Title

Genotypic differences in behavioural entropy: unpredictable genotypes are composed of unpredictable individuals

Journal

Animal Behaviour, 86(3)

ISSN

0003-3472

Authors

Stamps, Judy A
Saltz, Julia B
Krishnan, VV

Publication Date

2013-09-01

DOI

10.1016/j.anbehav.2013.07.012

Peer reviewed

Published in final edited form as:
Anim Behav. 2013 September ; 86(3): 641–649. doi:10.1016/j.anbehav.2013.07.012.

Genotypic differences in behavioural entropy: unpredictable genotypes are composed of unpredictable individuals

Judy A. Stamps,
Evolution and Ecology, University of California Davis

Julia B. Saltz, and
Molecular and Computational Biology, University of Southern California

V.V. Krishnan
School of Engineering, San Francisco State University

Judy A. Stamps: jastamps@ucdavis.edu; Julia B. Saltz: jsaltz@usc.edu; V.V. Krishnan: krishnan@sfsu.edu

Abstract

Intra-genotypic variability (IGV) occurs when individuals with the same genotype, raised in the same environment and then tested under the same conditions, express different trait values. Game theoretical and bet-hedging models have suggested two ways that a single genotype might generate variable behaviour when behavioural variation is discrete rather than continuous: behavioural polyphenism (a genotype produces different types of individuals, each of which consistently expresses a different type of behaviour) or stochastic variability (a genotype produces one type of individual who randomly expresses different types of behaviour over time). We first demonstrated significant differences across 14 natural genotypes of male *Drosophila melanogaster* in the variability (as measured by entropy) of their microhabitat choice, in an experiment in which each fly was allowed free access to four different types of habitat. We then tested four hypotheses about ways that within-individual variability might contribute to differences across genotypes in the variability of microhabitat choice. There was no empirical support for three hypotheses (behavioural polymorphism, consistent choice, or time-based choice), nor could our results be attributed to genotypic differences in activity levels. The stochastic variability hypothesis accurately predicted the slope and the intercept of the relationship across genotypes between entropy at the individual level and entropy at the genotype level. However, our initial version of the stochastic model slightly but significantly overestimated the values of individual entropy for each genotype, pointing to specific assumptions of this model that might need to be adjusted in future studies of the IGV of microhabitat choice. This is among a handful of recent studies to document genotypic differences in behavioural IGV, and the first to explore ways that genotypic differences in within-individual variability might contribute to differences among genotypes in the predictability of their behaviour.

Keywords

intra individual variability; Drosophila melanogaster; behavioural variability; behavioural predictability; personality

Introduction

Behaviour is among the most labile of traits, and for many years biologists have studied factors that contribute to individual differences in behaviour. Major sources of behavioural variability include genes, age, sex, experiences prior to the behavioural assay, and conditions during a behavioural assay (see Clark & Ehlinger 1987 for a review of the early literature, also Sih et al. 2004; Dingemanse et al. 2010; Stamps & Groothuis 2010a; Stamps & Groothuis 2010b; Groothuis & Trillmich 2011; Walker & Mason 2011). However, even if researchers carefully control for variation in all of these factors, experimental subjects do not always behave the same way. For instance, despite decades of attempts to standardize the genomes, rearing and testing environments of laboratory mice and rats, considerable variability remains in the behaviour of virtually isogenic strains (Lewejohann et al. 2011). Similarly, individuals from the same inbred line of *Drosophila melanogaster* may express different types of behaviour or make different choices, even if they are raised under highly standardized conditions, and then tested at the same age using a stringently controlled behavioural assay (e.g. Miller et al. 2011; Del Pino et al. 2012; Kain et al. 2012).

Here, we use the term intra-genotypic variability, or IGV, to denote the variability of isogenic subjects, all of which have been reared under the same, carefully controlled conditions, and then measured or tested at the same age in the same context (where 'context' here indicates the external stimuli that surround an individual when its trait values are measured, see Stamps & Groothuis 2010a). Several recent studies have shown that IGV in behaviour can vary across different genotypes from the same species (Perry et al. 2010; Miller et al. 2011; Schuett et al. 2011; Kain et al. 2012). The notion that genotypes might differ with respect to the variability of their phenotypes is not new: morphologists have for many years documented genotypic differences in phenotypic variability, and gone on to consider the proximate mechanisms that contribute to these differences. For instance, in *Drosophila melanogaster*, significant differences among genotypes in the variability of traits such as sternopleural bristle number (Dworkin 2005) and wing shape (Breuker et al. 2006) have been used to estimate genotypic differences in canalization, where canalization indicates the sensitivity of a genotype to environmental perturbations during ontogeny (Willmore et al. 2007). More recently, geneticists have begun to investigate loci that contribute to heterogeneity among genotypes in the variability of morphological, life history and behavioural traits (e.g. Kain et al. 2012; Shen et al. 2012).

However, there is one key difference between IGV for behaviour and IGV for traits that are temporally stable within individuals. In the case of temporally stable traits (i.e. most morphological and many life history traits), each individual need only be measured once, and the IGV of each genotype is equivalent to the inter-individual variability of that genotype. However, behaviour can vary across time within as well as across individuals, as a result of many different proximate mechanisms. Some of these processes generate systematic temporal changes in behaviour over time, e.g. circadian rhythms which affect the time of day that animals respond to particular stimuli or express particular types of behaviour, or gradual increases or decreases in behaviour over time in response to initially novel stimuli (e.g. habituation, sensitization, acclimation). Other processes generate short term, pseudo-random temporal fluctuations in behaviour. In humans, this type of stochastic variability in behaviour within individuals has been termed intra-individual variability or IIV (Nesselroade 1991; Ram & Gerstorf 2009). Recent studies of animals indicate that IIV can significantly vary across individuals (Stamps et al. 2012); such individual differences in IIV are typically attributed to differences among individuals in fluctuations in neuronal or hormonal factors that affect the expression of behaviour (Brembs 2011). Hence, in the case of behaviour, IGV could be due to different types of within-individual variability, to inter-individual variability, or to some combination of these.

Intriguingly, theoreticians have for many years not only assumed that IGV in behaviour varies among genotypes, but also identified two ways that a given genotype might generate variable phenotypes. In behavioural ecology, classic game theory models discriminate between genotypes with fixed strategies (invariant behaviour) and genotypes with mixed strategies (variable behaviour) (Maynard Smith & Price 1973; Maynard Smith 1982). In addition, they differentiate mixed strategies based on multiple behavioural phenotypes, each of which consistently expresses the same behaviour (e.g. 40% of the individuals with a given genotype always play hawk, while the remaining 60% always play dove) from mixed strategies based on a single, stochastically variable phenotype (e.g. every individual randomly plays hawk 40% of the time and dove 60% of the time)(Bergstrom & Godfrey-Smith 1998; Orzack & Hines 2005). Similarly, evolutionary biologists studying bet-hedging begin by assuming that genotypes differ with respect to the variability of their phenotypes (Seger & Brockmann 1987; Simons & Johnston 1997; Donaldson-Matasci et al. 2008), and then consider two ways that a single genotype might generate high phenotypic variability: one in which the genotype generates several different phenotypes, each of which is fixed within individuals, and the other, called ‘adaptive coin flipping’, in which the individuals with a given genotype stochastically express different trait values at different times (reviewed in Childs et al. 2010). Hence, theory tells us that there are at least two ways that a genotype might generate variable behaviour. One, ‘behavioural polyphenism’, occurs when a genotype produces several different types of individuals, each of which consistently expresses a single type of behaviour. The other, ‘stochastic variability’, occurs when a genotype produces a single type of individual whose behaviour varies randomly (or pseudo-randomly) over time.

The behavioural polyphenism and stochastic variability options are illustrated in Figure 1 for two genotypes, A and B, for a situation in which animals are able to choose one of four possible items (I-IV). In Genotype A (behavioural polyphenism) there are four types of individuals, each of which consistently chooses a different type of item (e.g. individual 1 always chooses I, individual 4 always chooses II, and so on). In Genotype B (stochastic variability), there is only one type of individual, which chooses each of the four types of items 25% of the time. When choices are aggregated across individuals within genotypes, behavioural variability is identical for Genotype A and Genotype B: in each genotype, each of the four items is chosen 25% of the time (see bars at the right of the figure).

Thus, given evidence that IGV for behaviour does differ across genotypes from the same population, the key question is whether and how genotypic differences in variability at the individual level contribute to genotypic differences in IGV. The current study of microhabitat use in *Drosophila melanogaster* addresses this question. In our experiment, individual flies from 14 natural genotypes from the same population were allowed free access to four different types of microhabitat, and each fly's choice of perch site was recorded on five occasions over the course of a day. Because each individual had access to four different types of habitat, we used Shannon entropy to quantify the variability of choice for each genotype, and for each of the individuals within each genotype. We first established that IGV, as measured by Shannon entropy H (Shannon 1948; Shannon & Weaver 1963) varied across the genotypes. We then used indices of entropy for each of the genotypes and indices of the mean entropy for the individuals with each genotype to test four hypotheses about different ways that within-individual variability might contribute to genotypic differences in the IGV of behaviour in this and other species.

Methods

Flies

The genotypes were recurrent F_1's made by repeatedly crossing the same inbred parental lines, originally derived from a population in Raleigh, NC. The parental inbred lines are part of the *Drosophila* Population Genomics Project (DPGP.org). The direction of the crosses (i.e., maternal and paternal genotypes) was consistent, to control for maternal effects. For example, genotype A/B would be generated by crossing virgin females of genotype A to males of genotype B. The fly crosses were: 303×313, 208×712, 360×335, 639×517, 707×765, 732×775, 304×862, 306×391, 315×365, 357×714, 375×427, 437×324, 486×380,786×820. This experiment focused on the microhabitats used by focal males of these 14 genotypes; stimulus males and females from one genotype (303×313) were used to see whether space use patterns of the focal males changed as a function of social context.

With the exceptions indicated below, methods were the same as those described in detail in (Saltz 2011). Flies were housed and experiments conducted in an experimental room with a 12:12 L:D cycle, approximately 26°C and 98% humidity. Flies were reared on standard fly food (approximately 10 ml/vial) in vials founded by ten males and ten virgin females to ensure that all of the larvae were reared at low (non-competitive) densities. Within 8 hours of eclosion, focal males and stimulus males were housed individually in vials; stimulus females were housed in groups of 5 and mated on their first day of life to a standard genotype (genotype 852 from the same population). The next day, these males were removed and the stimulus females were housed in groups of 5 females for 5 additional days. Trials began when the focal and stimulus males were 3 days old and the (mated) stimulus females were 6 days old; no individual flies were used in more than one trial.

The microhabitat use of individually tested focal flies from each genotype was tested using experimental arenas. Each arena (height:22cm, width: 33cm, depth: 20cm) contained two patches, each of which was composed of a Petrie dish filled with grapefruit food, on top of which sat a single mesh ‘habitat’ (Height:7.7cm, Diameter: 7.7cm), made from a modified, green, medium-sized Finum Brewing Basket. The mesh habitats were designed to hold stimulus flies, and ensure that each focal male was able to see and smell the stimulus flies, but not physically contact them. Three experimental treatments differed with respect to the number of stimulus flies in the arena: in treatment 1, there were no stimulus flies in either mesh habitat (0 flies per arena), in treatment 2 there were a total of two stimulus flies in the arena (one male and one female stimulus fly in one of the two mesh habitats) and in treatment 3 there were a total of 12 stimulus flies in the arena (two males and two females in one mesh habitat and four males and four females in the second mesh habitat). In treatments 2 and 3 the location of the larger versus the smaller social group was alternated across trials to control for side biases.

Focal flies and stimulus flies were briefly anesthetized on ice, then one hour before the onset of darkness the appropriate number of stimulus flies were added to each mesh habitat, and a single focal male was introduced to the arena using an “individual entryway” that allowed him to walk into the experimental arena on his own schedule (c.f. Figure 2 in Saltz 2011). The following day (12 hr of acclimation time), each focal male's perch location was recorded at five different times: at 1, 2, 6, 10, and 11 hours after the lights were turned on. These times were chosen to focus on behaviour at dawn and dusk, when flies are most active.

Each focal male was able to perch on four different types of microhabitat: food, dish, habitat and surfaces. Each microhabitat differed from the others with respect to many different stimuli (visual, olfactory, tactile) that might have been relevant to the flies. Feeding and

most social interactions occur when flies are perched on food (Harshman et al. 1988; Saltz & Foley 2011), but when not engaged in these activities, males could perch on any of the four types of microhabitat. Since we had no justification for arranging the microhabitats along a continuum or for dividing them into 2 categories, we simply scored the type of microhabitat on which each male perched at each observation. Focal flies were scored as 'on food' when they were on the agar, 'on dish' when they perched on the lip or sides of the petri dish, 'on habitat' when they perched on the mesh habitat, and 'on surfaces' when they perched on the walls, floor or ceiling of the arena. We assumed that a male's microhabitat choice at one hour was independent of his microhabitat choice an hour or more earlier, i.e. that a male's decision to remain on the same microhabitat where he had been observed earlier the same day was equivalent to a male's decision to land on that type of microhabitat after moving there from somewhere else. This approach avoids confounding differences among genotypes or individuals in activity rates (which would affect the rate at which flies switched among different types of microhabitat over the course of a day) with differences among genotypes or individuals in microhabitat choice (which would affect the types of microhabitats used by flies over the course of the day). Later, we show why our results can not be attributed to genotypic differences in activity rates (see Discussion).

For each of the 14 genotypes, we conducted 15 trials for treatment 1, and 30 trials each for treatment 2 and 3; analyses were based on trials for which a male's location was scored for all five time periods. For one set of trials, the genotype of the focal male and the stimulus flies were the same (303×313), but omitting these trials from the analyses had no qualitative effect on any of the results, so here we present the results of analyses based on all 14 of the focal genotypes. No permits or animal care protocols were required for these experiments, but they conformed to the ABS/ASAB ethical guidelines for the treatment of animals in research.

Measuring behavioural variability

When scores for behaviour are distributed along a continuum, familiar indices of dispersion (typically, standard deviation or variance) can be used to estimate the variability of behaviour for genotypes or for individuals (Breuker et al. 2006; Ram & Gerstorf 2009; Miller et al. 2011; Stamps et al. 2012). Alternatively, if each subject can only express two types of behaviour (or equivalently, make one of two possible choices) then standard statistical methods can be used to analyse variability at the level of genotypes or individuals (e.g. Kain et al. 2012). The situation is more complicated, however, when individuals are able to express three or more different types of behaviour, or choose from among three or more different items. In this situation, other methods must be used to estimate the variability of behaviour (Rosengren & Braswell 2001; Ram & Gerstorf 2009; Stamps et al. 2012). Of the many indices that could be used for this purpose, we chose the Shannon entropy index, H (Shannon 1948; Shannon & Weaver 1963). For many years, Shannon entropy has been used in other contexts to estimate variability when items of interest fall into discrete categories (e.g. species diversity, Jost 2007; De'ath 2012). Entropy is ideal for studying behavioural variability when animals express a limited set of discrete behaviours or choose from among a limited number of options, as it provides a way to estimate of the degree of uncertainty in predicting the behaviour expressed by a particular genotype, individual, or class of individuals (Shannon 1948; Jost 2006; Gorelick & Bertram 2010; Song et al. 2010).

The Shannon entropy index, H is calculated as follows:

$$H=\sum_{i=1}^{R} - p_i \log(p_i)$$

where H is the entropy, R is the total number of different types of behaviour that can be expressed in a given context, and p_i is the proportion of the behavioural observations that belong to the i-th type of behaviour; here we used $\log_{10}$ to compute H.

In our study, $R = 4$, since there were four different perch locations (food, dish, habitat and surfaces). When $R = 4$, the lowest possible value of H is 0.0, and the maximum possible value of H is 0.603. A value of H of 0.0 for a genotype would indicate that the perch choice of individuals with that genotype does not vary (it is completely predictable), i.e., the members of that genotype always choose one of the four locations. Conversely, a value of H of 0.603 for a given genotype would indicate that the perch choice of individuals with that genotype is highly variable (completely unpredictable), i.e., the members of that genotype are equally likely to choose each of the four locations. We first computed the entropy for each combination of genotype-treatment-time (14 genotypes × 3 treatments × 5 time periods = 210 groups). Because these entropy scores were not normally distributed (Shapiro-Wilk test $p<0.05$ for all time points), we used the non-parametric Kruskal-Wallis test to evaluate the effects of experiment, time and genotype on entropy. Analyses were implemented in SAS (version 9.2), using Proc Npar1way. This procedure allowed us to test the hypothesis that H varied across genotypes, when those genotypes were tested in different social contexts (treatments) and at different times of day.

We then computed an overall measure of entropy for each genotype (H_G), based on all of the observations of individuals with that genotype (aggregated across individuals, time and treatments). H_G provides an estimate of the uncertainty in predicting the location of a fly with a given genotype, if a fly with that genotype were sampled at random, at a randomly selected time of day, and in a randomly selected social context (treatment).

Based on initial results indicating strongly significant differences among genotypes in entropy (see Results), we developed four hypotheses to address different ways that within-individual variability in behaviour might contribute to genotypic differences in IGV. Our approach utilized two indices of variability: the entropy for each genotype, aggregated across all individuals (H_G, see above) and the average entropy for the individuals within each genotype (H_{BAR}, see below). We first show that when IGV differs across genotypes, the four hypotheses make different predictions about the relationship between H_G and H_{BAR} across genotypes. Then, we tested each of these hypotheses using data collected from the flies. It was not possible to partition behavioural variability into components reflecting entropy at different levels (e.g. inter-genotypic, intra-genotypic, inter-individual, intra-individual), because statistical methods for hierarchically partitioning entropy and diversity are still under development (de Bello et al. 2010; Ricotta 2010; Schmera & Podani 2013).

For each fly, j, we estimated its individual entropy, H_j, based on the five observations of the perch locations used by that individual over the course of the day. For individual, H_j is an estimate of the uncertainty in predicting the location of that individual, if it were sampled at random over the course of a day. Then, for each genotype we computed H_{BAR}, the average value of H_j for individuals with that genotype.

We used the relationship between H_{BAR} and H_G across the 14 genotypes to test four hypotheses about ways that behavioural variability at the individual level might contribute to behavioural variability at the genotype level. The first hypothesis (behavioural polyphenism)

provides a 'benchmark' for the study: it predicts the relationship between H_{BAR} and H_G if there were no within-individual variability in microhabitat choice. It assumes that each fly uses only one type of perch, and that differences among genotypes in entropy occur because of genotypic differences in the proportion of individuals that use each type of perch. For instance, a genotype in which 90% of the individuals always perched on the surfaces and the other 10% always perched on the food would have lower entropy (H_G) than a genotype in which 25% of the individuals always perched on the food, 25% always perched on the surfaces, 25% always perched on the dish and 25% always perched on the habitat. This hypothesis predicts that the expected value of H_j would be zero for every fly, that H_{BAR} would be zero for every genotype, and hence, that across genotypes, the slope of the relationship between H_G and H_{BAR} would be zero.

The polyphenism hypothesis is unrealistic, because most flies would be expected to spend some time on the food, not only in order to feed, but also to engage in social activities, which typically occur on food substrates (Harshman et al. 1988; Saltz & Foley 2011). The second hypothesis (consistent choice) allows for some within-individual variability in perch use, by assuming that every fly spends a certain proportion of its time (x) on food, but then spends the rest of its time on its preferred type of microhabitat (which could either be food, dish, habitat or surfaces). Hence, we again have four types of individuals: prefer-food, prefer-dish, prefer-habitat and prefer-surfaces. In this case, individuals whose preferred perches were dish, surface or habitat would have the same value of H_j, the value of which depends on x. We initially used 0.14 as our estimate of x, based on data showing that flies, on average, were on the food for 14% of the observations (see also Figure 2). This value of x yielded an expected value of H_j of 0.17 for flies whose preferred perches were dish, surface or habitat. For individuals whose preferred perch was food, the expected value of H_j would be 0.0 (see previous section). Hence, this hypothesis predicts that values of H_j would range from 0 to 0.17, and that the values of H_{BAR} would range from 0 and 0.17 across genotypes, depending on the proportion of prefer-food individuals in that genotype. Note, however, that this hypothesis does not predict a positive relationship between H_{BAR} and H_G across the genotypes. We repeated this procedure using values of x ranging from 0 to 0.5 (the value of x that generates the highest value of H_j possible under this hypothesis), but since all of the results were qualitatively the same as those for $x = 0.14$, we just report those results below.

The third hypothesis (time-based choice) assumes that genotypic differences in IGV are a result of predictable differences across genotypes in their temporal patterns of microhabitat use. That is, it assumes that genotypes differ with respect to the type of perches individuals use across the day, but that within each genotype, individuals use the same types of perch at a given time of day. For instance, a genotype whose individuals perched on the surfaces in the morning, afternoon and evening and on the food at midday would have a lower entropy score than a genotype whose individuals perched on the surfaces in the morning, the habitat in the middle of the day, the food in the afternoon, and the dish in the evening. This hypothesis predicts that the value of H_{BAR} would be equal to the value of H_G for each genotype (see online Appendix A). That is, it predicts that across genotypes, H_{BAR} would be positively related to H_G, with a slope of 1.0 and an intercept of 0.

The fourth hypothesis (stochastic variability) assumes that behaviour varies within individuals over time, but in contrast to the previous hypothesis, it assumes that each individual randomly chooses a perch on each occasion based on a set of genotype-specific probabilities. In this situation, differences among genotypes in entropy would occur if genotypes differed with respect to the probabilities that individuals choose particular types of perches. For instance, a genotype in which, at any given observation, every individual had a probability $p_F = .9$ of perching on the food, and a probability $p_S = .1$ of perching on the surfaces, would have a lower entropy than another genotype in which every individual had

an equal probability of perching on the food, the surfaces, the dish and the habitat (p_F = .25, p_S = .25, p_D = .25, p_H = .25). This hypothesis predicts that H_{BAR} would be positively related to H_G across the genotypes, but for each genotype, H_{BAR} would usually be lower than H_G (see online Appendix A).

Based on initial support for the stochastic variability hypothesis (see Results), we developed a formal model to predict the mathematical relationship between H_G and H_{BAR} under the assumptions of this hypothesis. For each genotype, this model assumes that every individual with a given genotype has identical values of the probability of choosing each type of location (i.e. identical values of p_F, p_D, p_H and p_S), and that each time an individual chooses a perch site, it randomly selects one of the four locations based on its genotype-specific set of p values. For instance, if a genotype had the following set of p values, p_F = .5, p_D = .3, p_H = .1 and p_S = .1, then whenever any individual with that genotype chooses a perch site, it would have a 50% chance of choosing the food, a 30% chance of choosing the dish, and a 10% chance each of choosing the habitat or the surfaces.

This model was translated into a Matlab program, which was used to simulate the locations that individual flies would choose over the course of a day, assuming that each fly made five independent choices of the four possible perch locations. The inputs to the model were the set of p_i values for the given genotype; these p_i values were generated based on the proportion of observations in which individuals of that genotype were observed perched at each of the four locations (data aggregated across all treatments, times and individuals, N = 375-390 location points per genotype). For each simulated fly, the model computed an estimate of its individual entropy score, H_j based on the locations it chose, and then repeated this process for 50,000 individuals to obtain a robust estimate of the predicted value of H_{BAR} for each genotype, based on the assumptions of the stochastic variability hypothesis and the conditions in our experiment.

Results

Entropy at the Genotypic level

There were significant differences in entropy among genotypes (χ^2 = 44.83, p<0.001), and as a function of time of day (χ^2 = 59.97, p<0.0001) but there were no discernible differences in entropy as a function of social context (treatment) (χ^2 =2.04, p=0.361). Entropy at the genotypic level, H_G (aggregated across all treatments, times and individuals), varied between 0.404 and 0.588 across the 14 genotypes (Figure 2).

Entropy at the individual level

There was no support for either of two hypotheses that assume that behavioural variability is negligible to low within individuals. The polyphenism hypothesis and the consistent choice hypothesis predicted that values of H_j would be low for all flies (0.0 in the case of the former, and between 0 and 0.17 in the case of the latter). However, across all of the genotypes in the study, the mean value of individual entropy was appreciably higher (Mean H_j = 0.318 (95% CI = 0.308 - 0.328, N = 1065 flies). These results confirm observations indicating that most flies shifted among different types of perches over the course of the day. In addition, neither of these hypotheses predicts a positive relationship between H_{BAR} and H_G across the genotypes. Contrary to this prediction, H_{BAR} was strongly positively related to H_G across the 14 genotypes in this study (r = 0.899, p < 0.0005, Figure 3). That is, genotypes with more variable (less predictable) behaviour were composed of individuals with more variable (less predictable) behaviour.

We next consider two more hypotheses (time-based choice and stochastic variability), both of which assume that perch choice varies across time within individuals. The time-based

choice hypothesis assumes that all individuals with a given genotype perch at the same location at any given time of day but perch at different locations at different times of day, whereas the stochastic variability hypothesis assumes that all of the individuals with a given genotype randomly choose perches throughout the day based on a set of genotype-specific probabilities (see Methods). These two hypotheses differ with respect to their predictions about the relationship between H_{BAR} and H_G. The time-based choice model predicts that $H_{BAR} = H_G$. Hence, it predicts a slope of 1.0 and an intercept of 0.0 when H_{BAR} is regressed against H_G. In contrast, the stochastic variability model predicts that H_{BAR} will be lower than H_G (online Appendix A). In addition, simulations of the stochastic variability model, parameterized for the current data set, predict a slope significantly lower than 1.0 (slope = 0.834, 95% CI = 0.778-0.889), and an intercept of less than 0.0 (intercept = -0.061, 95% CI = -0.09 - -0.03) when H_{BAR} is regressed against H_G.

In fact, H_{BAR} was significantly lower than H_G for all of the genotypes in this study (mean difference = 0.21, S.E. = 0.006, matched pairs t test, t = 35.9, 13 df, $p < 0.001$, Figure 3). In addition, the observed slope of the relationship between H_{BAR} and H_G was lower than 1.0, and not significantly different from the slope predicted by the stochastic variability model (observed slope = 0.731 (95% CI = 0.507- 0.995, Figure 3). The intercept for the observed relationship between H_{BAR} and H_G was virtually identical to the intercept predicted by the simulations (observed intercept = - 0.07, 95% CI = - 0.19 - 0.049). Based on these results, we were able to reject the time-based choice hypothesis, and provisionally accept the stochastic variability hypothesis.

However, although the relationship between H_{BAR} and H_G predicted by the stochastic variability hypothesis was quite similar to the relationship observed in the flies, closer inspection revealed that the values of H_{BAR} observed in our flies were slightly (on average, by 16%) but significantly lower than the values of H_{BAR} predicted by the simulations (paired samples t test, t = 12.18, 13 df, $p < 0.0005$, Figure 3). Hence, although the stochastic variability model was by far the most successful of the initial group of four hypotheses in predicting the patterns in the data, our results pointed to particular changes in the assumptions of the stochastic variability model might improve its fit (see Discussion).

Discussion

We first demonstrated that behavioural variability (as measured by entropy) of microhabitat choice in male *D. melanogaster* significantly differed across genotypes: the perch choices of some genotypes were more variable (less predictable) than those of other genotypes. This is one of only a handful of empirical studies to date to demonstrate genotypic differences in the intra-genotypic variability (IGV) in behaviour (see Introduction), and the first, to our knowledge, to demonstrate significant differences among genotypes in the variability of behaviour. More important, we used relationships, across genotypes, between entropy at the genotype level (H_G) and average entropy at the individual level (H_{BAR}) to test four hypotheses about ways that behavioural variability at the individual level might contribute to the differences in the variability we observed at the genotypic level. We found that the stochastic variability hypothesis (which assumes that genotypic differences in IGV are due to genotypic differences in stochastic temporal variation in behaviour within individuals) accurately predicted the observed slope and intercept when H_{BAR} was regressed against H_G across the genotypes. In contrast, there was no support for three other hypotheses: behavioural polyphenism, consistent choice, and time-based choice. Taken together, these results suggest that differences among genotypes in stochastic variability at the individual level contributed to genotypic differences in behavioural variability at the genotype level.

However, we also found that the initial version of the stochastic model slightly, but significantly, overestimated the values of H_{BAR} for each value of H_G. This discrepancy points to specific assumptions that might be adjusted in a next generation of stochastic models. First, we assumed that the probability of choosing any given type of location, p_i, was the same at every observation period for the individuals with a given genotype. If instead, the probability of choosing each type of perch varied within the individuals with a given genotype as a function of time of day (e.g., if individuals with a given genotype were slightly more likely to perch on the food at midday than at other times of day), then our model would overestimate the value of H_{BAR} for that genotype (see online Appendix B). Second, we assumed that every individual with a given genotype had the same set of probabilities of perching at each of the four locations. Recently Kain et al. (2012) studied phototactic behaviour in several laboratory strains of *Drosophila*, and were able to reject the hypothesis that all of the individuals within each strain had the same probability of approaching the light. They found that a minority of the flies in each strain behaved as if they had different probabilities of approaching the light than did the rest of the individuals from their strain. A similar pattern in our data (i.e., a minority of flies with lower entropy than others in their line) could also account for our overestimates of H_{BAR}. Unfortunately, neither of these modified versions of the stochastic variability hypotheses could be tested using the current data set. Each of our flies was sampled over the course of a single day, so we could not determine whether p values vary as a function of time of day within individuals, and 5 samples per individual is insufficient to determine whether p values significantly vary across individuals with the same genotype. However, these questions could be addressed in future studies, involving fewer genotypes but more samples per individual, distributed across a series of days.

Another important question is whether the genotypic differences in the variability of microhabitat use observed in this study might simply be an artifact of genotypic differences in locomotory activity in male *D. melanogaster* (e.g. Martin et al. 1999; Stamps et al. 2005a). The answer is no. Consider a situation in which genotypes differed with respect to their activity rates, but in which every individual in every genotype had the same set of probabilities of perching at the four different types of microhabitat. In that case, the genotypes would differ with respect to their rate of movement from one location to another, but every fly, regardless of genotype, would rely on the same set of probabilities each time it chose a microhabitat. As a result, the proportion of perches chosen, aggregated across all of the individuals with each genotype, would be comparable across the genotypes, and H_G would be comparable across genotypes. Of course, genotypic differences in activity might affect the perches used by an individual fly over the course of a day, thus affecting entropy at the individual level. For instance, if individuals with low activity rates were more likely to remain in the same location throughout the day than high-activity individuals, then genotypes whose individuals were relatively inactive would have lower values of H_{BAR} than genotypes whose individuals were more active. However, significant differences among genotypes in H_G, as well as the relationships across genotypes between H_G and H_{BAR} reported here, could only occur if the genotypes also varied with respect to their probabilities of selecting each of the four types of perches.

The mechanisms that led to genotypic differences in stochastic variability in our flies might be genetic and/or epigenetic. Our natural genotypes differ at many loci, making direct inference of genes that affect genotypic differences in IGV impossible. Kain et al. 2012 (see above), reported that the variability of phototactic behaviour in *Drosophila* (see above) was influenced by the *white* gene, which is involved in serotonin synthesis. Their results suggest that the ability of a genotype to generate individuals with the same level of stochastic within-individual variability might be influenced by variation in genes affecting neurotransmitter abundance. It remains to be seen if this mechanism contributes to genotypic

differences in other types of choice, e.g. microhabitat use in natural genotypes, such as those used in the current study.

Epigenetic differences among the genotypes may have also contributed to the genotypic differences in IGV that we observed. Although all of the flies were raised in uniform physical conditions prior to testing, their behaviour as adults might have been influenced by maternal effects, sibling interactions, inherited epigenetic markers, or any of the other factors affecting behavioural development that differed more across than within genotypes. For instance, differences among lines in female fecundity, and resulting differences in larval densities, might have contributed to the development of genotypic differences in behavioural variability later in life. In flies, epigenetic histone methylation has been shown to affect larval locomotion and adult learning (Kramer et al. 2011), but to our knowledge no study has investigated the role of epigenetics in generating behavioural IGV in flies.

Understanding the adaptive significance of genotypic differences in the variability of microhabitat use in *D. melanogaster*, would require more information, preferably from the field or semi-natural enclosures, about the costs and benefits to males of using different perch locations. Given that ambushing predators such as spiders are attracted to cues associated with high prey densities, to kairomones emitted by prey, or to locations where they have previously encountered prey (e.g. Chien & Morse 1998; Morse 2000; Persons & Rypstra 2000), perches on or near a food substrate might be more dangerous than perches on surfaces farther from the food. Conversely, of course, since female *D. melanogaster* are attracted to food substrates and to conspecifics on those substrates, and since most mating occurs on food (Wertheim et al. 2002; Stamps et al. 2005b; Saltz & Foley 2011), perching on or adjacent to a food substrate might enhance a male's mating success, as compared to perching on surfaces far from food. Our results hint that different genotypes might resolve potential microhabitat-specific tradeoffs between predation risk and reproductive success differently. For instance, some individuals with some genotypes might spend most of their time in safer locations (on surfaces, away from food, c.f. genotype 639, Figure 2), whereas individuals with other genotypes might spend their time shuttling between perches at different proximities to food and mating opportunities (c.f. genotype 375, Figure 2). By extension, given the difficulties that prey have in detecting cryptic predators (Troscianko et al. 2009; Ings et al. 2012), low levels of within-individual predictability in perch use might be viewed as a bet-hedging strategy, in which the individuals in some genotypes handle unknown levels of predation risk in different types of microhabitat by randomly shifting among those microhabitats. In addition, since males compete with other males for mating opportunities at or near food (Hoffmann & Cacoyianni 1990; Saltz & Foley 2011), frequency-dependent social interactions among males, or among males and females, might also affect the potential benefits and costs of perching at different locations. Thus, the results of this study set the stage for further studies of the adaptive significance of variability of microhabitat choice in this species.

Overall, we suggest that the time is overdue for empirical studies of the ways that different types of within-individual variability contribute to genotypic differences in the IGV of behavioural traits. For years, theoreticians have devised models based on untested assumptions about this question. The current study suggests ways to test hypotheses about relationships between within-individual variability and genotypic variability when individuals express multiple, discrete types of behaviour, or choose from among multiple discrete items. Standard statistical techniques can be used to study the same questions when behavioural traits are continuously distributed, or when animals express one of two behaviours. Genetic tools are available to identify loci that might contribute to genotypic differences in behavioural variability, and lines or clones derived from natural populations can be used to investigate the mechanistic bases and adaptive significance of genotypic

differences in behavioural variability. More generally, future work on this topic will shed light on the reasons why, at both proximate and ultimate levels, behaviour is so often unpredictable, even after stringent controls for genotypes, rearing and testing conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank P. Biro, Tom Turner, and an anonymous reviewer for comments on a previous version of this manuscript. This work was supported by NIH grant #MH091561 (PI S. Nuhzdin)

References

Bergstrom CT, Godfrey-Smith P. On the evolution of behavioral heterogeneity in individuals and populations. Biology & Philosophy. 1998; 13:205–231.

Brembs B. Towards a scientific concept of free will as a biological trait: spontaneous actions and decision-making in invertebrates. Proceedings of the Royal Society B-Biological Sciences. 2011; 278:930–939.

Breuker CJ, Patterson JS, Klingenberg CP. A single basis for developmental buffering of Drosophila wing shape. PLoS ONE. 2006; 1:e7.10.1371/journal.pone.0000007 [PubMed: 17183701]

Chien SA, Morse DH. The roles of prey and flower quality in the choice of hunting sites by adult male crab spiders Misumena vatia (Araneae, Thomisidae). Journal of Arachnology. 1998; 26:238–243.

Childs DZ, Metcalf CJE, Rees M. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. Proceedings of the Royal Society B-Biological Sciences. 2010; 277:3055–3064.

Clark, AB.; Ehlinger, TJ. Pattern and adaptation in individual behavioural differences. In: Bateson, PPG.; Klopfer, PH., editors. Perspectives in Ethology. New York: Plenum Press; 1987. p. 1-47.

De'ath G. The multinomial diversity model: linking Shannon diversity to multiple predictors. Ecology. 2012; 93:2286–2296. [PubMed: 23185889]

de Bello F, Lavergne S, Meynard CN, Leps J, Thuiller W. The partitioning of diversity: showing Theseus a way out of the labyrinth. Journal of Vegetation Science. 2010; 21:992–1000.

Del Pino F, Salgado E, Godoy-Herrera R. Plasticity and genotype × environment interactions for locomotion in Drosophila melanogaster larvae. Behavior Genetics. 2012; 42:162–169. [PubMed: 21818661]

Dingemanse NJ, Kazem AJN, Réale D, Wright J. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology and Evolution. 2010; 25:81–89. [PubMed: 19748700]

Donaldson-Matasci MC, Lachmann M, Bergstrom CT. Phenotypic diversity as an adaptation to environmental uncertainty. Evolutionary Ecology Research. 2008; 10:493–515.

Dworkin I. A study of canalization and developmental stability in the sternopleural bristle system of Drosophila melanogaster. Evolution. 2005; 59:1500–1509. [PubMed: 16153035]

Gorelick R, Bertram SM. Multi-way multi-group segregation and diversity indices. PLoS ONE. 2010; 5:e10912.10.1371/journal.pone [PubMed: 20532222]

Groothuis TGG, Trillmich F. Unfolding personalities: The importance of studying ontogeny. Developmental Psychobiology. 2011; 53:641–655. [PubMed: 21866544]

Harshman LG, Hoffmann AA, Prout T. Environmental effects on remating in Drosophila melanogaster. Evolution. 1988; 42:312–321.

Hoffmann AA, Cacoyianni Z. Territoriality in Drosophila melanogaster as a conditional strategy. Animal Behaviour. 1990; 40:526–537.

Ings TC, Wang MY, Chittka L. Colour-independent shape recognition of cryptic predators by bumblebees. Behavioral Ecology and Sociobiology. 2012; 66:487–496.

Jost L. Entropy and diversity. Oikos. 2006; 113:363–375.

Jost L. Partitioning diversity into independent alpha and beta components. Ecology. 2007; 88:2427–2439. [PubMed: 18027744]

Kain JS, Stokes C, de Bivort BL. Phototactic personality in fruit flies and its suppression by serotonin and white. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:19834–19839. [PubMed: 23150588]

Kramer JM, Kochinke K, Oortveld MAW, Marks H, Kramer D, de Jong EK, Asztalos Z, Westwood JT, Stunnenberg HG, Sokolowski MB, Keleman K, Zhou HQ, van Bokhoven H, Schenck A. Epigenetic regulation of learning and memory by Drosophila EHMT/G9a. PLoS Biol. 2011; 9:e1000569.10.1371/journal.pbio.1000569 [PubMed: 21245904]

Lewejohann L, Zipser B, Sachser N. "Personality" in laboratory mice used for biomedical research: a way of understanding variability? Developmental Psychobiology. 2011; 53:624–630. [PubMed: 21866543]

Martin JR, Ernst R, Heisenberg M. Temporal pattern of locomotor activity in Drosophila melanogaster. Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology. 1999; 184:73–84.

Maynard Smith J, Price GR. Logic of animal conflict. Nature. 1973; 246:15–18.

Maynard Smith, J. Evolution and the theory of games. Cambridge: Cambridge University Press; 1982.

Miller PM, Saltz JB, Cochrane VA, Marcinkowski CM, Mobin R, Turner TL. Natural variation in decision-making behavior in Drosophila melanogaster. PLoS ONE. 2011; 6(1):e16436.10.1371/journal.pone.0016436 [PubMed: 21283727]

Morse DH. The role of experience in determining patch-use by adult crab spiders. Behaviour. 2000; 137:265–278.

Nesselroade, JR. The warp and woof of the developmental fabric. In: Downs, R.; Liben, L.; Palermo, D., editors. Visions of development, the environment, and aesthetics: The legacy of Joachim F Wohlwill. Hillsdale, N.J.: Erlbaum; 1991. p. 213-240.

Orzack SH, Hines WGS. The evolution of strategy variation: Will an ESS evolve? Evolution. 2005; 59:1183–1193. [PubMed: 16050096]

Perry GML, Sagvolden T, Faraone SV. Intra-individual variability in genetic and environmental models of attention-deficit/hyperactivity disorder. American Journal of Medical Genetics Part B-Neuropsychiatric Genetics. 2010; 153B:1094–1101.

Persons MH, Rypstra AL. Preference for chemical cues associated with recent prey in the wolf spider Hogna helluo (Araneae : Lycosidae). Ethology. 2000; 106:27–35.

Ram N, Gerstorf D. Time-structured and net intraindividual variability: Tools for examining the development of dynamic characteristics and processes. Psychology and Aging. 2009; 24:778–791. [PubMed: 20025395]

Ricotta C. On beta diversity decomposition: Trouble shared is not trouble halved. Ecology. 2010; 91:1981–1983. [PubMed: 20715619]

Rosengren, KS.; Braswell, GS. Variability in children's reasoning. In: Reese, HW.; Kail, R., editors. Advances in child development and behavior. 2001. p. 1-40.

Saltz JB. Natural genetic variation in social environment choice: context-dependent gene-environment correlation in Drosophila melanogaster. Evolution. 2011; 65:2325–2334. [PubMed: 21790578]

Saltz JB, Foley BR. Natural genetic variation in social niche construction: social effects of aggression drive disruptive sexual selection in Drosophila melanogaster. American Naturalist. 2011; 177:645–654.

Schmera D, Podani J. Components of beta diversity in hierarchical sampling designs: A new approach. Ecological Indicators. 2013; 26:126–136.

Schuett W, Dall SRX, Baeumer J, Kloesener MH, Nakagawa S, Beinlich F, Eggers T. "Personality" variation in a clonal Insect: The pea aphid, Acyrthosiphon pisum. Developmental Psychobiology. 2011; 53:631–640. [PubMed: 21365642]

Seger J, Brockmann HJ. What is bet-hedging? Oxford Surveys in Evolutionary Biology. 1987; 4:182.

Shannon CE. A mathematical theory of communication. The Bell System Technical Journal. 1948; 27:379–423.

Shannon CE, Weaver W. The mathematical theory of communication. 1963

Shen X, Pettersson M, Ronnegard L, Carlborg O. Inheritance beyond plain heritability: variance-controlling genes in Arabidopsis thaliana. PLoS Genet. 2012; 8:e1002839.10.1371/journal.pgen.1002839 [PubMed: 22876191]

Sih A, Bell AM, Johnson JC, Ziemba RE. Behavioral syndromes: An integrative overview. Quarterly Review of Biology. 2004; 79:241–277. [PubMed: 15529965]

Simons AM, Johnston MO. Developmental instability as a bet-hedging strategy. Oikos. 1997; 80:401–406.

Song CM, Qu ZH, Blumm N, Barabasi AL. Limits of predictability in human mobility. Science. 2010; 327:1018–1021. [PubMed: 20167789]

Stamps J, Buechner M, Alexander K, Davis J, Zuniga N. Genotypic differences in space use and movement patterns in Drosophila melanogaster. Animal Behaviour. 2005a; 70:609–618.

Stamps J, McElreath R, Eason P. Alternative models of conspecific attraction in flies and crabs. Behavioral Ecology. 2005b; 16:974–980.

Stamps J, Groothuis TGG. The development of animal personality: relevance, concepts and perspectives. Biological Reviews. 2010a; 85:301–325. [PubMed: 19961473]

Stamps JA, Groothuis TGG. Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differencese. Philosophical Transactions of the Royal Society B-Biological Sciences. 2010b; 365:4029–4041.

Stamps JA, Briffa M, Biro PA. Unpredictable animals: individual differences in intraindividual variability (IIV). Animal Behaviour. 2012; 83:1325–1334.

Troscianko T, Benton CP, Lovell PG, Tolhurst DJ, Pizlo Z. Camouflage and visual perception. Philosophical Transactions of the Royal Society B-Biological Sciences. 2009; 364:449–461.

Walker MD, Mason G. Female C57BL/6 mice show consistent individual differences in spontaneous interaction with environmental enrichment that are predicted by neophobia. Behavioural Brain Research. 2011; 224:207–212. [PubMed: 21689685]

Wertheim B, Dicke M, Vet LEM. Behavioural plasticity in support of a benefit for aggregation pheromone use in Drosophila melanogaster. Entomologia Experimentalis Et Applicata. 2002; 103:61–71.

Willmore KE, Young NM, Richtsmeier JT. Phenotypic variability: Its components, measurement and underlying developmental processes. Evolutionary Biology. 2007; 34:99–120.

Highlights

- Natural genotypes of Drosophila melanogaster express different levels of behavioural variability.
- We tested four hypotheses for the role of within-individual variability in genotype variability.
- We found no support for behavioural polyphenism, consistent choice or time-based choice hypotheses.
- There was strong support for the hypothesis that behaviour varies stochastically over time within individuals.
- Our results indicate that genotypes with unpredictable behaviour contain unpredictable individuals.

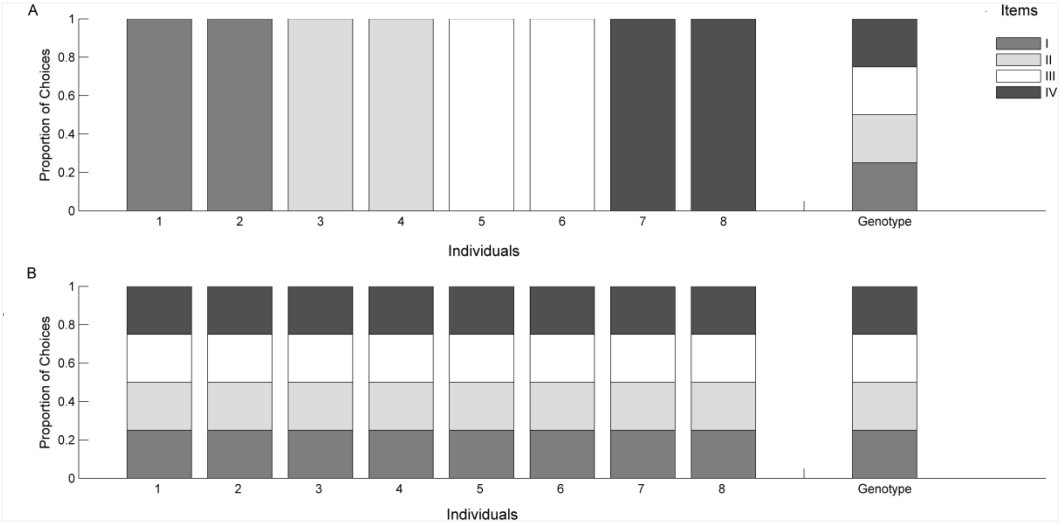

Figure 1.

Hypothetical patterns of behavioural variability for two genotypes (A and B). For each genotype, the behaviour of eight individuals (numbers 1-8) is repeatedly sampled over time; on each occasion, individuals can choose one of four different items (I, II, III or IV). A) Genotype A (behavioural polyphenism) generates four types of individuals, each of which consistently chooses one type of item. B) Genotype B (stochastic variability) generates a single type of individual, who randomly chooses each of the four items with the same probability (25%) on each occasion. When behaviour is aggregated across all of the individuals with each genotype (see Genotype, at the right of each graph), Genotype A and Genotype B have identical, high levels of intra-genotypic variability (IGV).

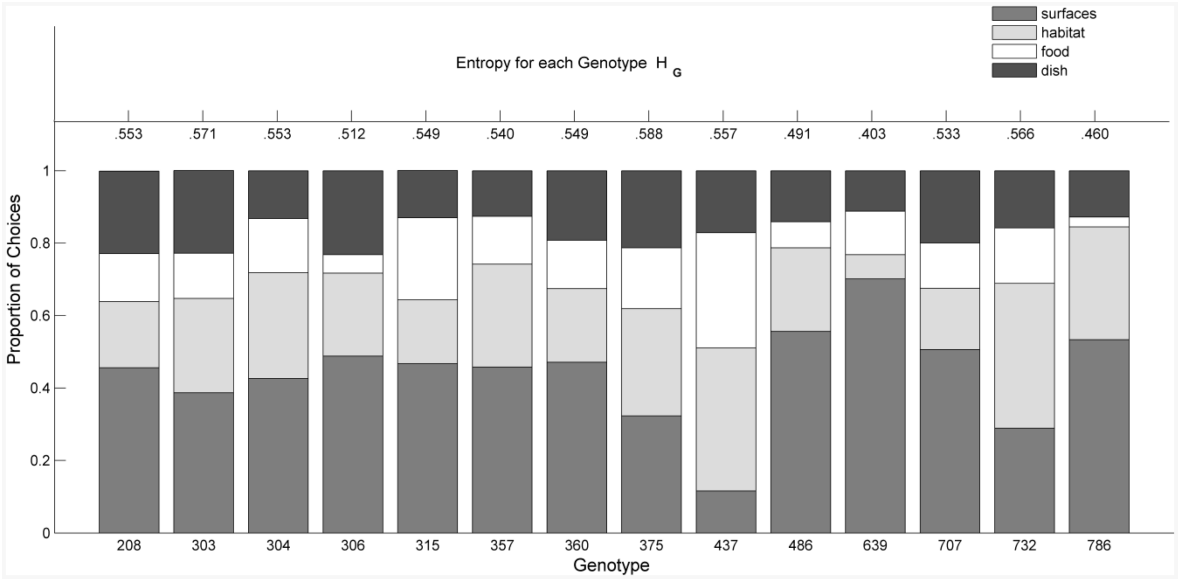

Figure 2.

Entropy at the genotype level as a function of microhabitat choice in male *D. melanogaster*. Each fly can perch at four different locations (surfaces, habitat, food and dish). For each genotype, the proportion of observations in which flies were observed at of the four possible locations, aggregated across individuals, times, and treatments, was used to estimate an entropy score for that genotype (H_G). Genotypes with lower entropy values have less variable (more predictable) patterns of microhabitat use than genotypes with higher entropy values.

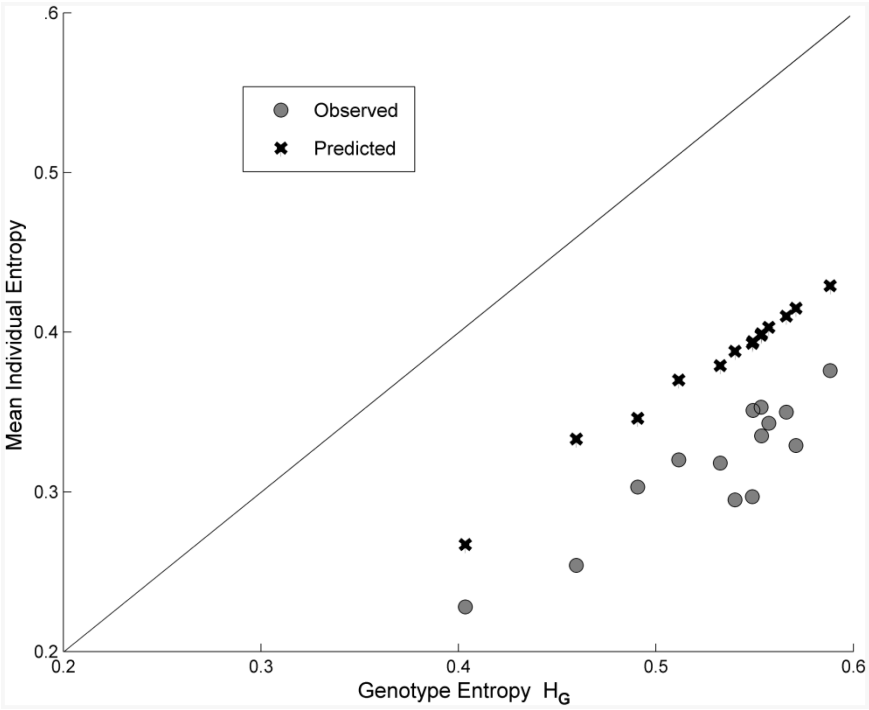

Figure 3.

Test of predictions of the stochastic variability hypothesis. For the 14 genotypes in our data set, the values of mean individual entropy (H_{BAR}) predicted by simulations based on the stochastic variability hypothesis (crosses) are compared with the observed values of H_{BAR} (circles). The model accurately predicted that the values of H_{BAR} would be lower than the values of H_G (equal values of H_{BAR} and H_G are indicated by the line), and accurately predicted the slope and intercept of the relationship between these two variables. However, across these 14 genotypes, the predicted value of H_{BAR} was slightly, but significantly higher than the observed value of H_{BAR} (see text).