individuality, in particular to the excess of residual individuality observed in non-spontaneous behavior. The fitted models allowed us to estimate how the momentary task-relevant behavior of a given fly differs from other flies, or how it compares to other moments in time (Methods). We found that the individual behavior had changed significantly during the experiment (Figure 4E-F) and that this change was significantly bigger in the flies that learned (Figure 4F, S13). A visualization of these differences with multidimensional scaling revealed that the behavior of individual flies does not cluster according to genotype throughout the experiment (Figure 4E). This lack of clustering was also confirmed with a classification analysis: using a long-short term memory (LSTM) classifier applied to the behavioral data of individual flies, we could predict the genotype in only 3.4% of the cases (Chance level : 1.1%, 4-fold cross-validation).

In conclusion, our experimental data, models, and simulations demonstrated that along with development, life-experience, and genetics, the measured individuality of momentary behavior is substantially and directly diversified by the process of learning that gives rise to individual, and genetically unpredictable behavior.

Discussion

We demonstrate that the presence of the core process of learning, dissected from the influence of genetics and life-history, directly contributes to diversification of individual behavior. Our study also reaffirms the known essential role of genetics, life experience and stochastic developmental processes in shaping the behavioral individuality. However, our summarized results are clearly at odds with often presumed predictive power of genetics on individual behavior. We found that genetics does not define behavior. Rather, it only provides a range of individual behavioral seeds that broadly influence the distribution of individual experiences. All genotypes could produce almost any individual behavioral phenotype within a shared environment. And yet, we also find these behaviors cannot be

faithfully modeled or reproduced without assuming diversity in genetic control. This, perhaps inconvenient truth has already been intuited by many experimentalists investigating the genetic basis of learning-dependent behaviors — even in experimental conditions, variability in cognitive performance is imminent. The source of this variability has been previously attributed to stochastic processes. We, too, find that minor events, such as the context of the first decision, often created by the behaving agent itself, can start a butterfly effect of continuous modification of future individual behavior. However, we demonstrate that the presence of learning is needed to achieve this effect. Because of learning, cognitive behavior becomes inherently individual, temporally dynamic, and non-ergodic. To understand the mechanistic intricacies, and especially the genetic basis of a free agent's cognitive behavior, we can no longer rely on averaged, linear or single momentary measures of behavior within a genotype or species, at least not within one generation. This will be especially important when we study cognitively complex animals, such as humans, where individual experiential complexity immeasurably exceeds that of a fly traversing a simple two-color Y-maze.

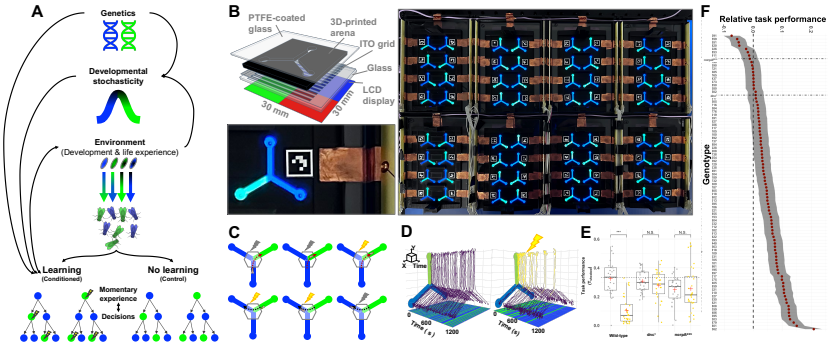

Figure 1: **Capturing sources of individual learning-dependent behavior.** (**A**) Sources of individual behavior. In a single environment, genetic, stochastic, and experiential sources of individuality interact with each other to produce behavior such as color bias. Behavioral biases can influence momentary experience and guide initial decisions. Variation in genotype can interact with all proposed source of individuality, directly or indirectly. Its effect is thus confounded with experience and learning. By dividing individuals with same genetics and life-experience in learning and non-learning groups, we can determine the role of learning on individuality. (**B**) Schematic of a Y-maze design (top left panel), top-down view of the 64-maze multiplexed platform with randomized visual stimuli (right), and a close-up photograph of a single Y-maze arena (bottom left). (**C**) Schematic of a shock delivery and shock removal timing upon a discrete decision in a visual place learning task. Shock is applied from the moment a fly has fully entered the shock arm (top right) until it has fully entered a new arm (bottom right). (**D**) Representative tracks of two flies traversing a Y-maze in the presence (right) or absence (left) of conditioning. Lines on the bottom represent color occupancy. (**E**) Task performance in green place learning task of a wild-type genotype compared to the memory- and learning-deficient mutant (dnc^1) and the visually impaired mutant ($norpA^{EE5}$). Measurements of individual control (grey) and conditioned (yellow) flies are shown as points. Boxes indicate the upper and lower quartiles. Mean and medians are represented as bar and red plus, respectively. (**F**) Relative task performance in place learning task across 90 genotypes uncovers genetic variability. Grey shading indicates standard error. A vertical line points to no change in performance upon conditioning (0) and horizontal lines indicate relative task performance for memory- and vision-deficient genotypes (dnc^1 and $norpA^{EE5}$).

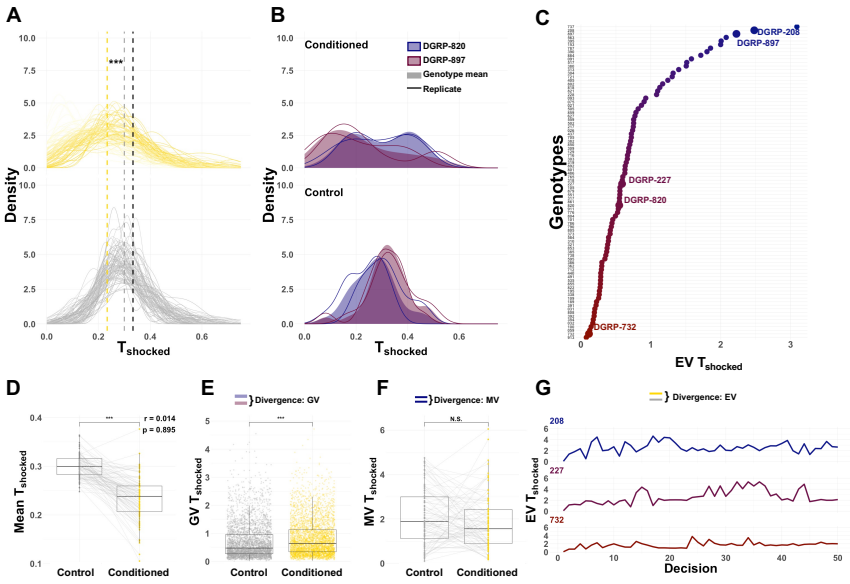

Figure 2: **Individuality and residual individuality in task performance.** (**A**) Distributions of individual task performance for each of the 88 wild-type genotypes in conditioned (yellow) and control (gray) task performance. Vertical lines show mean task performance for conditioned and control flies (yellow and gray), and chance expectation of $T_{shocked}$ at 1/3 (black). (**B**) Genotype-specific extent of individuality is replicable and dependent on conditioning. An example of individual $T_{shocked}$ distributions in two genotypes (shaded red and blue) is shown. When visualized as independent distributions (shown as lines), replicates exhibit consistent distributions of individual $T_{shocked}$ within a genotype, despite being raised independently. Unlike the control (lower panel), the conditioned flies (upper panel) tend to assume non-Gaussian distributions of $T_{shocked}$. (**C**) The change in residual individuality (comparison of shapes of same-colored distributions in B, and equivalent comparison across other genotypes) of $T_{shocked}$ upon conditioning compared to control is genotype-specific. (**D**) Mean task performance across genotypes in conditioned flies (yellow) is significantly different from task performance in control flies (gray). Light-gray lines connect the same genotype's value across the two treatments. Inset text shows correlation for mean $T_{shocked}$ between two treatments and the associated p-value. (**E**) Difference in expressed residual individuality between genotypes is significantly higher in conditioned (yellow) than in control treatment (gray). Each point represents *shape Div* of individual task performance distributions between two genotypes (*GV*). (**F**) Extent of expressed residual individuality in task performance is similar between replicates, pointing away from systematic experimental variability as the source of change in residual individuality. Each point represents *shape Div* between two replicates of a genotype (*MV*). (**G**) Divergence in expressed residual individuality of conditioned flies from control flies (*EV*) evolves with every decision and increases over time.

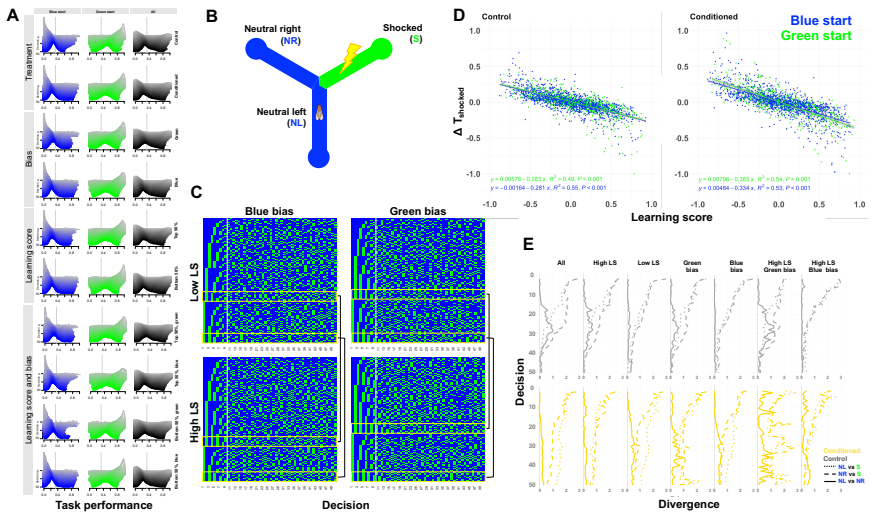

Figure 3: **Divergence in individual task performance depends on learning through experience.** (**A**) Evolution of individual task performance distributions over the first 50 decisions is dependent on starting color. Task performance is shown for all flies, those who share the same color bias, and those with above and below average learning score. Lighter colors indicate distributions at earlier decisions. For blue starting color, two distributions are overlaid, one for flies starting at neutral left and the other for neutral right arm of the maze (see B). Only flies that made at least 50 decisions are included. Distributions of all flies regardless of starting position is shown in black. (**B**) Schematic of the Y-maze and possible color and shock experiences. For the conditioned flies, the green colored arm (S) is associated with the shock, while the other two, blue arms are not (NL - neutral left and neutral right - NR). The green arm is also not associated with the shock in the control flies. (**C**) Bias and learning shape the sequence of decisions. First 50 decisions in flies with high and low learning score and different color bias are aligned between individual flies (rows) and sequentially sorted. The yellow rectangles bring attention to flies with most persistent color choice switching behavior at the beginning of the task. Black brackets indicate flies that share the same first decision and hence can be compared across groups. The group of flies with unfavorable bias and with high learning score (bottom right) are more prone to initial switching color decisions for a prolonged time. (**D**) Change in individual task performance from the start to the end of the experiment is more sensitive to experience at the first decision (blue or green color) due to learning. Note the change in slope of the association between learning score and the change in performance when the flies start the task at different colors in conditioned group (right panel). This effect is absent in the control (left panel). Each point is an individual fly. (**E**) Evolution of residual individuality in task performance is sensitive to initial experience and exacerbated by opposing forces of learning and unfavorable bias. Evolving *shape Div* in H_{resid} is shown for task performance of flies starting at different arms of the Y-maze that made at least 50 decisions.

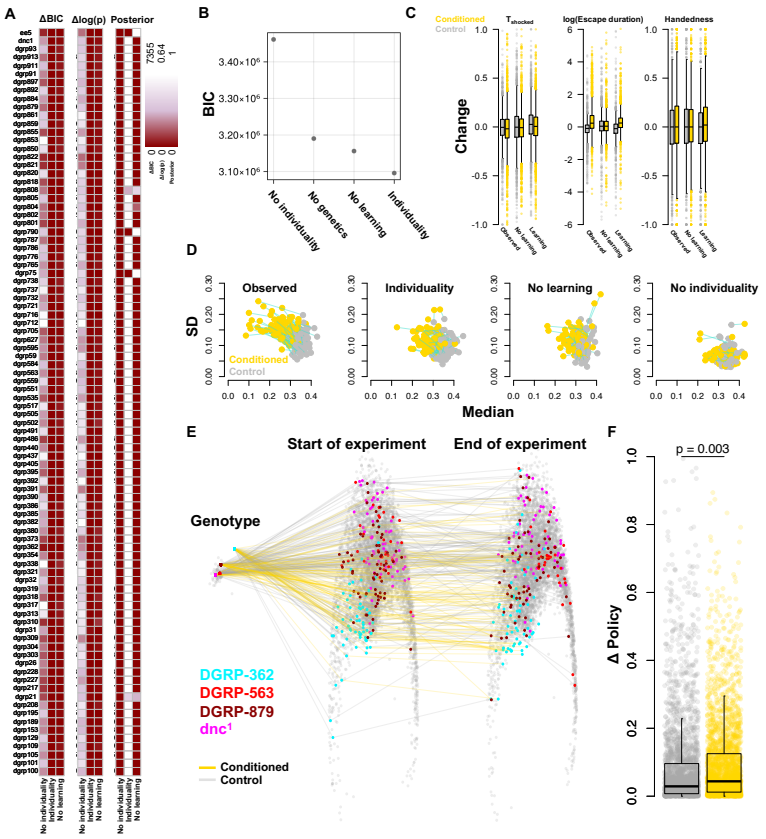

Figure 4: **Experience, genetics and learning shape individuality.** (**A**) Variation in behavior observed across genotypes is best explained by a model that includes genetically controlled but individually random prior life experience, and learning (*individuality*). (**B**) High Bayesian information criterion for models omitting the effects of prior individual variability, genetics, and learning demonstrate that genetic effects on trait mean and variance and learning, as modeled in the *individuality* model, are necessary for close *in silico* recapitulation of observed behavioral diversity. Behavioral individuality is affected by variability in behavior obtained prior to the task, effects of genetic on the mean and variance of the behavioral expression and the effects of learning, in that order. (**C**) Change in individual behaviors during conditioning can be attributed to the effect of learning. Boxplots summarize the behaviors of observed, real flies and of flies simulated with and without learning. (**D**) Flies simulated under the assumption of genetic control over past life experience and with learning can reproduce task performance behavior most consistently with the observed, real flies. (Other behaviors are shown in Figure S11.) (**E**) Genetic effect on behavioral policy projects to the entire individual behavior space. Mean genotype policies and overlapping individual policies measured at the start and at the end of the experiment are projected on a multidimensional scaling plot and demonstrate almost complete unpredictability of individual behavior given the genotype in a controlled environment. Highlighted points correspond to individuals from an outlier genotype (turquoise), two average genotypes (red and dark red) and a memory-deficient genotype (magenta). (**F**) Significant change from initial behavioral policy occurs in conditioned flies during the 20 minutes between the start and the end of the task experiment. Each data point in D) represents a genotype × condition. Each point in E) and F) represents an individual.

References and Notes

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Data and materials availability: Data and code used to produce the main results, as well as PCB and 3D print designs are available on a github repository https://github.com/jaksiclab/GeneticsOfLearningIndividuality and on Zenodo.

Supplementary materials

Materials and Methods

Figs. S1 to S14

Tables S1 to S6

References *(7-67)*

Video S1 to S2