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Drift in Individual Behavioral Phenotype as a Strategy for Unpredictable Worlds

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Ryan Maloney, **Athena Ye, Sam-Keny Saint-Pre, Tom Alisch, David Zimmerman, Nicole Pittoors, Benjamin L de Bivort**

Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States • Department of Psychology, Colorado College, Colorado Springs, United States • Tufts University, Medford, United States

eLife Assessment

Maloney et al. offer an **important** contribution to understanding the potential ecological mechanisms behind individual behavioral variation. By providing **compelling** theoretical data and **convincing** experimental data, the study bridges the gap between individual, apparently stochastic behavior with its evolutionary purpose and consequences. The work further provides a testable and generalizable model framework to explore behavioral drift in other behaviors.

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Abstract

Individuals, even with matched genetics and environment, show substantial phenotypic variability. This variability may be part of a bet-hedging strategy, where populations express a range of phenotypes to ensure survival in unpredictable environments. In addition phenotypic variability between individuals ("bet-hedging"), individuals also show variability in their phenotype across time, even absent external cues. There are few evolutionary theories that explain random shifts in phenotype across an animals life, which we term drift in individual phenotype. We use individuality in locomotor handedness in *Drosophila melanogaster* to characterize both bet-hedging and drift. We use a continuous circling assay to show that handedness spontaneously changes over timescales ranging from seconds to the lifespan of a fly. We compare the amount of drift and bet-hedging across a number of different fly strains and show independent strain specific differences in bet-hedging and drift. We show manipulation of serotonin changes the rate of drift, indicating a potential circuit substrate controlling drift. We then develop a theoretical framework for assessing the adaptive value of drift, demonstrating that drift may be adaptive for populations subject to selection pressures that fluctuate on timescales similar to the lifespan of an animal. We apply our model to real world environmental signals and find patterns of fluctuations that favor random drift in behavioral phenotype, suggesting that drift may be adaptive under some real world conditions. These results demonstrate that drift plays a role in driving variability in a population and may serve an adaptive role distinct from population level bet-hedging.

Significance Statement

Why do individuals animals spontaneously change their preferences over time? While stable idiosyncratic behavioral preferences have been proposed to help species survive unpredictable environments as part of a bet-hedging strategy, the role of intraindividual shifts in preferences is unclear. Using *Drosophila melanogaster*, we show the stability of individual preferences is influenced by genetic background and neuromodulation, and is therefore a regulated phenomenon. We use theoretical modeling to show that shifts in preferences may be adaptive to environments that change within an individual's lifespan, including many real world patterns of environmental fluctuations. Together, this work suggests that the stability of individual preferences may affect the survival of species in unpredictable worlds — understanding that may be increasingly important in the face of anthropogenic change.

Introduction

No two organisms of the same species, even when genetically identical, behave precisely the same. Individuality has been observed and measured in organisms ranging from bacteria(1) to plants(2), flies(3–7) to humans(8), even in the absence of genetic or environmental differences. This variability poses two major questions in biology—how does it arise and what, if any, evolutionary role does it serve.

Heritable phenotypic variation in a population allows for adaptive tracking, i.e., when alleles change in frequency as the selective pressure of the environment changes (9). A complementary strategy for fluctuating environments is phenotypic plasticity, in which organisms change their behavior (or morphology) in direct response to environmental changes. These two adaptive strategies share a basic outcome: the phenotype they produce matches the current or recent environment. Organisms that fail to match the environment can suffer deadly consequences.

Random differences in phenotype may reflect a "bet-hedging" strategy for species to deal with unpredictability in their environment (2, 10–16). Under this theory, variability allows some individuals to survive no matter what future environment arrives, increasing the odds that a population avoids extinction. Thus, bet-hedging species accept a lower arithmetic mean fitness for a higher geometric mean fitness (which equals zero if there is a single generation of no fitness). Theoretical work shows that bet-hedging strategies relying on random non-heritable variation outperform adaptive tracking in some environments (6, 16, 17). In particular, bet-hedging outperforms adaptive tracking when the environment fluctuations on timescales similar to the lifespan, as adaptive tracking requires multiple generations to respond to environmental cues (15, 18), and can lag behind fluctuating selective pressures. While phenotypic plasticity occurs on faster timescales, the ability to adapt and learn can be metabolically costly and provides limited buffer against sudden changes in selective pressures (19). Intrinsic variability in genetic control (1) has been identified as a source of phenotypic variation in microbes. In multicellular organisms, stochastic processes in development underlie variation (20), and, in the case of behavior, stochastic neuronal wiring (4, 21–23) has been shown to predict persistent individual differences.

Individuals, however, do not express a singular behavioral phenotype across their life. Individual biases in multiple behavioral settings are only partially consistent over time (3, 5, 24–27), showing spontaneous changes even in the absence of macroscopic cues that could trigger plasticity. These observations raise two questions: to what degree does individuality arise due to

developmental differences versus spontaneous fluctuations within the lifetime of the animal, and do fluctuations across the lifetime of an animal provide a evolutionary benefit? To answer these questions, we used isogenic animals to measure 1) the extent of variation present at the start of adulthood ("bet-hedging") and 2) the amount behavioral phenotypic change over time ("drift"). We use locomotor handedness in *Drosophila melanogaster* as a model to measure how behavior drifts over time and show that the extent of drift is influenced by genes and the neuromodulator serotonin. We then use a life-history model to test the hypothesis that drift is adaptive and assess whether real world environmental fluctuations may drive the evolution of drift as a phenotypic strategy. Taken together, these experiments and analyses suggest that phenotypic drift is plausibly an adaptive strategy to cope with environmental fluctuations within an organism's lifespan.

Results

Individual behavioral biases drift over time

To investigate the timescales on which individual preference spontaneously changes, we placed 252 flies in individual circular arenas and continuously tracked them for up to 30 days. At each time point, we computed the direction of circling (**Figure 1A**), a measure that exhibits idiosyncratic variation, when averaged over long timescales(3). To look for slow changes in bias, we examined the turning bias of individual flies at different timescales: first, we averaged their direction of circling over each hour and second we lowpass filtered their continuous turning behavior to discard frequency components faster than 24hrs (**Figure 1B**). Comparing both of these measures to the experiment-wide average preference for each fly shows substantial long-duration changes from their experiment-wide circling tendency. To quantify this across all flies we calculated the average power spectrum of turning across all measured flies (**Figure 1C**), showing substantial power in lower frequencies corresponding to hours- to days-long fluctuations in turning preferences. Interestingly, we did not see peaks at any specific frequencies (e.g., circadian), though there was a broad shoulder of power between 10^{-4} and 10^{-2} Hz. The overall trend across six orders of magnitude bore some resemblance to a power-law relationship between frequency and power. We saw similar patterns in other measures of behavior from this experiment, including speed, heading velocity, and distance from the center of the arena (Fig S1A-D).

Genes regulate the rate of behavioral drift

The extent of behavioral variability in a population differs between genotypes (28–30). We next looked to see if the rate of behavioral drift also differs between genotypes. This is a prerequisite if drift can evolve as a trait under natural selection. We used 10 different lines from the *Drosophila* Genetic Resource Panel (DGRP) (31). Using a Y-maze assay (**Figure 1D**) (3), we measured the locomotor handedness of flies in each of these lines three times weekly for three weeks. As in the circling assay (**Figure 1A-C**), individual flies exhibited substantial variation in their daily average turn biases (consistent with drifting biases) in all genotypes, as measured by the standard deviation in the daily fraction of right turns for each fly, showing substantial variation from their experiment-wise average (**Figure 1E**). We used a Bayesian autoregressive model (**Figure 1F**) to estimate the initial variability in turn bias for each genotype ($\sigma_{\text{Bet-Hedging}}$), the extent to which turn bias changed each day (σ_{Drift}), and the tendency of turn bias to revert to the mean (ϕ). We observed large differences in all three parameters between genotypes, as evidenced by non-overlapping 95% posterior distributions (**Figures 1G**, S1E). Interestingly, we saw independent variation across genotypes in the posteriors for $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift}, suggesting that different genetic mechanisms regulate bet-hedging and drift.

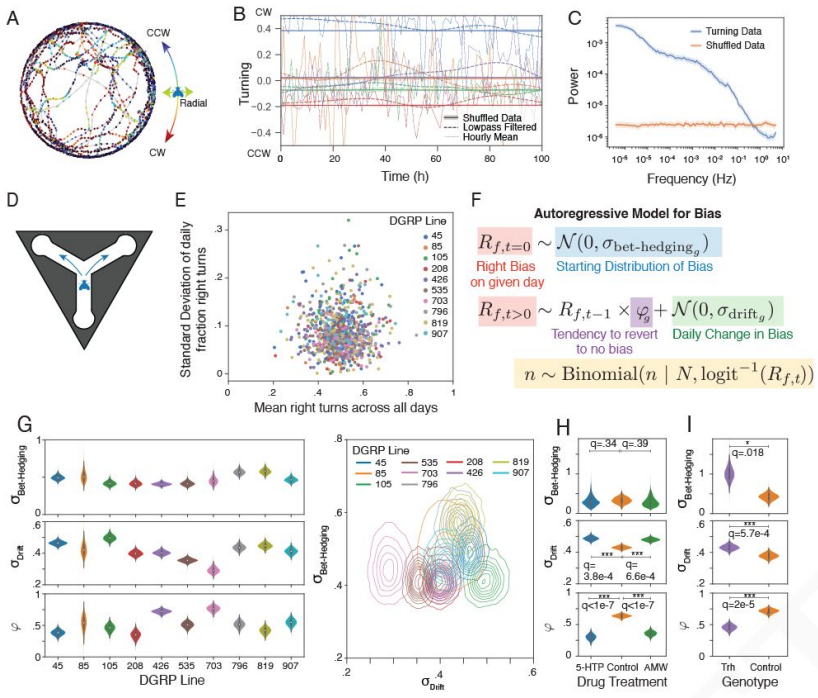

Fig. 1.

Characterizing changes in individual preferences in *Drosophila melanogaster*. **A**. 2 hr sample of centroid-tracking data for a fly in a circular arena. Each point is colored based on whether it is moving CCW, CW, or radially in the arena. **B**. Sample of 100h of continuous recording for 4 individual flies (colors). Hourly means of turning indices are shown in light lines. Dashed lines are lowpass filtered with a timescale cutoff of 24 h. Solid lines are lowpass-filtered shuffled data showing the average tendency across the experiment. **C**. Mean power spectrum of turning data for all flies (n=252) for actual and shuffled data. Shaded areas represent 95% confidence intervals generated via bootstrapping (n=1000). **D**. Schematic of Y-maze assay. Flies make either a left or right turn each time they walk through the intersection. **E**. Standard deviation of daily right biases vs average right bias across days for individual flies (points). Colors indicate DGRP genotype. **F**. Autoregressive model of individual right bias over time with parameters to estimate the initial right bias variability ($\sigma_{\text{Bet-Hedging}}$) and rate of daily change in right bias (σ_{Drift}). **G**. Posterior estimates of σ_{Drift}, $\sigma_{\text{Bet-Hedging}}$, and φ (the autoregressive parameter characterizing the rate of reversion to zero bias), for each DGRP genotype. Right: Countour plot of 2-dimensional joint posterior over σ_{Drift} and $\sigma_{\text{Bet-Hedging}}$ Lines represent deciles of the posterior distribution, with the outermost line representing 95% posterior density.. **H**. As in G-Left, posteriors estimates of right bias variability parameters for flies treated with 5-HTP, AMW, and controls. **I**. As in H, for control and mutant flies with a missense mutation in TrH generated by in vivo CRISPR.

Serotonin modulates the rate of behavioral drift

Neuro-modulators have been shown to play an important role in regulating individuality in a large range of organisms (8, 32–35), including flies (5, 36, 37). To test whether serotonin affects the rate of drift (and bet-hedging), we fed isogenized Oregon-R flies food supplemented with either the serotonin synthesis inhibitor aMW or the serotonin precursor 5HTP and measured their locomotor handedness for 3 days each week for 3 weeks, as in the previous experiment. While $\sigma_{\text{Bet-Hedging}}$ was similar in all three treatment groups, both pharmacological manipulations of serotonin decreased the stability of behavior over time, increasing σ_{Drift} and decreasing ϕ (**Figure 1H**, S1F). This was also evident in decreased correlation coefficients between handedness on successive days (Figure S1H-J) compared to control flies.

To assess the role of serotonin as a regulator of drift by a second approach, we generated a constitutive mutation in the tryptophan hydroxylase gene (*trh*), which is involved in the synthesis of serotonin. This approach also controls for off-target pharmaceutical effects. We used *in vivo* CRISPR (38) to knock out *trh* in an isogenized Oregon R background, yielding control flies of REFREF genotype with a closely matched genetic background. Knocking out *trh* led to an increase in both $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift} and decrease in ϕ compared to controls (**Figure 1I**, SG), as well as a decrease in correlation between control and *trh* null flies (Figure S1K-M).

A theoretical adaptive advantage for drift

The variation we see in σ_{Drift} among genotypes suggests that drift can potentially evolve to provide fitness advantages in some situations. To build an intuition for the ideal drift strategy and explore possible theoretical foundations, we formulated a simple two-state model (**Figure 2A**). Imagine an organism in an environment that shifts probabilistically between two states. The organism can exhibit two behavioral phenotypes. If the chosen phenotype matches the environment, the organism survives; if not, it dies. What is the optimal strategy for changing its behavior to survive probabilistic shifts in the environment that occur with probability p? We can prove, through methods analogous to classic findings on optimal betting solutions (39, 40) and previous analytical work on generational bet-hedging (41, 42), that in this two-state model, the optimal fraction of the population that should shift their behavioral phenotype in order to maximize long-term population growth equals p. This result extends to both the case of an arbitrary number of possible states as well as a continuous distribution of environmental states and behavioral phenotypes for all cases where the fitness narrowly depends on a tight match to a given environment (see Supplementary Materials for proofs). Interestingly, this holds true even when the fitness associated with successfully matching phenotype to environment A vs differs from matching environment B – in other words, matching the environment as much as possible is the most important thing, independent of the quality of specific environments.

A flexible model of drift shows a potential adaptive role under some patterns of environmental change

To model the effect of more realistic environmental fluctuations, we added dynamic environments, fitness effects and life history to the auto-regressive model we used to fit the experimental behavioral data (**Figure 2B**). In this model, individuals, tracked at the population level, exhibit different behavioral preferences that can potentially vary over their lifespans. The environment fluctuates, and the survival probability of an individual increases as the difference between their preference and the environment decreases. Two parameters determine the phenotypic strategy: $\sigma_{\text{Bet-Hedging}}$, captures the initial variability of the population, and σ_{Drift}, captures the amount each fly's preference changed per day. By integrating this model over time, we estimate the final population size of populations with varying levels of $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift} (**Figure 2C-D**, S2), thereby determining the optimal variability strategy for a given pattern of

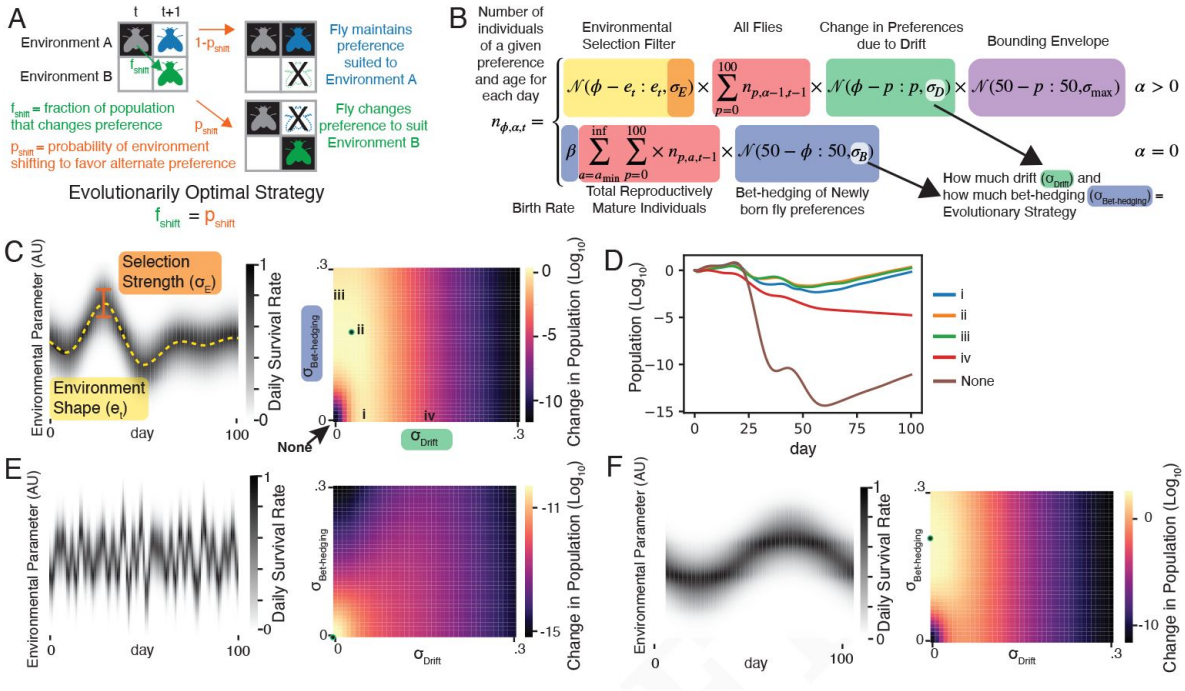

Fig. 2.

Modeling adaptive effects of drift. **A**. In a simplified model in which both phenotypes and environments have two states, the optimal fraction of the population that should change preference (f_{shift}) over a period of time equals the probability the environment changes (p_{shift}). See Supplementary text REFREF. **B**. Model for the number of individuals with a particular continuous preference as a function of time in a fluctuating environment and individual age. $n_{\phi,a,t}$, is the number of individuals with preference ϕ, age a at day t in the simulation. Two different cases determine this value, one for flies surviving from the previous timestep ($a > 0$), and one for flies born in a particular timestep ($a = 0$). The number of flies of a particular behavioral phenotype surviving on each successive day is determined by a function of how that preference is from the ideal preference on that day (orange), the total number of flies that already have, or drift into having that phenotype on that day (red), and a bounding term that stops the distribution of preferences from diffusing away from general range of what is adaptive (purple). The key behavioral strategy parameter from these terms is σ_d, which determines the rate at which flies' preferences drift over time. The number of new flies born each day is given by the total number of flies above the age of reproductive maturity a_{min} (red) times the birth rate β. New flies are born with an initial preference from a normal distribution centered on the long term environmental mean with a standard deviation given by $\sigma_{\text{bet-hedging}}$ (blue) **C**. Example environmental fluctuations and corresponding fitness landscape showing final populations for differing amounts of drift and bet-hedging. Green dot indicates ideal strategy (ii)**D**. Population over time for strategies marked with roman numerals in (C). **E, F**. As in (C) for two additional example environmental fluctuation patterns.

environmental fluctuations. Sampling a few randomly generated environments quickly revealed that different patterns of fluctuation favored different strategies (**Figure 2E-F**), so we began to systematically assess what characteristics of the environment and life history might favor drift, bet-hedging or combinations of both.

Drift is an effective response at shorter timescale fluctuations than bet-hedging

To characterize the adaptive value of bet-hedging and drift strategies as a function of statistics of environmental fluctuations, we created randomized environmental dynamics by filtering white noise in the time domain, leading to random fluctuations with a given temporal frequency content. We normalized and scaled the resulting environmental patterns, and used them in simulations of population survival for a range of bet-hedging ($\sigma_{\text{Bet-Hedging}}$) and drift ($\sigma_{\text{Drift}}$) values. From these simulations we constructed a fitness landscape over the four dimensions of $\sigma_{\text{Bet-Hedging}}$, σ_{Drift}, environmental fluctuation frequency and environmental fluctuation amplitude. At very high frequency fluctuations the most successful strategy was $\sigma_{\text{Bet-Hedging}} = \sigma_{\text{Drift}} = 0$, reflecting an optimal strategy of all individuals tightly matching the average conditions of the environment (**Figure 3C**). Similarly, low amplitude fluctuations favored low bet-hedging and low drift. These findings make sense: low amplitude fluctuations are effectively a static environment that does not require a variable phenotypic strategy, and very rapid fluctuations changing within the timescale of organismic response are averaged away (6, 15, 18). However, as the amplitude of the fluctuations increased and the frequency decreased, the optimal strategy showed increased amounts of drift and bet-hedging, as producing individuals with a preference far from the environmental mean became increasingly necessary for survival. While both drift and bet-hedging are preferred over no variability in these situations, higher frequency fluctuations favor drift, while lower frequency fluctuations favor bet-hedging. Increasing the time to sexual maturity increases the advantage of drift, suggesting that varying a phenotype dynamically provides a mechanism for organisms to survive long enough to reproduce. These results suggest that the relative benefit of a drift or bet-hedging strategy depends on the rate of fluctuation of the environment compared to the development time of an organism. These relationships hold for a wide range of β (Figure S3A) and a_{min} (Figure S3B-C).

Real world environmental time series may favor drift

To predict the adaptive value of drift strategies in somewhat less synthetic circumstances, we collected 43 types of environmental time series from publicly available NOAA (43) and NEON (44) datasets across 118,618 sites. We randomly sampled contiguous 1000-day time series from each of these datasets from the past 20 years, and normalized each sample's mean and standard deviation, scaled by σ_{mean} to represent different amplitudes of selective pressure. We then used these time series as model inputs to calculate the optimal amounts of drift and bet-hedging as a function of σ_{mean} and a_{min} (**Figure 4A-B**). Different patterns of environmental fluctuation, across differing measurement types and locales, showed differing optimal amounts of drift and bet-hedging. As in our previous analyses, increasing σ_{mean} increased the optimal σ_{Drift} and $\sigma_{\text{Bet-Hedging}}$ (**Figure 3**), and increasing the a_{min} increased the optimal magnitude of drift (**Figure 4A**, S4B,D).

To relate optimal strategies of drift and bet-hedging to climate and environmental factors, we used principle components analysis (PCA) to study variation in fitness landscapes over $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift} (**Figure 4C**). The first principle component of variability strategy landscapes had the vast majority of the variance (97.8%) and encoded the extent of bet-hedging; the second component (1.9%) encoded the extent of drift. Across time series, we observed a variety of optimal amounts of drift and bet-hedging. A substantial minority (12%) of environmental signals favored non-zero drift (**Figure 4D, E**), particularly those reflecting temperature and wind-speed measurements

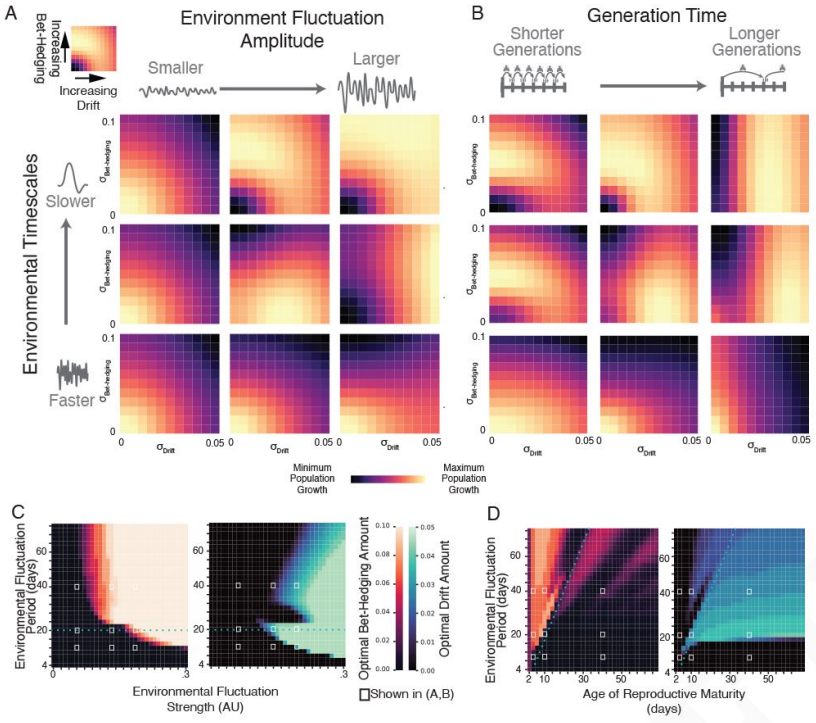

Fig. 3.

Effects of amplitude of environmental fluctuation, frequency of fluctuation, and generation time on ideal amounts of bet-hedging and drift. **A**. Fitness landscapes over the space of environmental fluctuation amplitude and frequency. Each heatmap shows the geometric mean of the log change in population for each combination of drift and bet-hedging over 100 randomized environments. Heatmaps are normalized to their maximum and minimum values. Rows of heatmaps have the same environmental fluctuation frequency and columns have the same environmental fluctuation amplitude (as measured by the standard deviation of all timepoints σ_{Mean}. The nine amplitude and frequency combinations in this panel correspond to values denoted with white boxes in (C). **B**. As (A), except columns of heat maps have the same generation times, as determined by a_{min}. **C**. Optimal amounts of bet-hedging (warm color scale; left) and drift (cool color scale; right) for each combination of environmental fluctuation amplitude and frequency. White squares indicate values associated with heatmaps in A. Dotted blue line corresponds to an environmental fluctuation period of 20 days, which is twice the (fixed) generation time in these simulations. **D**. As (C), except for optimal amounts of bet-hedging and drift for each combination of environmental fluctuation frequency and a_{min}. White squares indicate values associated with heatmaps in (B).

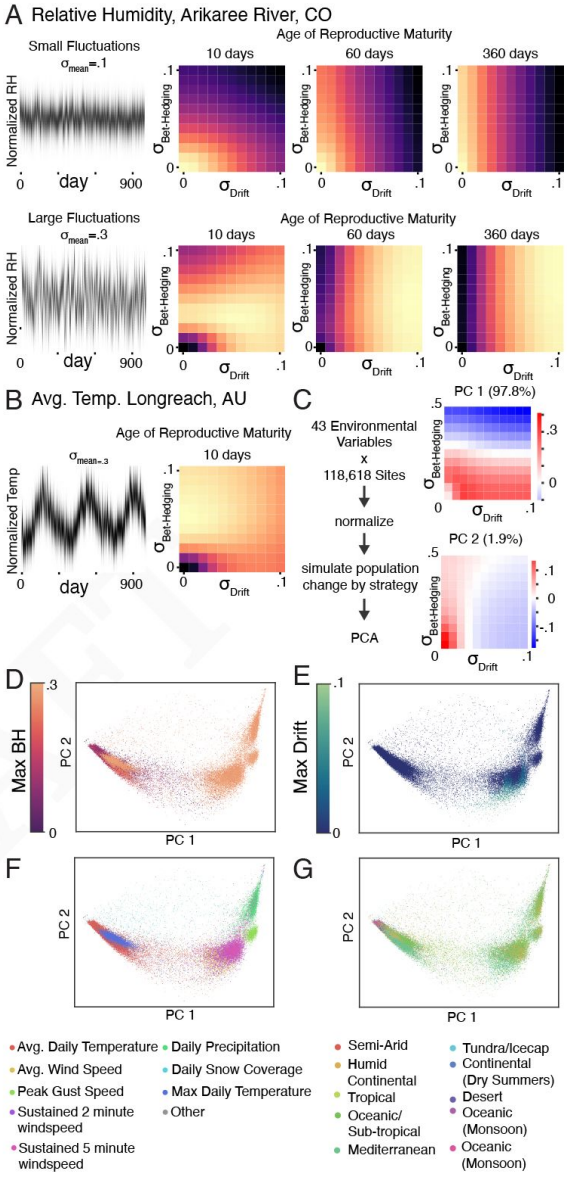

Fig. 4.

Optimal bet-hedging and drift strategies for real world environmental fluctuations. **A**. 1000 days of relative humidity data from the Arikaree River in Colorado, USA (left panels) were used to generate an environmental selection filter with low (top-left) or high (bottom-left) amplitude fluctuations (σ_{mean} in **Figure 2B**). Fitness landscape heatmaps over bet-hedging and drift strategies for different ages of reproductive maturity (a_{min}). **B**. As in (A), using average daily temperature data from Longreach, Australia. **C**. Pipeline for comparing the optimal variability strategies of organisms subject to real world environmental fluctuations. Daily environmental time series from many sites were collected, normalized, and used in the model to produce fitness landscapes over $\sigma_{Bet\text{-}Hedging}$ and σ_{Drift}. All landscapes were then subject to principle components analysis. These simulations held a_{min} and σ_{mean} constant. See Methods. The loadings of PC1 (97.8% of the variance; top-right) indicate that this component encodes the optimal amount of bet-hedging, while PC2 (1.9% of the variance; bottom-right) encodes optimal drift. **D**. Environmental time series from specific locations, plotted on PC2 vs PC1 axes, colored by optimal amount of bet-hedging. **E**. As in (D), except color indicates optimal amount of drift. **F**. As in (D), except color indicates the type of environmental measurement. **G**. As in (D), except color indicates the the Köppen climate classification of their location.

and a subset of locations with tropical, sub-tropical, Mediterranean and continental climates (**Figure 4F, G**). These results depend on the a_{min} in our model, with later ages favoring drift (Figure S4).

Discussion

We found that individual behavioral biases are not stable over an animal's lifetime, and that the degree of stability in behavioral bias is influenced by genetics and neuromodulation. Inspired by these findings, we provide a theoretical rationale for how instability in preferences could be adaptive for the long-term survival of individuals and species.

The behavioral preferences of individual flies change continuously over their life on days-long timescales (**Figure 1 A-C**; S1A-D). While indications of this phenomenon have been seen previously (3, 5, 24, 45–47), these studies examined a small number of time points or recorded behavior continuously for up to a week. By measuring behavior continuously for up to four weeks (roughly the lifespan of a fly), we were able to estimate the complete power spectrum of behavior: the continuous distribution of timescales at which it changes. Across multiple behaviors (**Figure 1C**, Figure S1A-D), we show that there is no specific timescale over which behavioral preferences change (e.g., between bouts of movement), and suggests that individuality both quickly and slowly (and on intermediate timescales).

Examining several different inbred strains derived from a natural fly population, we found that the stability of preferences depends on genotype (**Figure 1H**). This implies that there is natural allelic variation that affects the rate of behavioral drift. In turn, this suggests that drift is not strongly maladaptive, in which case we would expect alleles involved in promoting drift to have been selected out of the population. Genetic variation for drift implies that the rate of drift could evolve to increase the fitness of animals subject to different kinds of environmental fluctuations. However, genetic variation for drift does not, *per se*, demonstrate that drift is subject to selection; drift could be a neutral epiphenomenon of some other trait under selection.

Importantly, some lines with differing magnitudes of initial preference variability ($\sigma_{bet\text{-}hedging}$) exhibited similar amounts of preference stability (σ_{drift}) (e.g., lines DGRP 819 and 426). This suggests that mechanisms that drive differences in population preferences developmentally incompletely overlap with mechanisms that drive stability in preferences as adults.

Serotonin has been implicated as a regulator of the extent of in individuality in fly behavior (5, 36, 37) and stability of idiosyncratic preferences in *C. elegans* (33, 48). Bidirectional pharamacological perturbation of serotonin signaling (increasing it with serotonin precursors, or decreasing it with synthesis inhibitors or genetic perturbations) decreases the stability of preferences over time (or conversely increases the amount of drift) (**Figures 1H,I**, S1F-M). Constitutive mutations in the serotonin synthesis pathway increase both the amount of drift and the amount of variability we observe, suggesting that serotonin has a role in regulating preferences both during and after development.

The combination of genetic and neuromodulatory control suggests that extent of behavioral drift could evolve if it confers a fitness advantage in natural settings. The bethedging framework provides a theoretical basis for the adaptive value of stable idiosyncrasy in behavior(2, 49): namely, that variability in progeny phenotypes increases the chances that at least some offspring are fit when the environment fluctuates unpredictably. We hypothesize that drift may be advantageous for similar reasons, just operating within the lifespan of each individual. We provide two arguments in support of this hypothesis. First, we show in several analytically

tractable cases (**Fig 2A**, Supplemental Materials) that it is evolutionarily optimal for an individual to switch behavioral preference phenotypes with a probability equal to the probability the environment changes.

This analytical result matches what we find using a more biologically realistic computational model of drifting individual preferences in fluctuating environments. We find that drift is adaptive when the environment changes faster than the generation time of an animal. This finding suggests that behaviors that confer fitness with respect to aspects of the environment that change quickly should correspondingly change more quickly. Conversely, stable behavioral preferences may confer fitness with respect to stable aspects of the environment. This is borne out by simulating populations in different environments: quickly changing environmental parameters such as relative humidity (**Figures 4A**, S4D) favor a drift strategy, while environments that vary at longer (e.g., seasonal) time scales, such as average daily temperature, favor a bet-hedging strategy (**Figures 4B**, S4D). A key parameter of this model is the age of reproductive maturity ($a_{\min}$). Drift provides a mechanism for organisms with slow development times to survive fluctuating environments long enough to reproduce. Thus, a key quantity is the time scale of environmental fluctuations relative to maturation time. We predict that organisms with more delayed onsets of reproduction will be more likely to exhibit drift compared to organisms with more rapid development (subject to the same environmental fluctuations).

Organisms employ many strategies to survive changing environments. While our study focused on the respective advantages of bet-hedging (stable variability at the individual level) and drift (variability within an individual's lifespan), these findings complement previous work comparing bethedging and adaptive tracking (selection-induced changes in allelic frequency over time(50)). Previous work on thermal preference in flies suggested that adaptive tracking offers an advantage when the environment fluctuates on approximately years-long time scales, whereas bet-hedging offers an advantage for months-long fluctuations (6, 30). These time scales roughly correspond to several fly lifespans and one lifespan, respectively. This study finds that drift may be an adaptive strategy for environmental fluctuations within a lifespan. Thus, adaptive tracking, bet-hedging and drift appear to be complementary strategies for the challenges of environmental fluctuation over a wide range of time scales.

Importantly, random phenotypic drift and bet-hedging comes at potentially high costs. If organisms could detect (or predict) environmental fluctuations and deterministically change their phenotype to be optimal for the realized environment, they could avoid the losses of randomly choosing the wrong phenotypes. Nonetheless, drift may play an adaptive role in some systems for two key reasons. Firstly, sensing and predicting future environments may be metabolically costly (19), especially for all possible environments. Random changes in preferences may therefore be more efficient than evolving the ability to reliably adapt to any fluctuation.

Second, random strategies may be inherently optimal. In a game theoretic context, finite games always have an optimal strategy (a Nash equilibrium), but this strategy may have to be random. For instance playing randomly in Rock Paper Scissors is optimal in so far as your opponent cannot learn to predict your play and exploit it. The randomness of stochastic evolutionary strategies, such as bet-hedging and drift, may thus be truly optimal(41, 42). This may particularly likely if the fitness effect of a particular behavioral phenotype depends on fluctuating game-theoretic interactions.

This paper characterizes changes in individual behavior within a fly's lifetime, and proposes a theoretical framework in which such changes are evolutionarily adaptive. Together, these results motivate continued study of the biological mechanisms underpinning behavioral drift as well as empirical studies to test the hypothesis that behavioral drift helps organisms survive rapidly changing environments.

Materials and methods

See SI Materials and Methods for details. All Raw Data, Data Acquisition Software, and analysis scripts are available at *http://lab.debivort.org/drift-in-individual-preference/* and *https://zenodo.org/doi/10.5281/zenodo.13698148*. Analysis scripts are available at *https://github.com/Maloney-Lab/Drift-in-Individual-Preference.git*

Fly Care

Flies were raised at room temperature and maintained at room temperature. Flies were fed cornmeal/dextrose medium, as previously described (5). Flies were maintained in communal housing before the first time point (Age 2-5), and then maintained in individual housing to maintain identity at later timepoints. Flies were kept on normal 12:12h light dark cycles.

Behavior

All behavioral experiments were tracked using MARGO, as described previously (51).

Continuous Circling Experiments

For the 24hr continuous circling experiments (**Fig 1 A-C**, Fig S1A-D), flies were placed in a 28mm radius circular arena filled with standard fly allowing 3mm of space between food floor and lid. Arenas were illuminated with white light from 9am to 9pm.

Handedness

For y-maze experiments, flies were placed in y-mazes as described previously (3, 51). Flies were allowed to roam freely in the y-maze for two hours, and the number of left and right turns were computed for each fly. Y-maze experiments were done illuminated in white light.

Statistics and Modeling

Data were analyzed in Matlab and Python. Bayesian analysis was performed using the STAN programming language (52). All simulations were done in Python.

Real World Data

Data was collected from publicly available sources (NEON(44) and NOAA(43)) for 43 environmental variables across 118,618 sites sampled from the last 20 years.

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Author information

Ryan Maloney
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States, Department of Psychology, Colorado College, Colorado Springs, United States
ORCID iD: 0000-0002-6111-7822

For correspondence: rtmaloney@coloradocollege.edu

Athena Ye
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States

Sam-Keny Saint-Pre
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States, Tufts University, Medford, United States
ORCID iD: 0009-0008-9352-1484

Tom Alisch
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States
ORCID iD: 0000-0001-7884-7031

David Zimmerman
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States
ORCID iD: 0000-0002-8344-7072

Nicole Pittoors
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States
ORCID iD: 0000-0001-6581-9568

Benjamin L de Bivort
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, United States
ORCID iD: 0000-0001-6165-7696

Editors

Reviewing Editor
P Robin Hiesinger
Institute for Biology Free University Berlin, Berlin, Germany

Senior Editor
Albert Cardona
University of Cambridge, Cambridge, United Kingdom

Reviewer #1 (Public review):

Summary:

In "Drift in Individual Behavioral Phenotype as a Strategy for Unpredictable Worlds," Maloney et al. (2024) investigate changes in individual responses over time, referred to as behavioral drift within the lifespan of an animal. Drift, as defined in the paper, complements stable behavioral variation (animal individuality/personality within a lifetime) over shorter timeframes, which the authors associate with an underlying bet-hedging strategy. The third timeframe of behavioral variability that the authors discuss occurs within seasons (across several generations of some insects), termed "adaptive tracking." This division of "adaptive" behavioral variability over different timeframes is intuitively logical and adds valuable depth to the theoretical framework concerning the ecological role of individual behavioral differences in animals.

Strengths:

While the theoretical foundations of the study are strong, the connection between the experimental data (Figure 1) and the modeling work (Figure 2-4) is less convincing.

Weaknesses:

In the experimental data (Figure 1), the authors describe the changes in behavioral preferences over time. While generally plausible, I identify three significant issues with the experiments:

(1) All of the subsequent theoretical/simulation data is based on changing environments, yet all the experiments are conducted in unchanging environments. While this may suffice to demonstrate the phenomenon of behavioral instability (drift) over time, it does not properly link to the theory-driven work in changing environments. An experiment conducted in a changing environment and its effects on behavioral drift would improve the manuscript's internal consistency and clarify some points related to (3) below.

(2) The temporal aspect of behavioral instability. While the analysis demonstrates behavioral instability, the temporal dynamics remain unclear. It would be helpful for the authors to clarify (based on graphs and text) whether the behavioral changes occur randomly over time or follow a pattern (e.g., initially more right turns, then more left turns). A proper temporal analysis and clearer explanations are currently missing from the manuscript.

(3) The temporal dimension leads directly into the third issue: distinguishing between drift and learning (e.g., line 56). In the neutral stimuli used in the experimental data, changes should either occur randomly (drift) or purposefully, as in a neutral environment, previous strategies do not yield a favorable outcome. For instance, the animal might initially employ strategy A, but if no improvement in the food situation occurs, it later adopts strategy B (learning). In changing environments, this distinction between drift and learning should be even more pronounced (e.g., if bananas are available, I prefer bananas; once they are gone, I either change my preference or face negative consequences). Alternatively, is my random choice of grapes the substrate for the learning process towards grapes in a changing environment? Further clarification is needed to resolve these potential conflicts.

https://doi.org/10.7554/eLife.103585.1.sa3

Reviewer #2 (Public review):

Summary:

This is an inspired study that merges the concept of individuality with evolutionary processes to uncover a new strategy that diversifies individual behavior that is also potentially evolutionarily adaptive.

The authors use a time-resolved measurement of spontaneous, innate behavior, namely handedness or turn bias in individual, isogenic flies, across several genetic backgrounds.

They find that an individual's behavior changes over time, or drifts. This has been observed before, but what is interesting here is that by looking at multiple genotypes, the authors find the amount of drift is consistent within genotype i.e., genetically regulated, and thus not entirely stochastic. This is not in line with what is known about innate, spontaneous behaviors. Normally, fluctuations in behavior would be ascribed to a response to environmental noise. However, here, the authors go on to find what is the pattern or rule that determines the rate of change of the behavior over time within individuals. Using modeling of behavior and environment in the context of evolutionarily important timeframes such as lifespan or reproductive age, they could show when drift is favored over bet-hedging and that there is an evolutionary purpose to behavioral drift. Namely, drift diversifies behaviors across individuals of the same genotype within the timescale of lifespan, so that the genotype's chance for expressing beneficial behavior is optimally matched with potential variation of environment experienced prior to reproduction. This ultimately increases the fitness of the genotype. Because they find that behavioral drift is genetically variable, they argue it can also evolve.

Strengths:

Unlike most studies of individuality, in this study, the authors consider the impact of individuality on evolution. This is enabled by the use of multiple natural genetic backgrounds and an appropriately large number of individuals to come to the conclusions presented in the study. I thought it was really creative to study how individual behavior evolves over multiple timescales. And indeed this approach yielded interesting and important insight into individuality. Unlike most studies so far, this one highlights that behavioral individuality is not a static property of an individual, but it dynamically changes. Also, placing these findings in the evolutionary context was beneficial. The conclusion that individual drift and bet-hedging are differently favored over different timescales is, I think, a significant and exciting finding.

Overall, I think this study highlights how little we know about the fundamental, general concepts behind individuality and why behavioral individuality is an important trait. They also show that with simple but elegant behavioral experiments and appropriate modeling, we could uncover fundamental rules underlying the emergence of individual behavior. These rules may not at all be apparent using classical approaches to studying individuality, using individual variation within a single genotype or within a single timeframe.

Weaknesses:

I am unconvinced by the claim that serotonin neuron circuits regulate behavioral drift, especially because of its bidirectional effect and lack of relative results for other neuromodulators. Without testing other neuromodulators, it will remain unclear if serotonin intervention increases behavioral noise within individuals, or if any other pharmacological or genetic intervention would do the same. Another issue is that the amount of drugs that the individuals ingested was not tracked. Variable amounts can result in variable changes in behavior that are more consistent with the interpretation of environmental plasticity, rather than behavioral drift. With the current evidence presented, individual behavior may change upon serotonin perturbation, but this does not necessarily mean that it changes or regulates drift.

However, I think for the scope of this study, finding out whether serotonin regulates drift or not is less important. I understand that today there is a strong push to find molecular and circuit mechanisms of any behavior, and other peers may have asked for such experiments, perhaps even simply out of habit. Fortunately, the main conclusions derived from behavioral data across multiple genetic backgrounds and the modeling are anyway novel, interesting, and in fact more fundamental than showing if it is serotonin that does it or not.

To this point, one thing that was unclear from the methods section is whether genotypes that were tested were raised in replicate vials and how was replication accounted for in the analyses. This is a crucial point - the conclusion that genotypes have different amounts of behavioral drift cannot be drawn without showing that the difference in behavioral drift does not stem from differences in developmental environment.

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Reviewer #3 (Public review):

Summary:

The paper begins by analyzing the drift in individual behavior over time. Specifically, it quantifies the circling direction of freely walking flies in an arena. The main takeaway from this dataset is that while flies exhibit an individual turning bias (when averaged over time), their preferences fluctuate over slow timescales.

To understand whether genetic or neuromodulatory mechanisms influence the drift in individual preference, the authors test different fly strains concluding that both genetic background and the neuromodulator serotonin contribute to the degree of drift.

Finally, the authors use theoretical approaches to identify the range of environmental conditions under which drift in individual bias supports population growth.

Strengths:

The model provides a clear prediction of the environmental fluctuations under which a drift in bias should be beneficial for population growth.

The approach attempts to identify genetic and neurophysiological mechanisms underlying drift in bias.

Weaknesses:

Different behavioral assays are used and are differently analysed, with little discussion on how these behaviors and analyses compare to each other.

Some of the model assumptions should be made more explicit to better understand which aspects of the behaviors are covered.

https://doi.org/10.7554/eLife.103585.1.sa1

Author response:

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*

> *In "Drift in Individual Behavioral Phenotype as a Strategy for Unpredictable Worlds," Maloy et al. (2024) investigate changes in individual responses over time, referred to as behavioral drift within the lifespan of an animal. Drift, as defined in the paper, complements stable behavioral variation (animal individuality/personality within a lifetime) over shorter timeframes, which the authors associate with an underlying bet-hedging strategy. The third timeframe of behavioral variability that the authors discuss occurs within seasons (across several generations of some insects), termed "adaptive tracking." This division of "adaptive" behavioral variability over different timeframes is intuitively logical and adds valuable depth to the theoretical framework concerning the ecological role of individual behavioral differences in animals.*
>
> *Strengths:*
>
> *While the theoretical foundations of the study are strong, the connection between the experimental data (Figure 1) and the modeling work (Figure 2-4) is less convincing.*
>
> *Weaknesses:*
>
> *In the experimental data (Figure 1), the authors describe the changes in behavioral preferences over time. While generally plausible, I identify three significant issues with the experiments:*
>
> *(1) All of the subsequent theoretical/simulation data is based on changing environments, yet all the experiments are conducted in unchanging environments. While this may suffice to demonstrate the phenomenon of behavioral instability (drift) over time, it does not properly link to the theory-driven work in changing environments. An experiment conducted in a changing environment and its effects on behavioral drift would improve the manuscript's internal consistency and clarify some points related to (3) below.*

In our framework, we posit that the amount of drift has been shaped by evolution to maximize fitness in the environments that the population has experienced, and this drift is observed independent of environment. While we agree that exploring the role of changing environments on the measure of drift would be interesting, we would anticipate the effects may be nuanced and beyond the scope of the current paper (and the scope of our theoretical work, which assumes that the individual phenotype is unaffected by change of environment except as mediated by death due to fitness effects). For example, it would be difficult to differentiate drift from idiosyncratic differences in learning (Smith et al., 2022), and non-adaptive plasticity to unrelated cues has been posited as a method of producing diverse phenotypes (Maxwell and Magwene, 2017), so “learning” to uncorrelated stimuli could conceivably be a mechanism for drift. Given the scope of the current study, we prioritized eliminating potential confounds for measuring drift, but remain interested in the interaction between learning and drift.

> *(2) The temporal aspect of behavioral instability. While the analysis demonstrates behavioral instability, the temporal dynamics remain unclear. It would be helpful for the authors to clarify (based on graphs and text) whether the behavioral changes occur randomly over time or follow a pattern (e.g., initially more right turns, then more left turns). A proper temporal analysis and clearer explanations are currently missing from the manuscript.*

We agree it would be helpful to have more description of the dynamics over time aside from the power spectrum and autoregressive model fits. We hope to address this in more detail to provide more description of the changes over time in a revision.

> *(3) The temporal dimension leads directly into the third issue: distinguishing between drift and learning (e.g., line 56). In the neutral stimuli used in the experimental data, changes should either occur randomly (drift) or purposefully, as in a neutral environment, previous strategies do not yield a favorable outcome. For instance, the animal might initially employ strategy A, but if no improvement in the food situation occurs, it later adopts strategy B (learning). In changing environments, this distinction between drift and learning should be even more pronounced (e.g., if bananas are available, I prefer bananas; once they are gone, I either change my preference or face negative consequences). Alternatively, is my random choice of grapes the substrate for the learning process towards grapes in a changing environment? Further clarification is needed to resolve these potential conflicts.*

As in our response to point 1, we believe this is a crucial distinction, and we intend to further highlight it in the discussion in the revision and further expand our discussion of how the two strategies may interact.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *This is an inspired study that merges the concept of individuality with evolutionary processes to uncover a new strategy that diversifies individual behavior that is also potentially evolutionarily adaptive.*
>
> *The authors use a time-resolved measurement of spontaneous, innate behavior, namely handedness or turn bias in individual, isogenic flies, across several genetic backgrounds.*
>
> *They find that an individual's behavior changes over time, or drifts. This has been observed before, but what is interesting here is that by looking at multiple genotypes, the authors find the amount of drift is consistent within genotype i.e., genetically regulated, and thus not entirely stochastic. This is not in line with what is known about innate, spontaneous behaviors. Normally, fluctuations in behavior would be ascribed to a response to environmental noise. However, here, the authors go on to find what is the pattern or rule that determines the rate of change of the behavior over time within individuals. Using modeling of behavior and environment in the context of evolutionarily important timeframes such as lifespan or reproductive age, they could show when drift is favored over bet-hedging and that there is an evolutionary purpose to behavioral drift. Namely, drift diversifies behaviors across individuals of the same genotype within the timescale of lifespan, so that the genotype's chance for expressing beneficial behavior is optimally matched with potential variation of environment experienced prior to reproduction. This ultimately increases the fitness of the genotype. Because they find that behavioral drift is genetically variable, they argue it can also evolve.*
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> *Strengths:*
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> *Unlike most studies of individuality, in this study, the authors consider the impact of individuality on evolution. This is enabled by the use of multiple natural genetic backgrounds and an appropriately large number of individuals to come to the conclusions presented in the study. I thought it was really creative to study how individual behavior evolves over multiple timescales. And indeed this approach yielded interesting and important insight into individuality. Unlike most studies so far, this one highlights that behavioral individuality is not a static property of an individual, but it dynamically changes. Also, placing these findings in the evolutionary context was beneficial. The conclusion that individual drift and bet-hedging are differently favored over different timescales is, I think, a significant and exciting finding.*

> *Overall, I think this study highlights how little we know about the fundamental, general concepts behind individuality and why behavioral individuality is an important trait. They also show that with simple but elegant behavioral experiments and appropriate modeling, we could uncover fundamental rules underlying the emergence of individual behavior. These rules may not at all be apparent using classical approaches to studying individuality, using individual variation within a single genotype or within a single timeframe.*
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> *Weaknesses:*
>
> *I am unconvinced by the claim that serotonin neuron circuits regulate behavioral drift, especially because of its bidirectional effect and lack of relative results for other neuromodulators. Without testing other neuromodulators, it will remain unclear if serotonin intervention increases behavioral noise within individuals, or if any other pharmacological or genetic intervention would do the same. Another issue is that the amount of drugs that the individuals ingested was not tracked. Variable amounts can result in variable changes in behavior that are more consistent with the interpretation of environmental plasticity, rather than behavioral drift. With the current evidence presented, individual behavior may change upon serotonin perturbation, but this does not necessarily mean that it changes or regulates drift.*
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> *However, I think for the scope of this study, finding out whether serotonin regulates drift or not is less important. I understand that today there is a strong push to find molecular and circuit mechanisms of any behavior, and other peers may have asked for such experiments, perhaps even simply out of habit. Fortunately, the main conclusions derived from behavioral data across multiple genetic backgrounds and the modeling are anyway novel, interesting, and in fact more fundamental than showing if it is serotonin that does it or not.*

We agree that our data do not support a strong conclusion that serotonin plays a privileged role in regulating drift. Based on previous literature (e.g. Kain et al., 2014, where identical pharmacological manipulations had an effect on variability while dopaminergic and octopaminergic manipulations did not), we think it likely that large global perturbations in serotonin that we observe are likely to influence plasticity that might be involved in drift (and thus find the results we observe not particularly surprising). Nonetheless, we agree that the mechanism by which serotonin may affect drift could be indirect, and it is similarly plausible that many global perturbations could lead to some shift in the amount of drift. We intend to further discuss these issues in the revision.

> *To this point, one thing that was unclear from the methods section is whether genotypes that were tested were raised in replicate vials and how was replication accounted for in the analyses. This is a crucial point - the conclusion that genotypes have different amounts of behavioral drift cannot be drawn without showing that the difference in behavioral drift does not stem from differences in developmental environment.*

While a cursory inspection suggests that batch effects between different replicates was small, we intend to clarify this and more explicitly address the effects of replicates in revision.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *The paper begins by analyzing the drift in individual behavior over time. Specifically, it quantifies the circling direction of freely walking flies in an arena. The main takeaway*

> *from this dataset is that while flies exhibit an individual turning bias (when averaged over time), their preferences fluctuate over slow timescales.*
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> *To understand whether genetic or neuromodulatory mechanisms influence the drift in individual preference, the authors test different fly strains concluding that both genetic background and the neuromodulator serotonin contribute to the degree of drift.*
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>
> *The approach attempts to identify genetic and neurophysiological mechanisms underlying drift in bias.*
>
> *Weaknesses:*
>
> *Different behavioral assays are used and are differently analysed, with little discussion on how these behaviors and analyses compare to each other.*

We intend to address this in a revision of the discussion.

> *Some of the model assumptions should be made more explicit to better understand which aspects of the behaviors are covered.*

We will further clarify the assumptions of the model in revision.

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