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Drift in Individual Behavioral Phenotype as a Strategy for Unpredictable Worlds

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eLife Assessment

Maloney et al. offer an **important** contribution to understanding the potential ecological mechanisms behind individual behavioral variation. By providing **compelling** theoretical and 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 to phenotypic variability between individuals (“bet-hedging”), individuals also show variability in their behavioral phenotype across time, even absent obvious external cues. There are few evolutionary theories that explain random shifts in phenotype across an animal’s life, which we term phenotypic drift. 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 behavioral drift and bet-hedging across a number of different fly strains and show independent strain-specific differences in bet-hedging and behavioral drift. We show manipulation of serotonin changes the rate of behavioral drift, indicating a potential circuit substrate controlling behavioral drift. We then develop a theoretical framework for assessing the adaptive value of phenotypic 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 behavioral 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 fluctuates 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 an evolutionary benefit? To answer these questions, we used near-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 (“behavioral drift”). We use locomotor handedness in *Drosophila melanogaster* as a model to measure how behavior drifts over time and show that the extent of behavioral drift is influenced by genes and the neuromodulator serotonin. We then use a life-history model to test the hypothesis that random change in phenotype (“phenotypic drift”, as a more general form of behavioral drift) can be adaptive and assess whether real-world environmental fluctuations may drive the evolution of phenotypic drift. 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 low-pass filtered their continuous turning behavior to discard frequency components faster than 24 hrs (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 the raw turning data across 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 behavioral 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) that measures left-right choices that correlate with locomotor handedness in circling (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), and evident in plotting the fraction right turns for each day of all flies (Figure 1F). We used a hierarchical Bayesian autoregressive model (Figure 1G) 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 persist over time (ϕ) (Figure 1G). We observed large differences in all three parameters between genotypes, as evidenced by minimally overlapping posterior distributions of estimates of these parameters (Figure 1H). All genotypes showed ϕ above zero, demonstrating some level of persistence in individual biases over time, consistent with our circling experiments and previous studies of Y-maze handedness (3). Interestingly, we saw independent variation across genotypes in the posteriors for $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift} , suggesting that different genetic mechanisms regulate behavioral bet-hedging and drift.

Manipulating serotonin modulates the rate of behavioral drift

Neuromodulators 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 behavioral drift (and/or bet-hedging), we fed adult, 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 $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift} were 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 1I, S1F). This was also evident in decreased correlation coefficients between handedness on successive days (Figure S1H–J) compared to control flies.

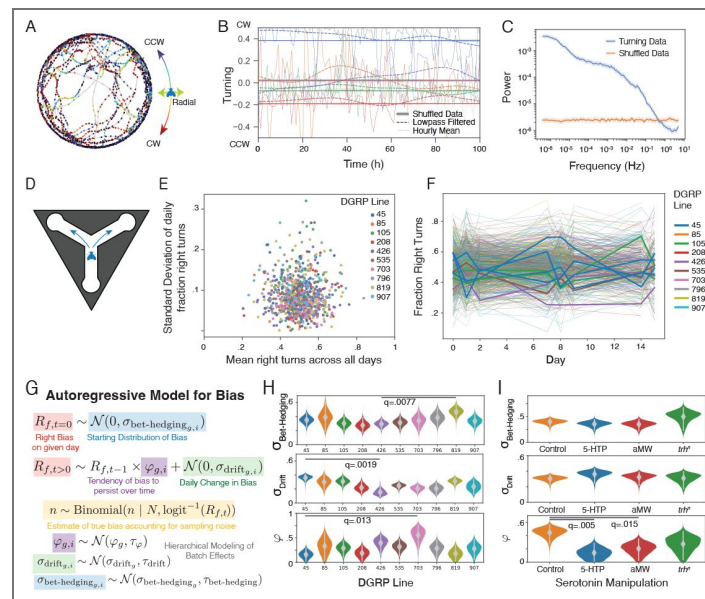


Fig. 1. Characterizing changes in individual preferences in *Drosophila melanogaster*.

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 low-pass filtered with a timescale cutoff of 24 h. Solid lines are low-pass filtered shuffled data showing the average tendency across the experiment. **C.** Mean power spectrum of turning data for all continuously monitored 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 ($n=48-235$ for each genotype (see Table S1)). **F.** Mean individual handedness per day across all DGRP lines, a random subset are thickened to show representative changes in individual handedness over time. **G.** 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}). **H.** 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. Grey bars represent 95% credible intervals. Lines indicate the smallest q value between populations. **I.** As in H, posterior estimates of right bias variability parameters for flies treated with 5-HTP, AMW, and controls, as well as mutant flies with a missense mutation in *trh* generated by in vivo CRISPR. $n=98$ or 192 for each condition (see Table S1). q values $<.05$ are indicated.

To assess the role of serotonin as a modulator of behavioral 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 pharmacological effects and, unlike the previous experiment, deprives the animals of serotonin during development as well as after eclosion. We used *in vivo* CRISPR (38) to knock out *trh* in an isogenized Oregon R background, yielding control flies of a closely matched genetic background. We did not see clear evidence of difference from control flies in the marginal distributions of $\sigma_{\text{Bet-Hedging}}$, σ_{Drift} or ϕ in our hierarchical model (Figure 1I, S1G), though we did see a significant decrease of day-to-day correlation between control and *trh* null flies (Figure S1K-M).

A theoretical adaptive advantage for phenotypic drift

The variation we see in σ_{Drift} between genotypes suggests that behavioral drift can potentially evolve to provide fitness advantages in some situations. To build an intuition for the ideal phenotypic 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 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 phenotypic 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 estimated the fold-change in population size 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 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 phenotypic drift, bet-hedging or combinations of both.

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

To characterize the adaptive value of bet-hedging and phenotypic 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

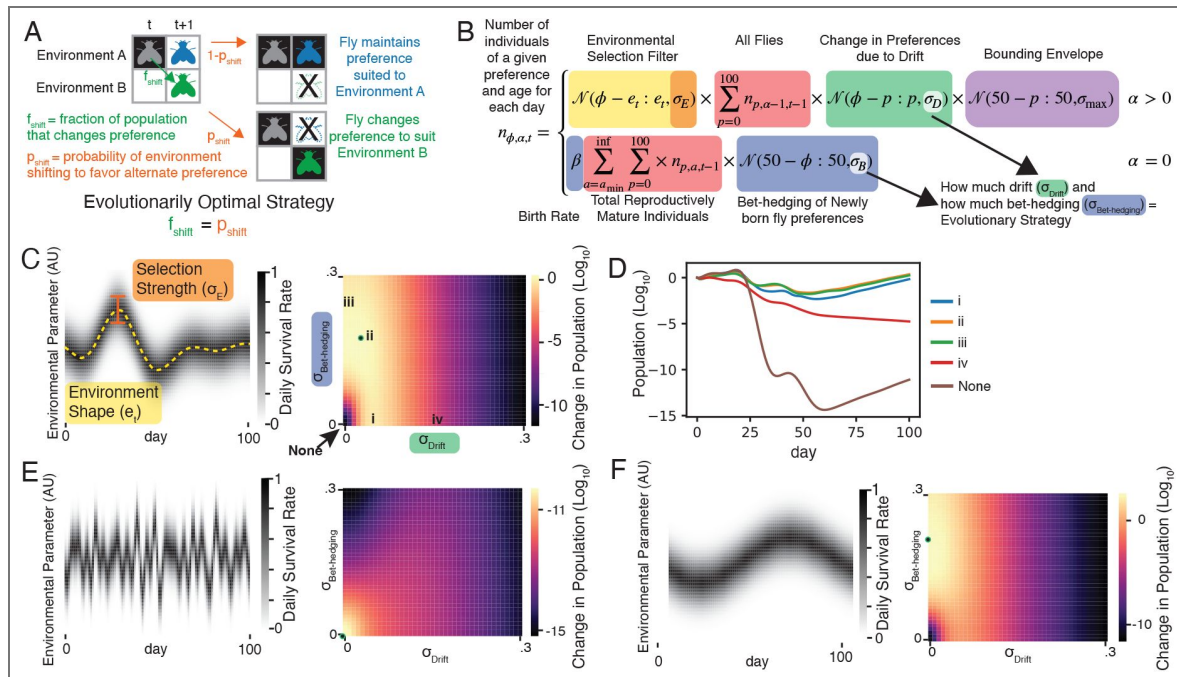


Fig. 2. Modeling adaptive scenarios of phenotypic drift.

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. **B.** Model for the number of individuals with a particular continuous preference as a function of time in a fluctuating environment and individual age. $n_{\phi, \alpha, t}$ is the number of individuals with preference ϕ , age α at day t in the simulation. Two different cases determine this value, one for flies surviving from the previous timestep ($\alpha > 0$), and one for flies born in a particular timestep ($\alpha = 0$). The number of flies of a particular behavioral phenotype surviving on each successive day is determined by a function of how far 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 (purple) that stops the distribution of preferences from diffusing away from general range of what is adaptive (adding some degree of reversion to the mean consistent with our observed values of ϕ , and via the Wiener-Kninchin theorem, consistent with the power spectrum observed in Fig 1C). 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 the change in population over 100 simulation days for differing amounts of phenotypic 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.

them in simulations of population survival for a range of bet-hedging ($\sigma_{\text{Bet-Hedging}}$) and phenotypic drift (σ_{Drift}) values. From these simulations we constructed a fitness landscape over the four dimensions of $\sigma_{\text{Bet-Hedging}}$, σ_{Drift} , environmental fluctuation period and environmental fluctuation amplitude (Figure 3A). 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 birthrates β (Figure S3A) and ages of reproductive maturity a_{min} (Figure S3B-C).

Real-world environmental fluctuations may favor phenotypic drift

To predict the adaptive value of phenotypic drift strategies in somewhat less synthetic circumstances, we collected 43 types of environmental time series from publicly available National Oceanography and Atmospheric Administration (NOAA) (43) and National Ecological Observatory Network (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, z-scored each time series, then scaled them to have standard deviations σ_{mean} to represent different amplitudes of selective pressure. We then used these time series as model inputs to calculate the optimal amounts of phenotypic 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 phenotypic drift and bet-hedging. As in our previous analyses, increasing σ_{mean} increased the optimal σ_{Drift} and $\sigma_{\text{Bet-Hedging}}$ (Figure 3C), and increasing the a_{min} increased the optimal magnitude of phenotypic drift (Figure 4A, S4B,D).

To relate optimal strategies of phenotypic drift and bet-hedging to climate and environmental factors, we used principal 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 phenotypic 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 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 higher ages of reproductive maturity favoring more phenotypic 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

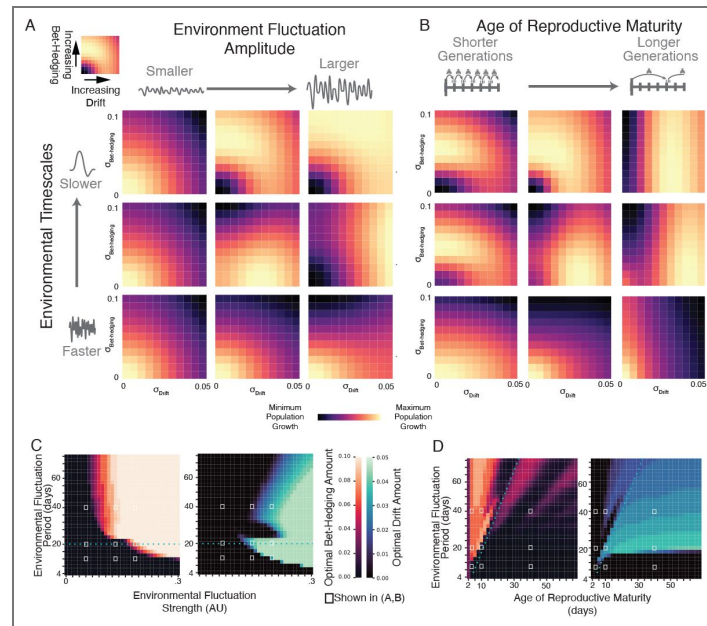


Fig. 3. Effects of amplitude of environmental fluctuation, frequency of fluctuation, and age of reproductive maturity on ideal amounts of bet-hedging and phenotypic drift.

A. Fitness landscapes over combinations 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 age of reproductive maturity, 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 age of reproductive maturity 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} . Dotted blue line corresponds to environmental fluctuations of twice the age of reproductive maturity. White squares indicate values associated with heatmaps in (B).

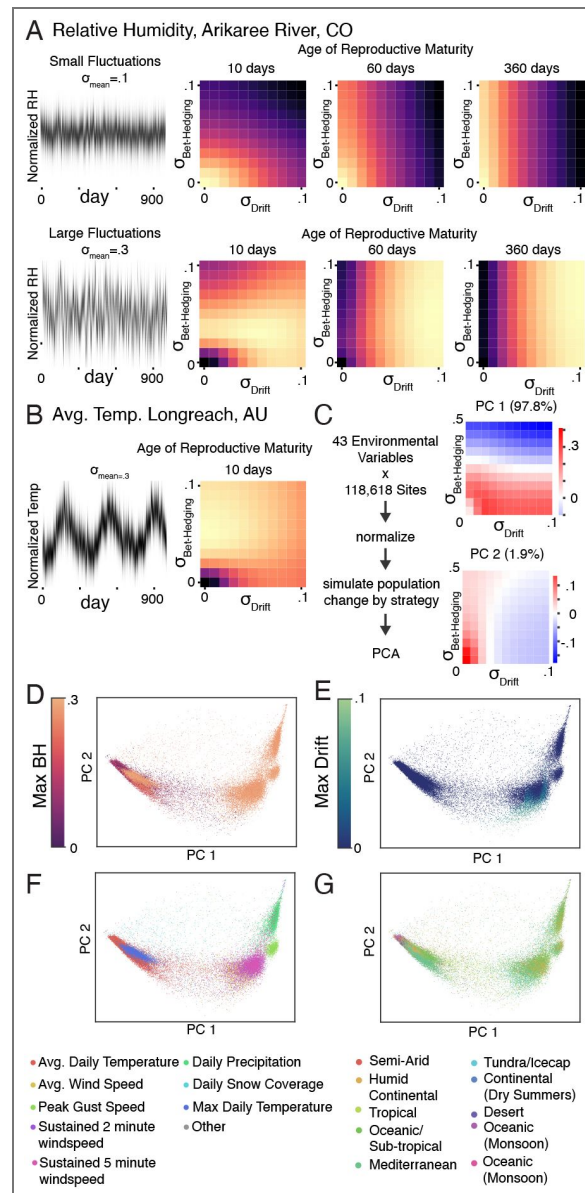


Fig. 4. Optimal bet-hedging and phenotypic drift strategies for real-world environmental fluctuations.

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 (σ_{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 $\sigma_{\text{Bet-Hedging}}$ and σ_{Drift} . All landscapes were then subject to principal components analysis. These simulations held σ_{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 Köppen climate classification of their location.

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., we did not see a circadian cadence to behavioral changes), and our data suggest that individuality shifts on a number of different time scales.

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 behavioral drift is not strongly maladaptive, in which case we would expect alleles involved in promoting behavioral drift to have been selected out of the population. Genetic variation for behavioral 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 behavioral 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 ($\sigma_{\text{bet-hedging}}$) 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 individuality in fly behavior (5, 36, 37) and stability of idiosyncratic preferences in *C. elegans* (33, 48). Bidirectional pharmacological 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 behavioral drift) (Figures 1I, S1F-M). Our results from constitutive mutations in the serotonin synthesis pathway are less compelling (though we still see a significant difference in the strength of day-to-day correlations). The more modest impact of constitutive *trh*ⁿ mutation may reflect compensatory effects playing out across development. Together, our serotonin manipulation experiments suggest that this neuromodulator reinforces stability in idiosyncratic individual behavior. Further study is necessary to determine if serotonin regulates drift through its role in synaptic plasticity or some other widespread effect on the *Drosophila melanogaster* nervous system.

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 bet-hedging 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 behavioral drift may be advantageous for similar reasons, but 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 of the environment changing to favor those behavioral phenotypes.

This analytical result matches what we find using a more biologically realistic computational model of drifting individual preferences in fluctuating environments. We find that phenotypic drift is beneficial when the environment changes faster than the time to reproductive maturity 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}). Phenotypic 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 phenotypic 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 phenotypic drift (variability within an individual's lifespan), these findings complement previous work comparing bet-hedging 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 phenotypic drift may be an adaptive strategy for environmental fluctuations within a lifespan. Thus, adaptive tracking, bet-hedging and phenotypic 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 come 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 phenotypic drift, may thus be truly optimal^(41, 42). This may be particularly likely if the fitness effect of a particular behavioral phenotype depends on fluctuating game-theoretic interactions.

The interaction between behavioral drift and learning is likely complicated. Studies have observed idiosyncratic differences in learning that help shape the population-level distribution^(51, 52). Specifically, variability in response to identical cues may lead to variation in the population. Similarly, non-adaptive plasticity in response to unrelated cues has been shown to be a mechanism for generating differences in a population as part of a bet-hedging strategy⁽⁵³⁾. While we limit our analysis in this paper to random shifts in phenotype rather than adaptive plasticity and learning, all these mechanisms of change likely co-exist in natural behaviors.

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.

Continuous Circling Experiments

For the 24 hr continuous circling experiments (Fig 1 A-C [4](#), Fig S1A-D), flies were placed in a 28mm radius circular arena filled with standard fly food allowing 3mm of space between food floor and lid. Arenas were illuminated with white light from 9am to 9pm. Flies were tracked with MARGO, as described previously ([54](#)).

Handedness

For Y-maze experiments, flies were placed in Y-mazes as described previously ([3](#), [54](#)). 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-mazes were illuminated in white light and flies were tracked with MARGO([54](#)).

Statistics and Modeling

Data were analyzed in Matlab and Python. Bayesian analysis was performed using the STAN programming language ([55](#)). 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.

Data availability

All Raw Data, Data Acquisition Software, and analysis scripts are available at <http://lab.debivort.org/drift-in-individual-preference/> [4](#) and <https://zenodo.org/doi/10.5281/zenodo.13698148> [4](#). Analysis scripts are available at <https://github.com/Maloney-Lab/Drift-in-Individual-Preference.git>

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Additional information

Author contributions

RM and BdB conceived this study, RM, AY, SS, TA, DZ, and NP performed experiments. RM and DZ produced proofs.

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Additional files

[Supplemental Material](#) 

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Peer reviews

Reviewer #1 (Public review):

Summary:

In "Drift in Individual Behavioral Phenotype as a Strategy for Unpredictable Worlds," Maloney et al. (2026) 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 compelling, the connection between the experimental data (Fig. 1) and the modeling work (Fig. 2-4) is convincing.

Weaknesses:

In the experimental data (Fig. 1), the authors describe the changes in behavioral preferences over time. While generally plausible, I had identified three significant issues with the experiments that were addressed in the revision:

(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 fully link to the theory-driven work in changing environments. A full experimental investigation of this would be beyond the scope of the current work.

(2) The temporal aspect of behavioral instability has been addressed in Figure 1F.

(3) The temporal dimension leads directly into the third issue: distinguishing between drift and learning (e.g., line 56). This issue has been further discussed in the revised manuscript.

<https://doi.org/10.7554/eLife.103585.2.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 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 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, 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 are regulating 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.

Comments on the latest version:

The changes to the manuscript sufficiently addressed my few comments. I do not have anything else substantial to add to my review and I am comfortable with my initial assessment.

<https://doi.org/10.7554/eLife.103585.2.sa2>

Reviewer #3 (Public review):

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), yet 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 in a Y maze concluding that both genetic background and the neuromodulator serotonin contribute to the degree of drift (although with some contrasting results). The use of a different assay for this different dataset (Y maze instead of wide arena) is justified by previous observation of similar behavioral biases in these assay. Yet the conceptual link between the spectral power analysis used for the first dataset and the autoregressive model used for the second remains unclear.

Finally, the authors use theoretical approaches to show the potential advantage of individual drift for survival in unpredictable, fluctuating environments. They demonstrate that while bet-hedging provides an advantage over timescales matching the generation time (since reproduction is required), it offers less benefit on shorter timescales, where an increased individual drift could be advantageous.

<https://doi.org/10.7554/eLife.103585.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

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.

We have added further discussion of this to the discussion section.

(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 have added a figure (1F to better visualize the changes in handedness over days). We have also pointed out the connection between the power spectrum and the autoregressive model given by the Wiener-Khinchin theorem (which states that the autocorrelation function of a wide-sense stationary process has a spectral decomposition of its power spectrum).

(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.

We have discussed this further in the discussion.

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.

We have adjusted our wording and contextualized our claims based on previous literature.

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.

We have reanalyzed the behavioral data in a hierarchical model to account for batch effects. Accounting for batch effects (Fig 1G, S1G) we still observe differences between genotypes and for pharmaceutical manipulations of serotonin, though our data provides more equivocal evidence for the effects of *trh*¹ on drift.

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.

We have added text indicating that these two behavioral responses have previously been shown to be correlated to each other and that the spectral power analysis and autoregressive model are conceptually linked.

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

We have added a table in the supplemental clarifying all of the parameters of modeling for each figure.

Recommendations for the authors:

Reviewing Editor Comments:

Highlights of the Consultation Session of 3 Reviewers

In the consultation session, the reviewers discussed as particularly important the relative contribution of genotype and variable environment. Further analyses of the replicates of the genotypes were suggested to exclude the environment as the source of difference in the extent of drift between genotypes. If the difference in the extent of drift between replicates is greater than the difference in the extent of drift between genotypes, then one cannot really say that there is a genetic control over drift and that it would evolve (which is still an interesting result, but would be less exciting for a follow-up evolution experiment). If replicates differ, testing whether the relative difference in the extent of drift between genotypes is maintained across environments would also be strong evidence that the extent of behavioral drift is a property of a genotype and not a mere result of a fluctuating/variable environment. The authors do present two behavior paradigms that can serve the purpose of comparing the relative extent of drift between genotypes across the two paradigms that they already have. The authors might consider whether experimental data could be brought closer to theory by including an experiment in a variable environment (e.g temp or diet changes etc.).

Reviewers also agreed in the consultation session that methods and definitions were somewhat cryptic, and it would be very helpful if they were more detailed. For example, linking the free walking analysis to the Ymaze and then the model1 to the model2 was not straightforward.

We have added text to make more explicit the theoretical connection between the freewalking analysis, the ymaze analysis, and the model. We have added text and a supplemental table to clarify the methods.

Reviewer #1 (Recommendations for the authors):

(1) Line 161: The authors state in the supplement that they used DGRP strains, which are inbred and not isogenic. According to the original authors, they possess 99.3% genetic identity. The isoD1 strain has no known crossing scheme, so complete chromosome isogeneity remains questionable, especially after 12 or more years since its creation. The authors should refer to the strains as "near-isogenic" or a similar term.

We have adjusted the language as suggested to be more accurate.

(2) Lines 276, 338: The manuscript contains some unfinished sentences or remnants from the drafting process (e.g., "REFREF"). A thorough editorial review is recommended to

eliminate such errors.

We have cleaned up all references and made additional passes to adjust text.

Reviewer #2 (Recommendations for the authors):

(1) If the authors want to claim that serotonin is a regulator of drift, they should provide a negative control experiment, using equivalent perturbations of another neuromodulator and non-modulator. Alternatively, they could simply soften the claims revolving around serotonin and its putative direct role in modulating drift.

We have softened the claims as suggested to avoid claiming our results show a specific role for serotonin.

(2) I would suggest always using "behavioral drift" when referring to drift, especially in the context of modeling, because it can be easily confused with genetic drift and cause confusion when reading.

We have adjusted the language throughout the manuscript per this suggestion.

(3) It would be good to see in the methods if the 2-hour assays were always done at the same time of the fly's subjective day and when (e.g. how many hours after lights on).

We have clarified this.

(4) I understand that many experiments use methodology replicated from the group's previous work, but I would recommend elaborating the experimental methods section in the supplementary such that the reader can understand and reproduce the methods without having to sift through and look for them in previous papers.

We have expanded on our discussion of the methodology in the methods section.

Reviewer #3 (Recommendations for the authors):

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 flies exhibit an individual turning bias (when averaged over time), yet their preferences fluctuate over slow timescales. However, it's unclear why the authors chose to switch to a different assay to compare strains. In particular, it's ambiguous whether the behavioral measure in one setup is comparable to that in the other; specifically, whether a bias in one setup reflects the same type of bias in the other. The behavior is also sampled differently across setups (though the details are unclear; see comments below) and analyzed using different methods. Consequently, it remains uncertain whether the slow fluctuations observed in the arena setup are also present in the Y maze. It appears that the analysis of the Y maze data only addresses individual behavioral variance or, at most, day-to-day changes, without accounting for longer-term correlations in bias-which I understood to be the primary interest in the arena setup. Some clarification is needed here (see specific comments below).

In Figure 2, the authors attempt to show the potential advantage of individual drift for survival in unpredictable, fluctuating environments. They demonstrate that while bet-hedging provides an advantage over timescales matching the generation time (since reproduction is required), it offers less benefit on shorter timescales, where an increased individual drift could be advantageous. This approach is well-conceived, and the findings are convincing, though the model would benefit from further clarification and additional explanation in the text.

Here are some more specific comments:

PART 1:

(1) L 223 one probably cannot see a circadian peak at 24h if the data were filtered at 24h, did they look with another low pass cutoff?

We clarified in the text that the power spectrum analysis was performed on unfiltered data.

(2) L 243 the spread in standard deviation is said to be consistent with drifting bias, however, I do not agree with this. The variation could be stochastic but independent across days, and show no temporal correlation. As done with the circular arena, a drift should be estimated as a temporal correlation in the behavior.

It is consistent insofar as seeing a non-zero standard deviation is a necessary condition for drift. While it does not show that there is any consistency over time, this can be inferred from the autoregressive model (as well as previous work). We have added text to make this clearer.

(3) In the autoregressive model this temporal aspect seems to be incorporated only to the first order (from day to day). Therefore, from what I understand, the drift term is not correlated over time. This seems very different from the spectral analysis done in the circular assay, and I wonder if it fits at all the initial definition of drift. For example, is the model compatible with a fixed mean and a similar power spectrum as in Figure 1C? The text should clarify that.

can be made clear in the case of $\sigma = 0$ and $\Phi = 1$, where values would $\Phi \neq 0$. In an AR(1) process, datapoints day to day are correlated as long as $\Phi \neq 0$. This perfectly correlated with each other across time. The AR(1) model and the PSD of circling can be related via the Wiener-Khinchin theorem. We have added text to make this connection clear.

(4) Did serotonin have no role in turning bias? My understanding of previous work was that serotonin should affect the bet-hedg variance as well - the authors should discuss what is expected or not, especially given that the pharmacological and genetic approaches do not have the same effect on bet-edging (Figure 1H-I).

As the pharmacological methods were only applied after eclosion, we do not find it surprising that we do not measure differences in the initially measured distribution of handedness in that case. We do see more evidence of it in the mutations, though the trh^N experiments provide a less clear effect after our adjustments to account for batch effects.

(5) Methods: It is unclear how flies were handled across days; e.g. in Y mazes: 2h each day for how many days? In the arena flies were imaged either twice daily for 2h per session, or continuously for 24h (L138) - but which data are used where?

We will make this more clear, but all data in figure 1 was the continuous 24h data

This part of the methods is not well explained and I think it should be described in more detail.

(6) How many flies per genotype were tested in fig 1E?

Information was added to the caption to duplicate information in the table.

PART 2:

(7) In Figure 2B I do not understand the formulation $N(50-\Phi: 50, \sigma)$, $N(\phi-\epsilon: \epsilon, \sigma)$ or in general $N(x: m, s)$: does this mean that the variable x has normal distribution with mean m and variance s ? Usually this would be written as $N(x|m, s)$ or $N(x; m, s)$

If so then: $N(50 - \Phi: 50, \sigma) = N(\Phi: 0, \sigma)$ which has mean=0 while the figure caption says "from a normal distribution centred on the long term environmental mean" - what is the long term environmental mean?

If this is correct, and, therefore, we are just centering the mean, what about $N(\text{et-}\phi: \text{et}, \sigma)$?

Et is the environment at the time, not the mean of the environment (which is 50). We have added more detail in supplementary methods to address this.

(8) Should Φ vary between 1-100? And is the environmental parameter in Figure 2C also varying between 1-100? These ranges should be written somewhere.

While implied in the sigma notation, we have added more detail in supplementary methods to explain the situation.

(9) As far as I understand the bounding envelope in Figure 2B is necessary to contain the drift model. In Figure 1F, a bounding effect was generated by the "tendency to revert to no bias." It is unclear to me whether these two formulations are equivalent. Moreover, none of these two models might be able to recapitulate the correlations observed in the circular arena and analyzed spectrally in Figure 1C. It would be necessary that the author make an effort to relate these models/quantifications one to another. My understanding of Figure 1B is that there are slow fluctuations around the mean. Is the bounded drift model in 2B not returning to the same mean? And do these models generate slow fluctuations? Further explanation could help clarify these points.

We have added additional explanation to explain the connection between the power spectrum and the two methods of (phi and bounding envelop) of establishing stationarity.

(10) Expanding on the above: I thought that the definition of individuality is based on some degree of stability over days. However, both models assume drift to occur from day to day (and also the analysis of the DGRP lines assumes so). Some clarification here could help: is the initial bet-hedging variation maintained in the population? And is the mean individual bias still a thing or it is just drifting away all the time?

The initial bet-hedging is maintained to some degree, based on the parameter of phi and the bounding envelope. We have added text to make this clearer.

(11) In both Figures 2C and 2E the populations are always shrinking, is that correct? And if so, is it expected? Does the model allow growth in a constant environment?

As the plotted values are the log, the optimal environments do allow growth (visible more clearly in 2D). We have added some text to make this clearer.

(12) Growth is quantified only across 100 days (Figure 2D) but at day 100 there is not something like a steady state, how is 100 chosen? Would it make sense to check longer times to see if the system eventually takes off? And if not, why?

(13) Related to the above: what is the growth range achieved in Figure 3A-B? Is the heatmap normalized to the same value across conditions? I think it would be important to consider the absolute range of variation of growth or at least the upper value across conditions.

Moreover: is growth quantified at day 100? What happens at longer times? Does the temporal profile of the growth curve differ across environmental conditions? (I'm referring to a Figure as 2D).

As we are plotting the log change, we are ultimately showing the growth rate. While a more realistic model would involve carrying capacity, we believe a simplified model showing growth or no growth captures the difference in growth rate between different strategies. We have added some text to make this clearer.

(14) Suddenly at line 502, sexual maturity is introduced as a parameter, which was never mentioned before, called $a_{\min}$ in the figure legend of panel 3a, but it is unclear where this is in the model. And please also clarify if sex maturity is the same as generation time.

Sexual maturity is the same as generation time, we have standardized terminology throughout the paper.

(15) Regarding lines 505-508, could one simply conclude that in this model formulation, the generation time has the effect of a low pass filter on environmental fluctuation? The question is: is this filtering effect the only effect of generation time?

While this seems to capture the high-frequency effect we see, it does not explain the shift from bet-hedging->drift we see at lower-frequency environmental fluctuations.

(16) What reproductive rate is used for the PCA analysis? Is the variance associated with the drift so low because of choosing a fast reproductive rate? A comment in the main text would be helpful.

We have clarified that these plots were done at 10 days.

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