The genetic basis of fitness in *Drosophila*: A genome-wide association study

A Preprint

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February 5, 2021

Abstract

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***K*eywords** blah · blee · bloo · these are optional and can be removed

Introduction

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the *Drosophila* Genetic Reference Panel (DGRP), a collection of almost entirely homozygous lines that represent a snapshot of natural genetic variation from a population in North Carolina (REF: Mackay et al., 2012)

Methods

Fly stocks and husbandry

Our study focused on 125 lines randomly selected from the DGRP. All flies were reared in 25mm vials with Hoff food medium (REF?), lightly sprinkled with dried yeast, at a temperature of 25°C. We verified the genotype of each DGRP line using the restriction-based assay PCR described in (Mackay et al., 2012) for the eight most diagnostic markers, and verifed the genotypes of the lines prior to data collection. In addition to the DGRP, we used two stocks carrying the visible markers, *brown*^{1} (*bw*^{1}) and *PFRT(w^{hs})G13 PUbi-GFP.nls2R1 PUbi-GFP.nls2R2* as mates and competitors for the DGRP flies, which are hereafter referred to as *bw* and *GFP* respectively (*GFP*: green fluorescent protein).

Measuring male and female fitness

We measured sex-specific fitness for each DGRP line using a protocol modified from Innocenti and Morrow (REF). In brief, fitness for both sexes was defined as the quantity of offspring produced when the focal flies were placed in a vial with some bw females and GFP males. For females, we recorded the absolute number

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of offspring produced, and for males we recorded the proportion of offspring sired by the focal males. We measured fitness twice for both sexes: one fitness measurement was performed on 2- to 5-day-old flies (referred to as 'early-life fitness'), and one was performed on 14- to 17-day-old flies ('late-life fitness'). Focal flies and their same-sex competitors were not replaced when they died, such that our fitness assays incorporate variance in relative mortality, as well as offspring siring/production rate. For both the male and female fitness assays, we ran six replicate vials per line. Each vial contained 5 DGRP flies, such that we measured a total of 30 flies per line in six groups. Importantly, we measured the number of offspring produced by the focal flies by counting first instar larvae, and (for the male assay) recording whether each larva expressed GFP. The advantage of this method is that we avoid confounding inter-line variation in male mating success or female productivity with inter-line variation in egg-to-adult survival.

To ensure consistent larval density across the experiments, all experimental lines were reared at a standardised density. For each DGRP line, mated females were placed onto grape-agar medium to oviposit for 24h, and 100 first instar larvae were transferred into yeasted vials. For the bw and GFP flies, we placed 15 mated females (1 to 4 days old) into yeasted vials, allowed them to oviposit for 36h, then collected virgin offspring on days 10-13.

To measure male fitness, we placed 5 males from the focal DGRP line in a standard vial (the "interaction vial") with 10 GFP males and 15 bw females. All flies were virgin and were between 2 and 3 days old post-eclosion when first added to the interaction vial. After allowing the flies to interact and mate for 3 days, the females were moved to a 20mm vial with 8ml of grape juice (the "egg collection vial") and allowed to oviposit for 24h. The females were then transferred back to their original interaction vial, containing the surviving DGRP males and GFP males. The collected embryos were allowed to develop for 24h, and a random sample of up to 200 first instar larvae was collected and scored for GFP as a measure of male early-life fitness. The flies were then left to age in the interaction vial for 8 days, and were tipped into a fresh vial once during this time. Then, when the DGRP and GFP males were approximately 14 days old, the old bw females were replaced with 15 new 2- to 3-day-old virgin bw females, and the flies left to interact for 3 days (note that dead DGRP males or their GFP competitors were not replaced). The females were then placed in a new egg collection vial to oviposit for 24h. The GFP status of these first instars were scored as a measure of male late-life fitness.

To measure female fitness, 5 females from the focal DGRP line were placed in an interaction vial with 15 GFP males and 10 bw females, and allowed to interact and mate for 3 days (all flies were 2-3 days-old virgins). To measure female early-life fitness, the 5 DGRP females were moved to an embryo collection vial and allowed to oviposit for 24h, before being returned to the original interaction vial. The eggs were allowed to develop into first instar larvae, and the total number of larvae was counted (thus, unhatched eggs were not counted towards female fitness). To measure female late-life fitness, we waited 8 days (tipping once into a fresh vial), and replaced the old GFP males with 15 new 2- to 3 days-old virgin GFP males, and allowed the flies to interact for 3 days (note that dead DGRP females or their bw competitors were not replaced). The DGRP females were then moved to the egg collection vials and allowed to oviposit for 24h, and we again counted the total number of 1st instar larvae that emerged.

The fitness assays were run in nine blocks, and DGRP line 352 was included in every block, providing a reference point to help estimate block effects on fitness. There were 8-17 lines per block, not including the reference line. To estimate the line average for each female early- and late-life fitness, we fit a model with offspring number as the response variable and line and block as crossed random factors. We then took the predicted values for the line mean, assuming that all the assay had taken place in block 1. These predicted values, which correct the fitness estimates for block effects, were used in all downstream analyses of the fitness data. We similarly obtained corrected values for line mean male fitness. For males, the response variable was the proportion of offspring sired (rather than the number), and we additionally corrected for the number of live competitor GFP males that were present at the time the females were removed for egg collection (by including the number of competitors as a covariate). Thus, we assume that the GFP males died randomly with respect to the genotype of the DGRP males, and adjusted the fitness of each line accordingly.

Quantifying genetic (co)variance

Identifying variants that affect male and female fitness

We estimated selection on each variant using linear regression (REFS). The statistical model used to test each variant was a simple linear model with the formula Y ~ Genotype, where Y is relative fitness (e.g. the predicted line mean for male early-life fitness, divided by the average of all the predicted line means), and

Genotype is the genotype of the focal line. Genotype was coded as a 0 for lines homozygous for the reference allele or 1 for lines homozygous the alternate allele for the focal variant - heterozygous loci are rare in the DGRP, and were excluded from analysis (because the genotype is unknown for these loci in our study). We defined the reference allele as the one that was most common across the entire panel of DGRP lines (n = 205), such that a positive slope means that the minor (i.e. rarer) allele confers higher fitness, and a negative slope means that the major (commoner) allele confers higher fitness. For each variant, we recorded the effect size, the associated standard error, and the t, df and p values for the test. Our approach is equivalent to performing a GWAS with relative fitness as the response variable. The reason we did not use the GWAS pipeline generously provided online by the creators of the DGRP is that we wished to obtain effect size for every variant, and the pipeline only provides effect size for statistically significant variants. However, we did compare the results of our analysis with the results obtained by the Mackay lab's pipeline, and obtained essentially identical results (e.g. our analysis identified the same statistically significant variant), suggesting that our approach were very similar. One difference is that the Mackay lab's pipeline estimates the effects of each variant after correcting for the presence/absence of Wolbachia and chromosomal inversions in each line. We conducted pilot analyses which showed that including these variables yielded very similar results (because Wolbachia and chromosomal inversions were both unassociated with fitness in our study; $p > 0.05$), and so we elected to leave them out of our models for simplicity.

Measuring the specificity of selection across sexes and age classes

We calculated a selection index, termed I, for each variant, by adapting the formula from Innocenti and Morrow (XX):

FORMULA

Innocenti and Morrow referred to I as an "index of sex-specific selection", but I is equally useful for any study that measured selection in two categories of individuals; in our case, we calculated I to compare the effects of each variant on A) male and female fitness, and B) early life and late life fitness.

When I is positive, selection is "concordant", meaning that there is selection in both sexes or age classes, in the same direction (i.e. the same variant is associated with elevated fitness in both cases). When I is close to zero, selection is absent in one or both cases. When I is negative, selection is "antagonistic" meaning that there is selection in both sexes or age classes, but the variant that is associated with higher fitness is different in each case. We calculated I to compare four kinds of selection: A) males vs females, in early life; B) males vs females, in late life; C) young vs old males; D) young vs old females.

To numerically estimate the uncertainty associated with each estimate of I, we generated 1000 independent samples of b_i and b_j by drawing random numbers from a normal distribution with a mean and standard deviation obtained from the models used to estimate b_i and b_j. This yielded 1000 estimates of I, from which we recorded the median and 95% quantiles, which approximate the 95% confidence limits on I given the uncertainty associated with b_i and b_j.

Annotations for each variant

We relied on the annotations generated by the creators of the DGRP, who used the software SnpEff to classify each variant by site class (e.g. whether the variant is in an intron, or a non-synonymous codon position, etc). Additionally, we assigned a list of KEGG and GO terms to each variant, which matched those associated with the gene (or genes) in which that variant resides (obtained from NCBI).

Results

Variance and covariance in fitness across lines

There was substantial variation in line mean relative fitness, for both sexes and both age classes (Figure 1). All correlations in Figure 1 are positive and significant ($p < 0.001$), indicating that lines with high male fitness tended to have high female fitness, and lines with high early-life fitness tended to have high late-life fitness.

In spite of the overall positive correlations between sexes and age classes, some lines ranked highly for female fitness had low male fitness, and some that ranked highly for early life fitness had low late-life fitness (and

vice versa; Figure 2). In sum, the data suggest that the majority of genetic variance in fitness is concordant across sexes and age classes, but alleles with antagonistic fitness effects may nevertheless exist.

Genetic variance and covariance in fitness

The estimated G matrix for our four traits is shown in Table 1. Fitness was highly heritable...

Distribution of fitness effects across variants

Figure 3 plots the estimated effects of each of the XX variants on each of the four fitness traits: positive numbers mean that the commonest variant was associated with elevated fitness, and negative numbers indicate the reverse.

Variants had smaller average effects on relative fitness in males relative to females, and in young individuals relative to older ones. There was positive covariance for all combinations, such that variants that positively affected one fitness component tended to positively affect another (Figure 3; Table XX). Additionally, we detected XX variants that affected fitness in two fitness components, but all of these affected fitness in a concordant rather than antagonistic fashion; for example, we did not find any alleles that elevated male fitness but reduced female fitness.

The mean effect on fitness across all variants was slightly negative for all traits, which means that the minor alleles were, on average, associated with lower fitness than the major alleles (Table XX). Additionally, the distribution of fitness effects was significantly positively skewed for all four fitness measures, such that there were was an excess of loci with highly positive fitness effects relative to the number with highly negative effects (Figure 3; Table XX). This means that among the subset of loci with extreme effects on fitness, the beneficial allele tended to be the minor rather than major allele.

Almost all loci affect fitness, or are close to a locus that does

Inspired by Boyle et al. (xx), we sorted all of the variants by their fitness effects, placed them in bins of 1000, and then calculated the average fitness effect for each bin. Figure 4 shows that there was a very tight correlation (XXX) between the average effects of the variants in each bin on male and female fitness. As well as reaffirming our earlier results that there is a positive genetic correlation between male and female fitness, these results reveal that fitness is an extremely polygenic trait. The male and female fitness measurements were collected independently, and so Figure 4 allows us to distinguish small but genuine effects from statistical noise, despite the low power of our study (and most GWAS) to detect variants with weak effects. To see why, consider an alternative hypothesis, in which the great majority of variants have no effect on fitness, and the genetic (co)variance in fitness reported above resulted from a small subset (dozens or hundreds) of variants with comparatively large average effects. The plot in Figure 4 would then be flat in the centre with steep inflections at each end. The straight line that we see instead suggests that there a large number of variants that each affect fitness - typically in both sexes, in the same direction - whose effect sizes range from tiny to moderate (see Boyle et al. XXX). Put another way, the effect size of each 1000-variant bin on female fitness was replicated for male fitness, suggesting that the effects are real, as opposed to being deviations from a true effect of zero caused by statistical uncertainty.

Selection estimates and allele frequencies are correlated

At loci for which the minor allele was associated with higher fitness, the minor allele tended to be more common across the DGRP. This confirms the intuitive prediction that...

Distribution of fitness effects across chromosomes

To do...

Discussion

Tables

Table 1: List of variables, and their corresponding parameter(s) in the model, which were varied in order to study their effects on extinction. # `{r xtable, results="asis"} # print_table1() #`

Figures