Heritabilities and genetic correlations of Drosophila melanogaster locomotory behaviour traits: a high-throughput phenotyping approach

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Open Research

Code needed to reproduce the analyses and visualisations can be found on GitHub under the following link: Code and analyses. Data has been deposited on GitHub in the repository: Data and code repository.

Conflict of Interest

We have no conflict of interest to declare.

Abstract

Genetic variance forms the basis for evolutionary inferences as it describes the evolutionary potential of traits. The major limitation of quantitative genetic studies is achieving sufficient power and sample sizes to estimate heritabilities with sufficient precision. This issue is especially important in the case of traits that are inherently susceptible to stochastic, nonbiological variation. Behavioural traits have long been associated with this group, often yielding quantitative estimates of genetic parameters that are subject to broad estimation errors, thereby hampering the discovery of genetic variation underlying such characters. Here, we used a well-established panel of inbred genetic lines of the fruit fly, *Drosophila melanogaster*, to estimate relevant genetic parameters in a range of behavioural traits associated with mobility and exploration. Using a high-throughput phenotyping approach and automated scoring of large numbers of individual animals, we provide precise estimates of the quantitative genetic background behind some basic characters associated with animal behaviour. Fruit flies turn out to harbour significant genetic variance in traits directly associated with mobility and substantially lower heritabilities of traits describing the temporal variability of Y-maze movements. Mobility traits also appeared to be only moderately genetically correlated, except for movement distance vs. variability traits, where we estimated strongly negative genetic correlations. In general, our results demonstrate the existence of evolutionary potential in behavioural trait proxies measured by high-throughput methods, additionally hinting at the potential for sex-specific effects. They also emphasise the growing importance of high-throughput phenotyping in modern behavioural biology and ecology.

Keywords— fruit fly, *Drosophila melanogaster*, heritability, genetic correlation, phenomics

1 Introduction

The study of behaviour from a genetic and evolutionary perspective presents a rich but demanding field. Behavioural traits differ from most morphological or physiological traits in being more plastic, context-dependent, and often more strongly shaped by both internal states and external environments (Garamszegi and Herczeg, 2012; O'Dea et al., 2022). Despite this, behaviours are now widely recognised as heritable phenotypes subject to natural and sexual selection (Dingemanse and Dochtermann, 2013; Dingemanse et al., 2004; Johansson et al., 2024). Quantitative genetic parameters such as heritabilities, genetic variances, and genetic correlations are central to understanding the evolutionary potential of behaviour (Walsh and Lynch, 2018). Heritability estimates reveal the genetic contribution to behavioural variation, while genetic correlations inform us about possible trade-offs, constraints, or synergies in the joint evolution of traits. Together, such measures define the scope for behavioural responses to selection in natural and experimental populations.

However, robust estimates of quantitative genetic parameters for behaviour remain elusive. Behaviour is inherently dynamic: it depends on age, sex, circadian rhythms, previous experience, motivational state, and feeding history (Dingemanse and Dochtermann, 2013; Thys et al., 2021). These influences reduce repeatability, introduce substantial measurement error, and complicate the separation of genetic from environmental variance components. Traditional behavioural assays exacerbate these problems: they are labour intensive, often rely on direct observation or manual scoring, and are typically limited in scale (Carter et al., 2013; Groothuis and Taborsky, 2015). As a result, studies often lack sufficient replication or sample size to detect the underlying genetic signal, leading to inflated estimates of environmental variance, underestimated heritabilities, and large statistical uncertainty (Kruuk and Hadfield, 2007; Postma, 2014). Moreover, many assays have been criticised for lacking ecological realism, raising questions about how measured behaviours map onto natural histories and fitness consequences (Groothuis and Taborsky, 2015). These challenges help explain why behavioural quantitative genetics has historically produced estimates that are less consistent and less precise than those for morphological or physiological traits (Postma, 2014).

Technological and methodological advances now offer powerful solutions to these technical difficulties. Automated and high-throughput phenotyping systems allow behavioural traits to be measured with unprecedented resolution in large cohorts of individuals (Cleal et al., 2021; Werkhoven et al., 2021). Automated tracking allows for continuous quantification of locomotion and exploration, detecting subtle variations in activity patterns, trajectories, and space use that manual scoring would miss (Werkhoven et al., 2021, 2019; Macartney et al., 2025). Combined with machine learning, such systems can classify behavioural motifs, track state transitions, and reduce observer bias, thereby increasing both precision and repeatability. These advances expand the scale of behavioural datasets, which in turn enables more robust quantitative genetic analyses, including multivariate animal models that partition phenotypic variance into genetic and environmental components and estimate cross-trait genetic correlations (Walsh and Lynch, 2018).

The availability of model organisms further accelerates progress in behavioural quantitative genetics. *Drosophila melanogaster* has long been central in genetics and neurobiology (Anholt and Mackay, 2004), with its short generation time, ease of rearing, and well-annotated genome making it ideal for large-scale quantitative genetic studies (MacKay et al., 2012; Mackay and Anholt, 2024). Many Drosophila behaviours *Drosophila*, from circadian rhythms (Werkhoven et al., 2021) and learning (Stern et al., 2019) to aggression, courtship, and locomotor activity (Nandy et al., 2016; Kohlmeier et al., 2021; Macartney et al., 2025), show individual variation, fitness consequences, and measurable heritabilities (Harbison et al., 2013; Shorter et al., 2015; Rohde et al., 2017; Ivanov et al., 2015). The Drosophila Genetic Reference Panel (DGRP) provides a stable genome-sequenced collection of inbred lines that can be assayed repeatedly and in varying environments. This makes it possible to obtain precise estimates of heritability, dissect polygenic architectures, and quantify genotype-by-environment interactions. Crucially, multivariate analyses of DGRP data also allow the estimation of genetic correlations among behaviours, which can reveal constraints or trade-offs in behavioural evolution (Dingemanse and Dochtermann, 2013; Walsh and Lynch, 2018).

In this study, our objective was to estimate the heritabilities and genetic correlations of a set of locomotory and exploratory traits in *D. melanogaster* using the DGRP panel. The target behavioural traits were measured using a high-throughput phenotyping system, which allowed us to obtain precise and repeatable measurements of individual behaviours in an automated way. We focused on several key traits, including overall locomotor activity, Y-maze exploration patterns, and startle response. The choice of traits was already validated in an earlier experimental study (Macartney et al., 2025). Using a large sample size and robust statistical analyses, we provide reliable estimates of genetic parameters and explore the genetic architecture underlying these behaviours.

2 Methods

Throughout the Methods section, we use the MeRIT convention (Method Reporting with Initials for Transparency), following Nakagawa et al. (2023).

2.1 Fly colony

We used 50 lines from the Drosophila Genetic Reference Panel (DGRP). DGRP is a landmark development in the genetic analysis of complex traits in *Drosophila*. It is a collection of more than 200 inbred lines derived from a natural population in Raleigh, North Carolina (MacKay et al., 2012). Each line in the DGRP has been fully sequenced and, because the lines are homozygous, repeated measures of individuals from the same genotype can be used to estimate genetic variance with high precision. The DGRP provides an open resource for genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, and the estimation of quantitative genetic parameters across a wide range of phenotypes, including behaviour.

Fifty inbred lines from the core set of the DGRP were ordered by SMD in the second half of 2021 from the Bloomington Drosophila Stock Centre (Indiana University, Bloomington, IN, USA; see Supplementary Table 1 for the complete list of lines). All lines were shipped to the Charles Perkins Centre (University of Sydney) and their stocks were maintained there under the supervision of ZH. Initially, all lines underwent two rounds of the full reproductive cycle under quarantine. Afterwards, adults from the F3 generation were used in the expansion stage (intensive parallel breeding of flies) to establish the required number of experimental flies in each line (done by ZH, CH, RA and YQL).

DGRP flies were used in a larger study, and the current analysis represents its subset. For the purposes of the large study, 50 lines were grouped in batches of 10 lines; batching was used to create a staggered design, where each group of 10 lines would enter actual assays every few days, allowing phenotyping to be done within the allocated time. Within each line, the flies used in the current study were supplied in groups of 20-30 individuals of each sex. Thus, each batch consisted of 10 lines and two sexes per line, with up to 30 individuals per vial (600 adult flies in total per batch). Subsequent batches entered the assays as freshly emerged adult flies (up to 1 week of age) on the following days of 2022: 23/02, 25/02, 10/03, 15/03, 1/04. Adult flies were placed in acrylic vials with 55-65 mL of standard *D. melanogaster* food medium (by ZH, CH, RA and YQL) and delivered to the School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney by SMD or commercial shipping service. There, they were transferred to fresh food vials put on plastic flippers (Drosoflipper, London, UK) by SMD to facilitate the exchange of used food vials for new ones in later procedures not described here (irrelevant in this study as all fly assayed for the current analysis went through behavioural trials on the third day after arriving at the lab).

Each batch was split by SMD into two 'sessions', where half the batch was housed in an incubator where the 12h light cycle started at 9 am, and the other half of the batch was stored in an incubator where the 12h light cycle started at 1 pm. This was so that we could assess all flies from one batch on a single day (that is, in a morning session and an afternoon session) while standardising the circadian rhythm so that all sessions were conducted in the 'morning' activity period for the flies and avoided their low activity period of midday. General maintenance of the colony was done by SMD and CV.

2.2 Phenotyping

The assay methods are based on the detailed protocols reported elsewhere by Macartney et al. (2022, 2025). Behavioural assays were performed by SMD, ELM, SN, ML, SB, and PP. Briefly, we used high-throughput automated tracking units produced by Zantiks (Zantiks MWP unit, Cambridge, UK; firmware version 02/2021). These units are designed to track small animals, where each unit consists of i) an experimental chamber where the animals are placed (see below for details of the 'plates' in which individuals are loaded before being placed in the unit chamber), ii) a camera that tracks the animals, and iii) a computer controlling the units. All units were programmed to maintain 25°C in the chamber.

Using these units, we conducted three different behavioural assays: 1) locomotion tracking in which the overall

movement of individuals was measured within a 1 cm deep and round arena, 2) startle response to a light-off stimulus (also conducted in the same 1 cm deep and round arena as the locomotion assay), and 3) exploration in a Y-maze (see Macartney et al., (2025) for more details about the arenas). The locomotion tracking assay was run for 30 min (plus a 10-min habituation period), and the distance travelled by each individual was recorded in three intervals of 10 min each. The light-off startle response recorded the distance travelled in 1-second intervals following three consecutive 15-ms light-off pulses, allowing us to measure habituation across the pulses. The Y-maze assay recorded 'trigrams' of the turn direction flies took while travelling between the maze's arms (e.g., RRR, LLL RLR, LRL, etc, where R = right, L = left) (see Macartney et al. (2025)). See Macartney et al. (2025) for a discussion of the biological relevance and robustness of the assays.

For each session (i.e., morning and afternoon sessions per batch), we assayed 300 flies (10 lines/batch, 2 sexes, 5 flies/batch/sex in each of the three assays). In total, 3000 flies were scored; see Results for final sample size after accounting for deaths and lack of movement detected within arenas. At the beginning of each session, all flies were anaesthetised by briefly immersing the vials in a bucket of ice. All flies from each line and sex combination were then tipped into separate Petri dishes. Random flies from each line and sex combination were then selected from their respective Petri dishes and transferred by aspiration into a 48-well plate (only 45 wells were filled) or into a Y-maze plate (each session used 3 Y-maze plates, 15 mazes/plate). The flies were then given 10 min at 25°C to recover and transferred to four different assay units: one for the well-plate with 45 flies and three units for each of the three Y-maze plates. The unit with the well plate always recorded locomotion first, followed by the light-off startle response. In particular, each adult fly was used only once in an assay (taking advantage of the inbred property of the lines, we assumed that all flies from the given line represent the same genotype) and then discarded after a trial.

2.3 Phenotypic traits

The data returned by the units were used to calculate a number of phenotypic descriptors. For the locomotion assay, the measured trait was the total distance travelled in the arena in each of the 10-minute intervals; the distance was recorded in raw arbitrary units and then recalculated to millimetres. The startle response assay returned distances of movement after each light-off stimulus (see the previous subsection). In circular arenas, flies often follow the arena walls, and thus this behaviour is also referred to as arena circling. Finally, the Y-maze assay returned a series of left-right turn decisions, together with the timestamps of each turn.

To process the Y-maze data, we calculated several maze exploration metrics. First, a logged ratio of the number of right-turns to the number of left turns was calculated as a measure of movement handedness (the value of zero indicates lack of turn bias and no handedness; positive values measure increasing levels of right-handedness, negative values - left-handedness). Secondly, the turn-taking events were assembled into 'trigrams' (triplets of consecutive turns, e.g., LLL, RLR, RRL, etc.). The counts of specific triplets were used to calculate the numbers of repetitions (occurrences of three consecutive turns in the same direction, i.e., RRR or LLL) and alternations (turns involve a there-and-back movement, i.e., RLR or LRL) recorded in a given assay session. Finally, to quantify the variability of maze behaviour, we calculated two indices. Switchiness recorded the consistency of the turning behaviour (e.g., ...LLLLLRRRRR... represents low switchiness, ...LLRLRRLLLR... - moderate, ...LRLRLRLRLR... - highest possible). Switchiness was calculated as $(N_{L\rightarrow R} + N_{R\rightarrow L})/(2N_R N_L/N)$, where N describes the number of all turns, N_x - the number of x-type turns and $N_{x\rightarrow y}$ - the number of x-turns that are followed by y-turn. The second index, clumpiness, expressed the temporal uniformity of maze exploration. Low value describes individuals consistently exploring the maze without stopping, large values - flies that explored the maze in bursts of intense exploration separated by periods of no movement). The clumpiness was calculated using the median absolute deviation (MAD) of the intervals between turns T_i (measured in seconds): $\text{median}[|T_i - \text{median}(T_i)|]/(1800/N)$.

2.4 Statistical analysis

Data processing and analysis were performed by SMD. Each unit produced a data sheet per assay run. All data sheets were labelled with a unique ID, which means that we could match each data sheet to individuals coming from a specific line and sex. All data were cleaned and analysed in the R environment (version 4.2.2) using Visual Studio Code (Microsoft, USA). Genetic parameters were estimated using ASReml-R v. 4.2 (VSNi, UK) (Butler et al., 2023). Data processing and parsing was done using `dplyr` (Wickham et al., 2023). Visualisation was done using `ggplot2`

(Wickham, 2016) and `ggcorrplot` (Kassambara, 2023). Detailed versions and session data can be found in the paper's data supplement.

First, we ran univariate general linear mixed models (LMM) for each phenotypic character. The models included sex as a fixed effect (females as the reference level). Random terms included: line ID L_{ij} (genetic effect, its variance approximates the broad-sense genetic variance), batch ID B_i, and assay ID A_{ijk} (the latter two accounting for technical nonindependence introduced through assay clustering). The general form of any univariate model was:

$$y_{ijkl} = \mu + s_{male} + B_i + L_j + A_k + e_{ijkl}, \tag{1}$$

$$L_j \sim \mathcal{N}(0, \sigma^2_G), \tag{2}$$

$$B_i \sim \mathcal{N}(0, \sigma^2_B), \tag{3}$$

$$A_k \sim \mathcal{N}(0, \sigma^2_A), \tag{4}$$

$$e_{ijkl} \sim \mathcal{N}(0, \sigma^2_e). \tag{5}$$

In the above models, μ is the model intercept, s_{male} is the fixed effect of sex. All random effects and the residual error $_{ijkl}$ are assumed to be sampled from relevant normal distributions with zero mean and variance interpretable as (conditional) variability estimated between replication units at each level.

The random effect of line ID was used to calculate broad-sense heritability (as a fraction of the variance between lines in overall variability). The *sensu lato* nature of our heritability estimates is due to the fact that the variation between inbred lines cannot be decomposed to separate the dominance variance (Walsh and Lynch, 2018).

Cross-trait correlations were estimated using a multivariate model that allowed for heterogeneous genetic (line) and residual variance components between the traits, explicitly modelling the cross-trait genetic covariance matrix $\mathbf{G}$ and residual covariance matrix $\mathbf{R}$:

$$y^{(n)}_{ijkl} = \mu^{(n)} + s^{(n)}_{male} + B^{(n)}_i + L^{(n)}_j + A^{(n)}_k + e^{(n)}_{ijkl}, \tag{6}$$

$$\mathbf{L} \sim \mathcal{MVN}(\mathbf{0}, \mathbf{\Sigma_G}),\ \mathbf{\Sigma_G} = \mathbf{G}_{n\times n} \otimes \mathbf{I}, \tag{7}$$

$$\mathbf{e} \sim \mathcal{MVN}(\mathbf{0}, \mathbf{\Sigma_e}),\ \mathbf{\Sigma_e} = \mathbf{R}_{n\times n} \otimes \mathbf{I}. \tag{8}$$

The (n) indicator indexes the n phenotypic traits in a model. Both $\mathbf{\Sigma}_.$ matrices are built using the identity matrices of appropriate dimension $\mathbf{I}$ - i.e., we assume that, e.g., the j-th line effects for one trait may depend only on the effects of this line in other traits.

Finally, sex-specific estimates for the analysed traits were derived from models that assumed sex-specific heteroscedasticity of genetic and residual variances, with the cross-sex genetic correlations estimated rather than fixed at unity:

$$\mathbf{R}_{\times \text{sex}} = \sigma^2_{e_m} \oplus \sigma^2_{e_f}, \tag{9}$$

with $\sigma^2_{e_m}$ and $\sigma^2_{e_f}$ being the sex-specific residual variances. The direct product indicates that the cross-sex residual correlations are not estimated (they are not identifiable).

Hypothesis tests for variance components are based on a series of likelihood-ratio tests comparing target models with their simpler alternatives (i.e., for variance components, models without the focal random term, for genetic correlations, models with correlations fixed at values expected under H_0) (Lynch and Walsh, 1998). Thr logged ratios of model likelihoods were assumed to be χ^2-distributed with an appropriate number of degrees-of-freedom (Self and Liang, 1987).

3 Results

3.1 Locomotion and Y-maze behaviour

3.1.1 Genetic variances

All traits studied exhibited low to moderate levels of genetic variance (expressed as variance between genetically homogeneous lines of the DGRP panel; Fig. 1). Locomotory activity appeared to be the most heritable trait in the entire set. Overall locomotion activity (measured as arena circling distance covered during the test) harboured significant genetic variance (likelihood-ratio test (LRT): $\chi^2_{df=1} = 203.532$, $p < 0.001$; see also Tab. 1). The round effects (one of the three repetitions recorded for each individual) were marginally important (Tab. 1), highlighting that the repeatability of the trait was mainly due to genetic effects. Batch and Assay ID effects explained fractions of variance not distinguishable from zero (Tab. 1).

Excluding individuals that did not move throughout the test (but remained alive - see Methods) did not remove genetic variance; such data still resulted in heritability of $h^2 = 0.185$ significantly different from zero (see Tab. 1). Males tended to be more active than females (estimate from data including zeroes ±SE: $b_{M-F} = 0.163 \pm 0.101$; data without zeroes: $b_{M-F} = 0.226 \pm 0.101$) and in the latter case the difference was significant ($\chi^2_{df=1} = 5.034$, $p = 0.025$).

The sexes did not appear to have heterogeneous genetic variances in locomotor activity (males: $h^2_m = 0.230 \pm 0.052$, females: $h^2_f = 0.228 \pm 0.051$), but appeared independent in terms of the underlying genetic effects (low genetic correlation between sexes: $r_{g,mf} = 0.271 \pm 0.171$; 1-degree-of-freedom LRT for $H_0 : r_g = 1$, $\chi^2 = 88.278$, $p < 0.001$; for $H_0 : r_g = 0$, $\chi^2 = 2.288$, $p = 0.13$).

Y-maze exploration was measured from several perspectives. In all Y-maze behaviour descriptors (except for handedness; $\chi^2_{df=1} = 0.000$) there was significant genetic variance resulting in heritabilities ranging from low to moderate (see Tab. 2; LRT p-values: no. of repetitions $p < 0.001$, no. of alternations $p < 0.001$, switchiness $p < 0.001$, MAD clumpiness $p < 0.001$). Batch effects did not matter (in all cases estimates of variance were not different from zero; Tab. 2). Assay ID appeared more important in some measured traits (number of alternations and switchiness) indicating that their underlying determinants (e.g., the identity of the person operating the unit, time of day) may have some impact on measured traits; nonetheless, in the most heritable traits, all other random effects were relatively negligible.

In all traits measured with Y-maze, the sexes did not differ significantly from each other. Nonetheless, in traits that should be associated with overall locomotor activity, males tended to score higher, in line with the pattern seen in arena circling behaviour (no. of repetitions: $b_{M-F} = 0.195 \pm 0.167$, no. of alternations: $b_{M-F} = 0.103 \pm 0.124$). The only exception was MAD clumpiness, where males tended to explore the mazes in a "clumpier" way (i.e., more likely in bursts of activity separated by periods of inactivity, rather than continuously walking between the arms; $b_{M-F} = -0.340 \pm 0.135$).

The patterns of sex-specific genetic variance varied between traits. Both the number of repetitions and alternations were genetically integrated between the sexes (strong genetic correlation, in both cases fixed at $r_{g,mf} = 1$ by the model). Interestingly, while the number of repetitions did not exhibit sex-specific genetic variances ($h^2_m = 0.142 \pm 0.088$ vs. $h^2_f = 0.147 \pm 0.087$), heritability of no. of alternations was higher in females ($h^2_f = 0.144 \pm 0.088$) than in males (effectively $h^2_m = 0$). Handedness exhibited no detectable genetic variance (Tab. 2), and this pattern was not different

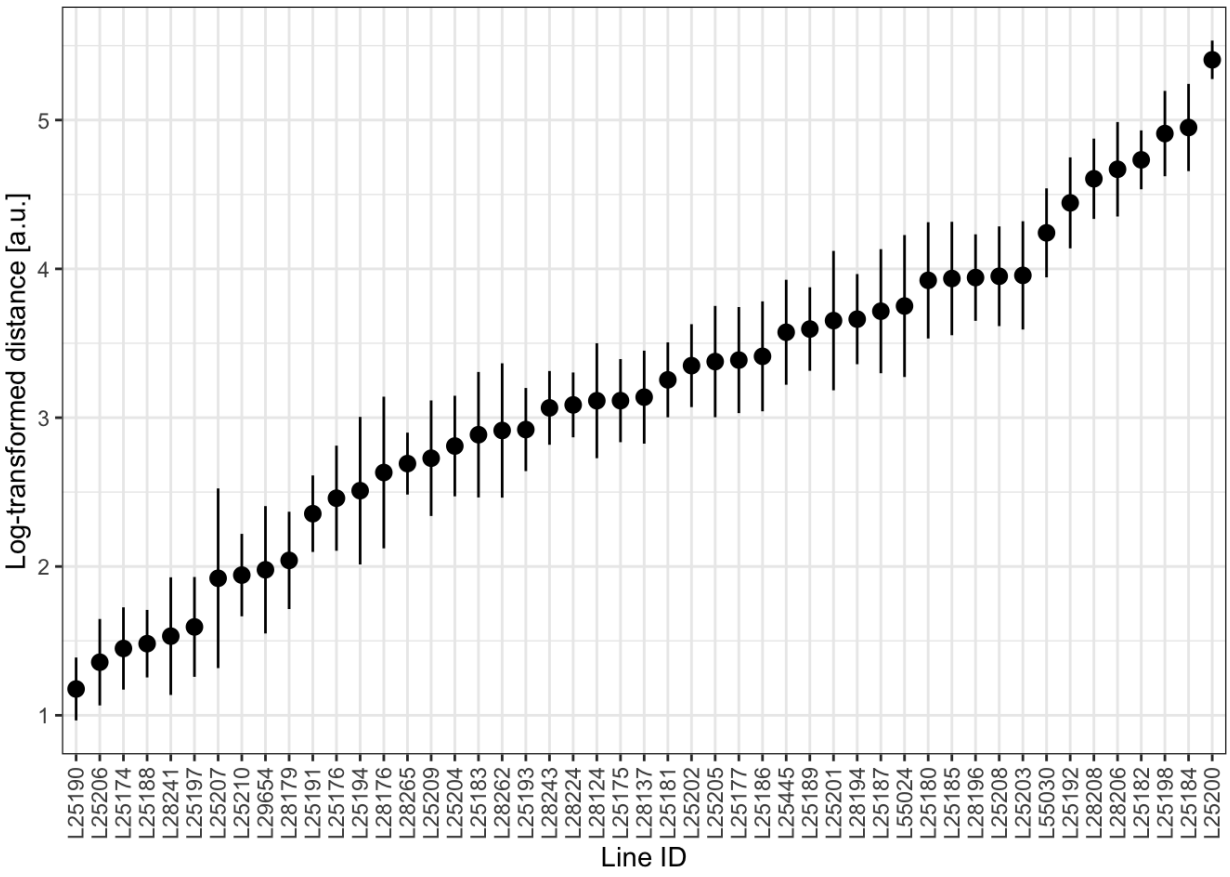

Figure 1: The distribution of average line-specific locomotory scores among the 50 included DGRP lines (transformed with a natural logarithm). The lines are sorted according to the mean trait value. Error bars indicate within-line standard errors; distance was measured in arbitrary units proportional to actual metric distances.

Table 1: Variance components of locomotory activity (arena circling). The "Fraction" column provides proportions of total variance explained by each random effect with its SE (Fraction for line effect is equal to heritability: $h^2 = \sigma^2_G/\sigma^2_{total}$)

Random effect	Variance	SE	Fraction
Locomotion data including zeroes			
Round	0.005	0.013	0.001 ± 0.003
Batch	0.239	0.314	0.053 ± 0.066
Assay ID	0.165	0.121	0.036 ± 0.026
Line	1.011	0.242	0.223 ± 0.046
Residual	3.110	0.125	0.686 ± 0.059
Locomotion data excluding zeroes			
Round	0.006	0.013	0.002 ± 0.003
Batch	0.285	0.310	0.074 ± 0.076
Assay ID	0.128	0.098	0.033 ± 0.025
Line	0.710	0.177	0.185 ± 0.042
Residual	2.701	0.115	0.705 ± 0.065

Table 2: Variance components of Y-maze behaviour. The last column provides proportions of total variance explained by each random effect with its SE. For definitions of variables - see Materials & methods. Estimates without SE (—) have been fixed by the model at the boundary of their parameter space

Random effect	Variance	SE	Fraction
Number of repetitions (log scale)			
Batch	0.360	0.356	0.137 ± 0.119
Assay ID	0.453	0.207	0.172 ± 0.078
Line	0.381	0.173	0.144 ± 0.067
Residual	1.443	0.139	0.547 ± 0.107
Number of alternations (log scale)			
Batch	0.028	0.075	0.022 ± 0.059
Assay ID	0.213	0.096	0.171 ± 0.072
Line	0.198	0.090	0.159 ± 0.07
Residual	0.809	0.078	0.649 ± 0.084
Handedness (log of N_R/N_L)			
Batch	0.000	—	0.000
Assay ID	0.015	0.021	0.026 ± 0.038
Line	0.000	—	0.000
Residual	0.546	0.050	0.974 ± 0.038
Switchiness			
Batch	0.018	0.020	0.125 ± 0.128
Assay ID	0.054	0.017	0.38 ± 0.101
Line	0.013	0.006	0.091 ± 0.045
Residual	0.057	0.006	0.404 ± 0.078
MAD Clumpiness (log scale)			
Batch	0.049	0.102	0.03 ± 0.061
Assay ID	0.139	0.099	0.085 ± 0.06
Line	0.430	0.164	0.262 ± 0.09
Residual	1.022	0.100	0.623 ± 0.09

in sex-specific parameters (not shown). MAD clumpiness and switchiness showed no strong sex-specificity on variances (MAD clumpiness: $h^2_m = 0.174 \pm 0.098$ vs. $h^2_f = 0.326 \pm 0.105$; switchiness: $h^2_m = 0.224 \pm 0.098$ vs. $h^2_f = 0.102 \pm 0.079$). Inter-sexual r_g was close to unity in MAD clumpiness, but relatively low in switchiness, indicating weaker cross-sex genetic integration ($r_{g,mf} = 0.139 \pm 0.411$).

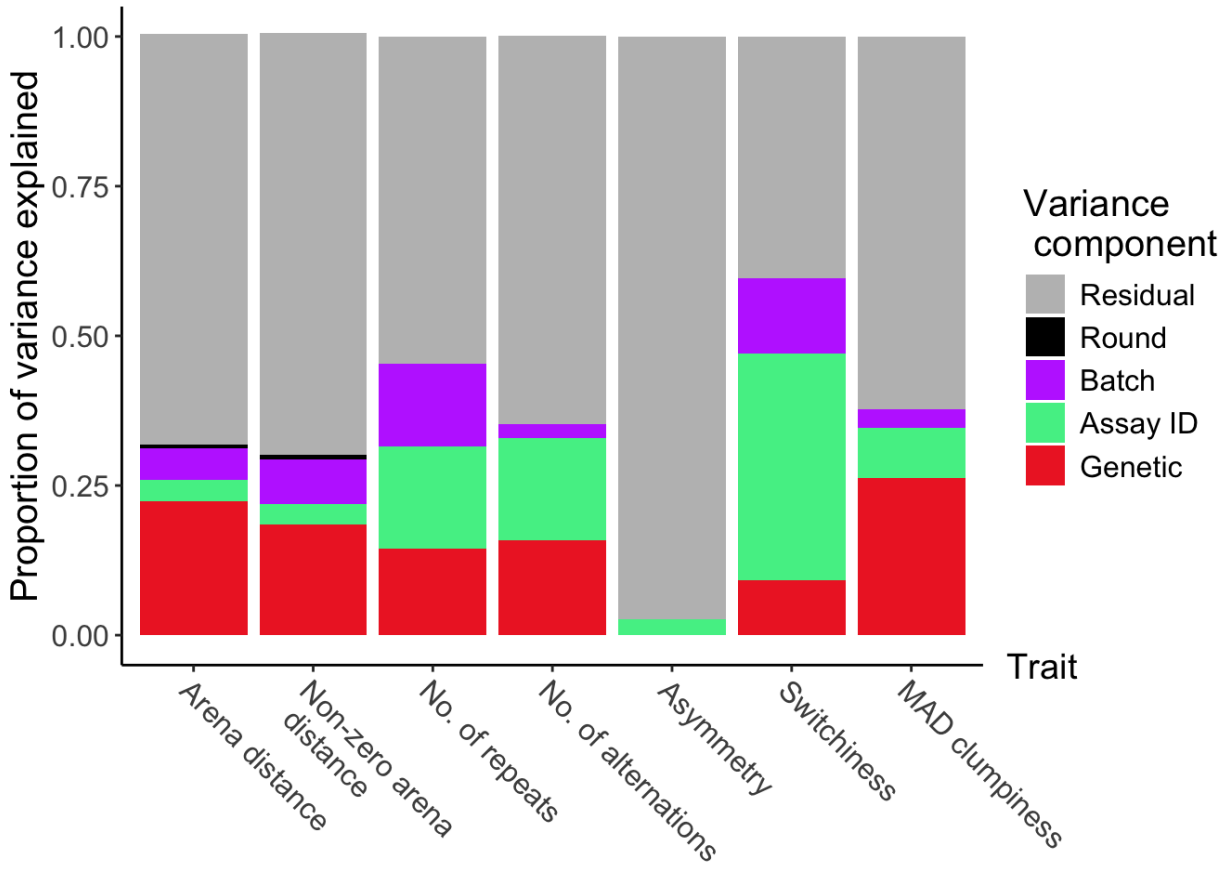

Figure 2: The fraction of overall trait variance explained by the random effects (colour-coded) of the inbred line (genetic), batch (grouping several lines) and assay (grouping multiple individuals in one assay run). The round effect was present only in locomotory data (within each assay, the measurements were grouped into three 10-minute rounds). The genetic (red) effect is estimated as the between-lines variance.

3.1.2 Genetic correlations

Instead of using raw numbers of repeats and alternations (thus avoiding unidentifiable correlations likely close to unity), we used a single measure of Y-maze behaviour (proportion of alternations in all trigrams) that can be regarded as a measure of working memory. Most traits measured in the locomotion and Y-maze trials were tightly genetically correlated - indicating their common underlying genetic underpinnings. In most cases, the cross-trait genetic correlations were fixed at the boundary of parameter space ($|r| = 1$; Fig. 3). In a few cases, we observed less strict genetic constraints (Fig. 3): relative number of alternations was weakly correlated with total arena circling distance, Y-maze clumpiness and Y-maze movement asymmetry (distance vs. fraction of alternations: $r_g = 0.216 \pm 0.305$; clumpiness vs. fraction of alternations: $r_g = -0.611 \pm 0.176$; asymmetry: $r_g = 0.198 \pm 0.602$). More active flies also had lower genetic scores for asymmetry in the Y-maze and Y-maze clumpiness ($r_g = -0.506 \pm 0.512$ and $r_g = -0.611 \pm 0.176$, respectively), but these negative correlations were estimated with errors, making them statistically non-significant. In general, the model clearly lacked the power to resolve all genetic correlations.

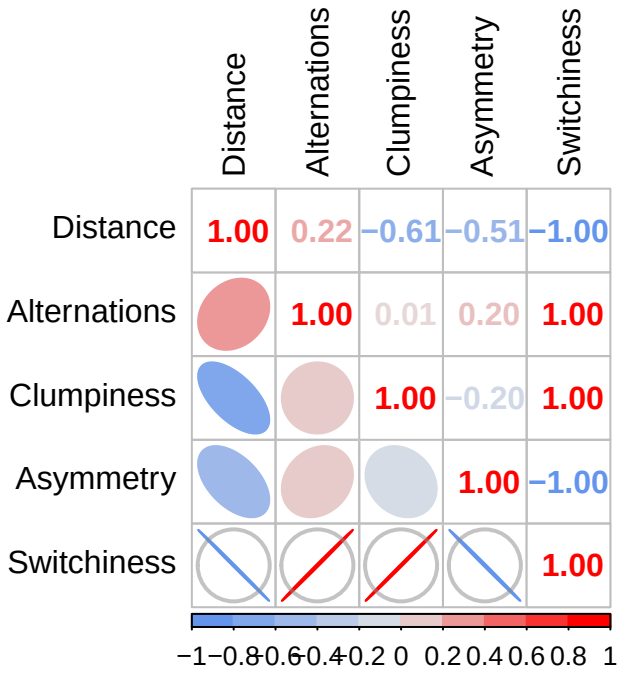

Figure 3: Genetic correlations between traits. Ellipses and their colouration depict the strength of correlation. Correlations close to 1 (or -1) take the form of diagonal lines. Correlations marked with open circles were estimated at the boundary of parameter space (fixed at 1 or -1). Diagonal elements are blank (variances, i.e., r=1)

3.2 Startle response

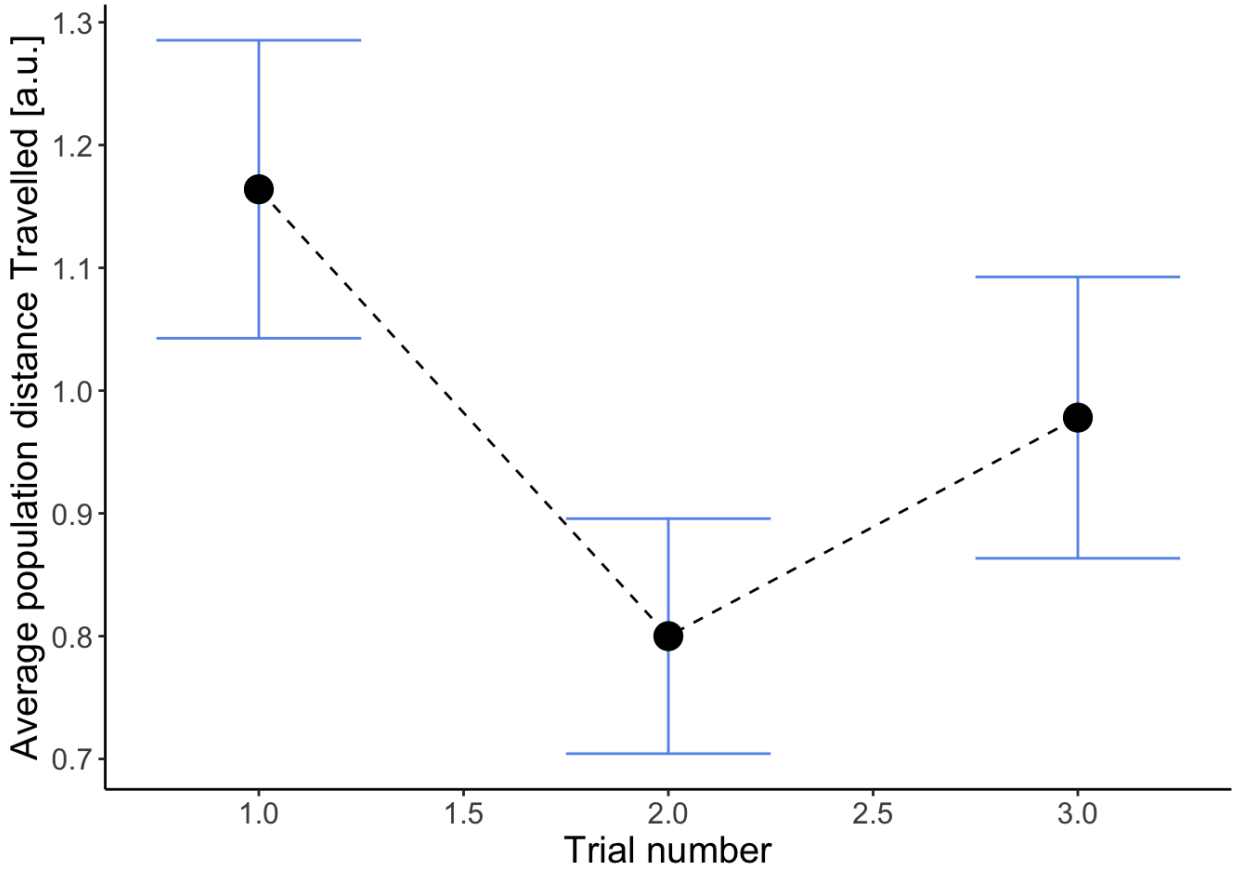

Figure 4: Startle response distance travelled by flies in the Y-maze. Each point represents the mean distance travelled by flies in a given trial. Error bars represent the standard error of the mean.

There was a marked average response to the startle stimulus (light-off) between the first and second exposure, and no significant difference between the second and third (Fig. 4). Inbred lines differed clearly not only in overall activity levels (Fig. S1; see also Fig. 1), but also in the slope and direction of the startle response. However, the genetic variation in startle response (line-linked variation in habituation slopes) was not statistically significant (model assuming continuous startle number variable: $\chi^2 = 1.800$, $p = 0.09$, variance estimate: 0.046 ± 0.037; model using the contrast convention: $\chi^2 = 0, p = 1.0$, boundary variance estimate ($var = 0$)). Estimation of non-zero genetic variation in the startle-response slopes was not possible even after constraining the random regression part to the first contrast (time point 1 vs. 2) only (i.e., interval where the response is more likely to be noted due to no prior habituation).

3.3 Sex-specific genetic effects

Sex specificity was absent in most genetic parameter estimates, except for the number of Y-maze alternations - in this trait, males exhibited no detectable genetic variance, contrary to females ($h^2 = 0.144$; see Tab. 3). Interestingly, despite no sex-specific heritabilities, overall locomotor activity was not correlated strongly between the sexes ($r_{mf} = 0.271$), indicating loose cross-sex genetic constraint and crossing intersexual reaction norms.

Table 3: Sex-specific genetic variances and heritabilities (in parentheses) of the analysed traits with relevant SE, together with their cross-sex genetic correlations (±SE). All estimates come form the `Line ID` random effect. Lack of SE indicates estimates at the parameter space boundary (variance close to 0 or correlation close to ±1)

Trait	Female	Male	Correlation
Locomotory activity (log)	0.483 ± 0.126 (0.228 ± 0.051)	0.487 ± 0.126 (0.23 ± 0.052)	0.271 ± 0.171
No. of repetitions (log)	0.27 ± 0.176 (0.147 ± 0.087)	0.259 ± 0.172 (0.142 ± 0.088)	0.999
No. of alternations (log)	0.223 ± 0.154 (0.144 ± 0.088)	0.000 0.000	0.999
Handedness (log)	0.000 0.000	0.000 0.000	0.997
Switchiness	0.246 ± 0.204 (0.102 ± 0.079)	0.623 ± 0.321 (0.224 ± 0.098)	0.139 ± 0.411
MAD clumpiness (log)	0.604 ± 0.263 (0.326 ± 0.105)	0.263 ± 0.166 (0.174 ± 0.098)	0.999

4 Discussion

Behavioural traits, though more variable and context-dependent than morphological or physiological characters, are crucial phenotypes with direct fitness consequences. Their genetic architecture has remained relatively underexplored compared to other trait classes, due to persistent challenges in consistent phenotyping and the inherently dynamic nature of behaviour (Dingemanse and Dochtermann, 2013). In this study, we examined the genetic basis of behavioural variation in *Drosophila melanogaster*, focusing on locomotion, space exploration (via Y-maze-based proxies), and habituation to a light-induced startle response. Our findings emphasise three key areas: heritability of behavioural traits and their evolutionary potential, sex-specific components of genetic variance, and the opportunities and limitations of laboratory-based behavioural assays.

4.1 Heritability and Evolutionary Potential

The consistent detection of genetic variance across multiple behaviours indicates measurable evolutionary potential (Lynch and Walsh, 1998). The strongest signals were observed in locomotor activity and exploratory Y-maze behaviours, with heritabilities of $\sim 20\%$ and $\sim 15\%$, respectively. These values are consistent with previous estimates of genetic effects in arthropods, including *D. melanogaster* (15–44%: Jordan et al., 2006, 2007; Noer et al., 2024), *Cydia pomonella* (37%: Schumacher et al., 1997), *Tribolium castaneum* (10–40%: Matsumura et al., 2022; Matsumura and Miyatake, 2015), and *Tetranychus urticae* (24%: Bitume et al., 2011). Importantly, these signals persisted after excluding immobile individuals, suggesting that heritability is not an artefact of extreme phenotypes but reflects underlying genetic architecture. Locomotor traits in particular are known to respond quickly to directional selection (Matsumura et al., 2022), and may be especially important in contexts such as behavioural thermoregulation under rapid climate change (Pottier et al., 2025).

Exploratory behaviour also showed robust line effects (indicative of significant genetic variance). Traits derived from alternation and movement irregularity in the Y-maze retained genetic components despite potential modulation by motivation or physiological state. The fact that traits describing behavioural variability (e.g. switchiness, median absolute deviation (MAD) clumpiness) were heritable highlights the capacity of high-throughput phenotyping to dissect not only mean-level traits but also variance traits (Ayroles et al., 2015). These results add to a growing recognition that even complex cognitive or sensorimotor behaviours, often dismissed as too plastic, can be rigorously quantified and genetically analysed (Werkhoven et al., 2019, 2021; Sauce et al., 2018). Together, they support the view that behaviour harbours substantial, evolvable genetic variation.

4.2 Sex Differences in Behavioural Genetics

A central question in behavioural genetics is the degree to which males and females share the underlying genetic architecture (Poissant et al., 2008, 2010). Our results suggest a mixed pattern. For many behaviours, heritabilities

were similar across sexes, implying shared architecture. Yet for certain exploratory strategies, we observed sex differences in the magnitude of genetic variance or cross-sex correlations, consistent with findings in other insects (Yarwood, 2022; Fuchikawa and Okada, 2013). In line with broader comparative evidence (Wyman and Rowe, 2014), most of the asymmetries referred to male-biassed genetic variance in locomotor traits. However, such biases can be assay- or population-specific: Videlier et al. (2021) reported a 40% male bias in locomotor variance in *D. melanogaster*, while in our data, overall activity variance was homogeneous, with sex differences emerging mainly in maze-derived traits.

Low cross-sex genetic correlations in some traits indicate reduced integration and the potential for sex-specific evolutionary trajectories. Such patterns open the door to sexual conflict over shared genetic variation and echo results on sociality and mating behaviours in flies (Scott et al., 2018). These results resonate with research on behavioural syndromes and personality, where genetic correlations within individuals may manifest differently between sexes (Matsumura, 2024). They highlight the role of sexual selection, dimorphism, and potentially sex-specific selection pressures in shaping behavioural evolution in *Drosophila*.

4.3 Genetic Correlations and Behavioural Integration

Genetic correlations can constrain or facilitate multivariate evolution by linking trait responses. We found a strong integration between certain behaviours, such as activity level and exploration of the maze (Fig. 3), suggesting a correlated evolution and a potential structure of behavioural syndromes (Garamszegi and Herczeg, 2012). Similar coupling has been documented in locomotor traits (Hamlin et al., 2025), although it remains uncertain whether such patterns extend to natural settings (Matsumura, 2024). At the same time, other traits appeared largely independent, despite phenotypic similarity, pointing to distinct underlying circuits or motivational bases.

Some genetic correlations were difficult to resolve due to limited statistical power—a common issue in multivariate models when traits differ in measurement reliability or sample distribution. Nonetheless, the evidence for both integrated and independent axes of variation demonstrates the value of automated phenotyping and multivariate approaches for uncovering behavioural architecture.

4.4 Startle Responses and the Challenge of Detecting Transient Behavioural Variation

The startle assays provided clear phenotypic responses but limited genetic variance, particularly in habituation. Similar patterns were previously observed in outbred *Drosophila* populations (Macartney et al., 2022). The transient state-dependent nature of startle responses may explain their low repeatability, which complicates genetic analysis. Alternatively, habituation plasticity may be shaped more by developmental or epigenetic mechanisms than by additive genetic effects (Huang et al., 2020). These challenges illustrate why detecting genetic variance in reactive behaviours requires larger datasets, alternative assay designs, or integrative approaches combining genetics with neural or physiological measurements.

4.5 Laboratory Environments and the Ecological Validity of Behavioural Assays

A recurring concern in behavioural genetics is the ecological validity of laboratory assays. Our paradigms—locomotor arenas, Y-mazes, and startle assays—were designed for consistency and repeatability and have been validated in previous work (Fenckova et al., 2019; Cleal et al., 2021; Werkhoven et al., 2021). However, they inevitably simplify natural challenges, such as predation, foraging, or social interactions. Y-maze exploration may capture novelty responses but not dispersal ecology, while arena locomotion omits environmental complexity. However, such assays are generally considered to quantify modular behavioural components that can evolve semi-independently (Berman et al., 2014; Seidenbecher et al., 2020; Vinicius, 2010; Eberhard, 2018). Our detection of robust heritabilities and correlations suggests that these modules represent genuine axes of genetic variation. Extending the analyses to semi-natural or enriched conditions would further clarify their ecological relevance (e.g. Macartney et al., 2025).

The trade-off between experimental tractability and ecological realism is well recognised. Automated and high-throughput assays offer precision and power, yet complementary field-based or semi-natural approaches will be crucial for connecting laboratory phenotypes with natural behaviour. Recent advances in computational ethology (see Anderson and Perona, 2014; Branson et al., 2009) provide exciting opportunities to bridge this gap, allowing behavioural assays to scale while retaining ecological relevance. Integrating such approaches will strengthen the link between behavioural genetics, ecology, and evolution (cf. Kruitwagen et al., 2018).

4.6 Concluding Remarks

Using DGRP in combination with automated phenotyping, we obtained robust estimates of quantitative genetic parameters and revealed patterns of sex specificity and genetic integration. Our study demonstrates that behavioural traits in *Drosophila melanogaster*, particularly those related to mobility and exploration, harbour substantial heritable variation, including general and sex-specific components. These findings confirm that behaviour is not purely plastic but an evolvable phenotype.

Beyond *Drosophila*, these results inform broader questions in behavioural ecology, evolutionary biology, and neurogenetics. As behavioural genetics incorporates genome editing, machine learning, and large-scale phenotyping, the ability to connect genes, circuits, and behaviour within an evolutionary framework becomes increasingly attainable. Our findings contribute to this larger effort, highlighting both the promise and the complexity of dissecting the genetic architecture of behaviour.

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P. Pottier, M. R. Kearney, N. C. Wu, A. R. Gunderson, J. E. Rej, A. N. Rivera-Villanueva, P. Pollo, S. Burke, S. M. Drobniak, and S. Nakagawa. Vulnerability of amphibians to global warming. *Nature*, 639(8056):954–961, Mar. 2025. ISSN 0028-0836, 1476-4687. doi: 10.1038/s41586-025-08665-0. URL https://www.nature.com/articles/s41586-025-08665-0. TLDR: This work used a phylogenetically informed data-imputation approach to predict the heat tolerance of 60% of amphibian species and assessed their vulnerability to daily temperature variations in thermal refugia to challenge evidence for a general latitudinal gradient in overheating risk.

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J. Shorter, C. Couch, W. Huang, M. A. Carbone, J. Peiffer, R. R. H. Anholt, and T. F. C. Mackay. Genetic architecture of natural variation in drosophila melanogaster aggressive behavior. *Proceedings of the National Academy of Sciences*, 112(27):E3555–E3563, July 2015. ISSN 10916490. doi: 10.1073/pnas.1510104112. URL https://www.pnas.org/doi/abs/10.1073/pnas.1510104112. Publisher: Proceedings of the National Academy of Sciences TLDR: This work performed genome-wide association analyses using the inbred, sequenced lines of the Drosophila melanogaster Genetic Reference Panel and an advanced intercross population derived from the most and least aggressive DGRP lines to identify genes that have been previously implicated in aggressive behavior as well as many novel loci.

U. Stern, H. Srivastava, H.-L. Chen, F. Mohammad, A. Claridge-Chang, and C.-H. Yang. Learning a Spatial Task by Trial and Error in Drosophila. *Current Biology*, 29(15):2517–2525.e5, Aug. 2019. ISSN 0960-9822. doi: 10.1016/j.cub.2019.06.045. URL https://www.cell.com/current-biology/abstract/S0960-9822(19)30772-9. Publisher: Elsevier TLDR: It is shown that Drosophila can remember a reward-baited location through reinforcement learning and do so quickly and without requiring vision, uncovered new depth in flies' ability to learn a spatial task and established an assay with a level of throughput that permits a systematic genetic interrogation of flies' able to learn spatial tasks.

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5 Supplementary materials

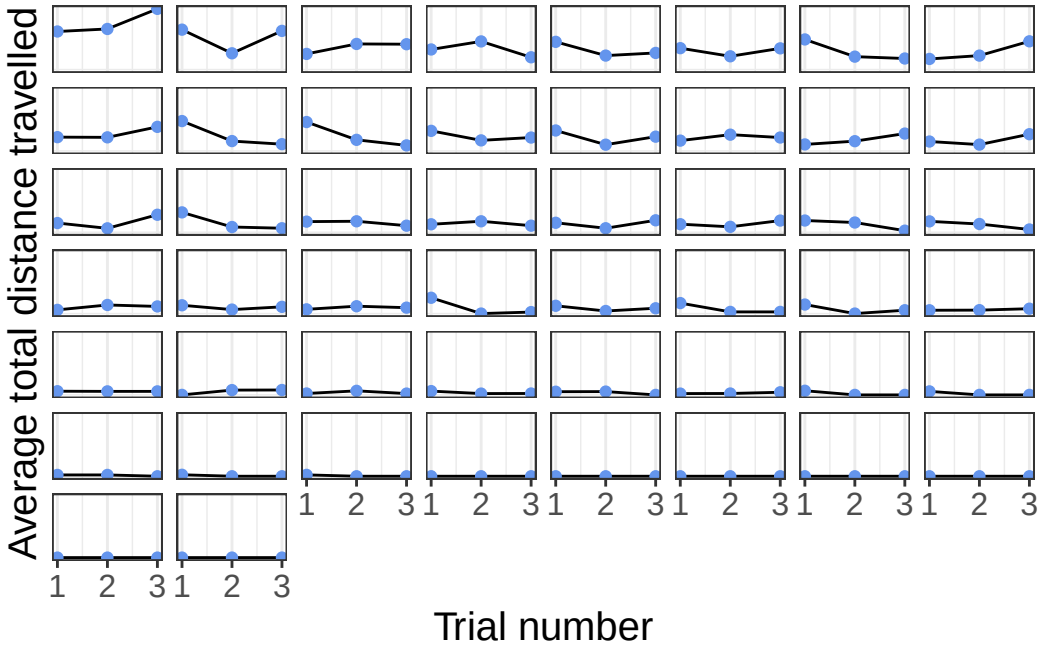

Figure S1: Average startle response distance travelled by flies in each line, sorted by overall level of locomotory activity.