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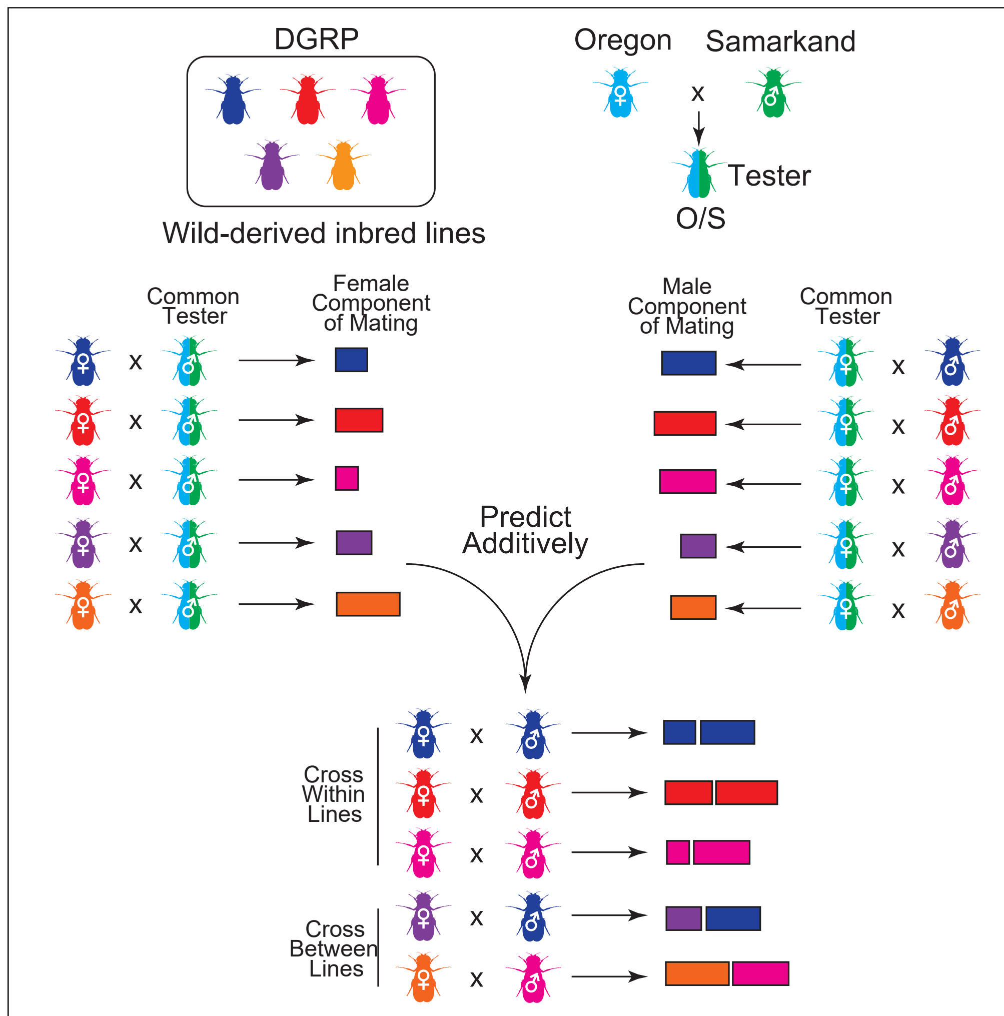

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Highlights

The mating component of fitness is genetically variable in *Drosophila melanogaster*

Independent genetic basis of mating success between sexes of the same genotype

Mating success of two genotypes predicted from their male and female mating success

RNAi confirms independent effects of candidate genes on mating success of both sexes

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The genetic basis of variation in *Drosophila melanogaster* mating behavior

Akihiko Yamamoto,[1,2] Wen Huang,[1,3] Robert R.H. Anholt,[1,4] and Trudy F.C. Mackay[1,4,5,*]

SUMMARY

Mating behavior is an essential fitness trait. We used the inbred, sequenced lines of the *Drosophila* Genetic Reference Panel (DGRP) to gain insights into the evolution of mating success and to evaluate the overlap in genetic architecture of mating behavior between the sexes. We found significant genetic variation for mating success when DGRP males and females from the same line were mated together, and when DGRP males and females were mated to an unrelated strain of the opposite sex. The mating success of DGRP males and females was not correlated when they were paired with the unrelated strain, suggesting independent genetic architecture of mating success in males and females that was confirmed by genome-wide association analyses. However, the mating success between pairs of the same or different DGRP lines was predicted accurately by the respective female and male mating success with the unrelated line.

INTRODUCTION

A major challenge in evolutionary quantitative genetics is to understand the mechanisms maintaining genetic variation for fitness traits in natural populations, in the face of reduction of segregating variation by directional natural selection.[1,2] There are several non-mutually exclusive explanations. Mutation-selection balance will always result in the maintenance of genetic variation for rare deleterious alleles,[3] but not alleles at intermediate frequency. Variation for fitness can be maintained at intermediate frequencies for loci experiencing heterozygote advantage (although there are few examples of such loci), or by apparent heterozygous advantage from a balance of selective forces, such as frequency-dependent selection, and opposite selection pressures in different conditions (fitness traits, environments, generations, genetic backgrounds, or sexes).[4–10]

Mating behavior of *Drosophila melanogaster* may be an example of a fitness component for which genetic variation is maintained by sexual antagonism. *Drosophila* courtship behavior is multimodal, involving visual, auditory, and chemical signals. When flies mate, males transfer seminal fluid proteins to the female reproductive tract that reduce female receptivity to other males, increase egg laying and decrease female lifespan, thus increasing male fitness at the expense of that of the female. In response, females can evolve resistance to seminal fluid proteins, setting up an antagonistic sexual evolutionary arms race.[11] There is evidence for sexually antagonistic genetic variation in *D. melanogaster* mating behavior. In laboratory populations where only males are genetically variable and can evolve, male overall competitive fitness and ability to remate increases rapidly, while female viability decreases.[12] If sexual selection is removed in laboratory populations, males become less harmful to females and females evolve resistance to males.[13] A negative genetic correlation for direct measures of adult reproductive success in males and females has been reported.[14] However, previous studies have not quantified the genetic variation in the same mating behavior in both males and females, nor has the cross-sex genetic correlation for mating behavior been estimated.

Here, we used the inbred, sequenced lines of the *Drosophila* Genetic Reference Panel (DGRP)[15,16] to assess DGRP male and female mating success with unrelated outbred F_1 hybrids of the opposite sex derived by crossing inbred Oregon females with inbred Samarkand males (O/S). In addition, we assessed DGRP male and female mating success with their own genotypes. We found significant genetic variation for mating success in all scenarios. The genetic correlation between DGRP female and male mating success with O/S males or females was not significantly different from zero. However, the mating success of pairs of DGRP lines could be accurately predicted from adding the mating success of the DGRP females and males with O/S males and females, respectively. Thus, male and female mating behavior can evolve independently. We performed genome-wide association (GWA) analyses for mating behavior and identified candidate genes and variants. Consistent with the combinability of polygenic effects, the addition of the effects of the variants from the GWA analyses of DGRP female and male mating success with O/S males and females predicts nearly perfectly the effect of the variants on mating behavior of DGRP females paired with DGRP males of the same genotype. We used RNA interference (RNAi) to reduce expression of 11 candidate genes associated with mating behavior and validated that nine of these genes affect mating of RNAi females and/or males with O/S males or females.

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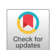

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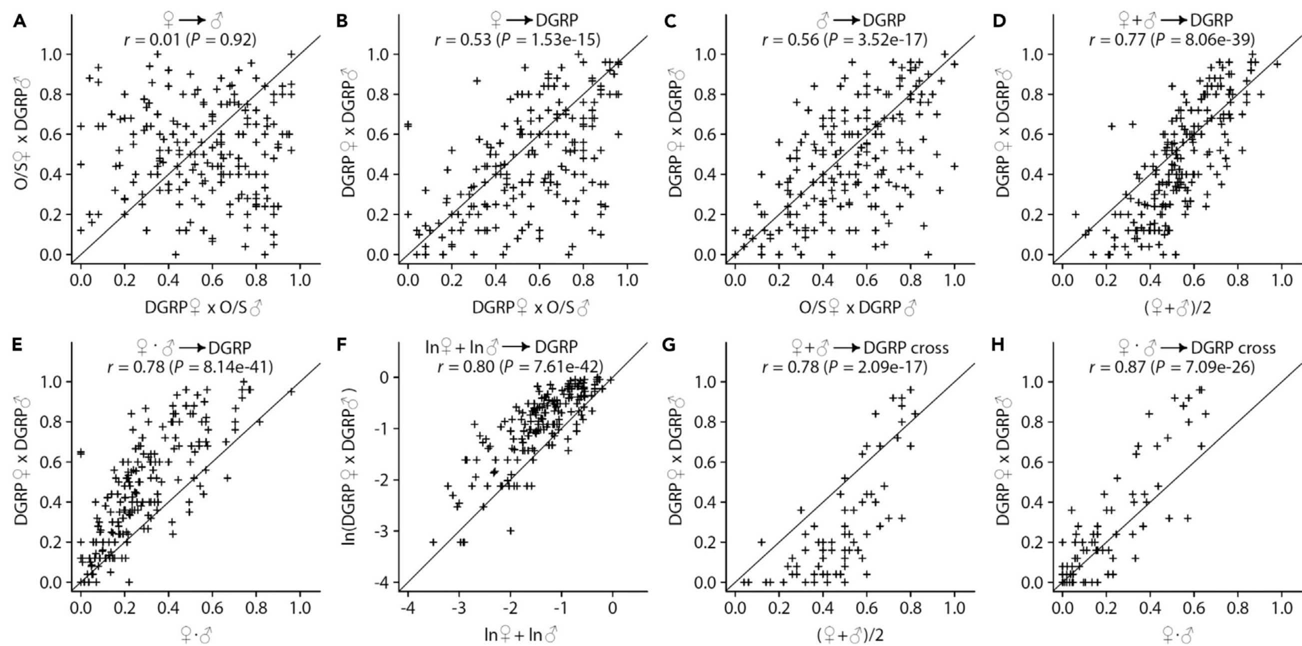

Figure 1. Phenotypic correlations of mating success between different mating types and predictions

(A) Scatterplot of the mating success of DGRP females × O/S males and that of the mating success of O/S females with DGRP males.

(B) Scatterplot of the mating success of DGRP females × O/S males and that of the mating success of DGRP × DGRP female/male pairs of the same genotype.

(C) Scatterplot of the mating success of DGRP females with O/S males and that of DGRP × DGRP female/male pairs of the same genotype.

(D) Scatterplot of the mating success predicted by an additive model and that of DGRP × DGRP female/male pairs of the same genotype.

(E) Scatterplot of the mating success predicted by a multiplicative model and that of DGRP × DGRP female/male pairs of the same genotype.

(F) Scatterplot of the mating success (log scale) predicted by an additive model and that of DGRP × DGRP female/male pairs of the same genotype.

(G) Scatterplot of the mating success predicted by an additive model and that of DGRP × DGRP female/male pairs of different genotypes.

(H) Scatterplot of the mating success predicted by a multiplicative model and that of DGRP × DGRP female/male pairs of different genotypes. Above each plot, the mating types and prediction model, correlation, and *p* value of the correlation are indicated.

RESULTS

Quantitative genetic analysis of mating behavior in the DGRP

We quantified the mating behavior in no choice assays for 205 DGRP lines for three pairs of genotypes: virgin DGRP females and Oregon/Samarkand (O/S) F_1 males (hereafter DGRP ×O/S), O/S virgin F_1 females and DGRP males (O/S × DGRP), and virgin DGRP females and DGRP males (DGRP × DGRP) from the same line (Table S1). Mating success was defined here as the proportion of females within each replicate vial that had successfully mated in a fixed amount of time. This definition includes male courtship vigor, female responsiveness, and mating speed and does not include re-mating behavior. We chose 30 min for DGRP × O/S but 1 h for O/S × DGRP and DGRP × DGRP to maximize variation between lines for subsequent analyses (Figure S1). These data enabled us to quantify genetic variation in DGRP female and DGRP male mating behavior when mated to a common tester genotype and the genetic correlation between female and male mating behavior. We found significant genetic variation among the DGRP females when they mated with O/S males (broad sense heritability H^2 = 0.48, p = 4.16 × 10^{-83}) and among DGRP males when they mated with O/S females (H^2 = 0.44, p = 1.94 × 10^{-68}).

The genetic correlation (r_{GFM}) between DGRP females and males mated to O/S flies of the opposite sex was effectively zero (r_{GFM} = 1.92 × 10^{-8}), suggesting that the polygenic genetic architecture of female and male mating behaviors may be distinct (Figure 1A). While female (r = 0.53) and male (r = 0.56) mating behaviors of DGRP flies as measured against the O/S tester genotype separately predicted the mating success of crosses between them to a substantial degree (Figures 1B and 1C), the combination of the two predicted the mating success to a far greater degree using both an additive (Figure 1D, r = 0.77) and a multiplicative (Figure 1E, r = 0.78) model, the latter of which is also additive on a logarithm scale (Figure 1F).

Remarkably, the combinability of female and male mating behaviors to predict mating success of specific crosses extended beyond mating with the same genotypes. We chose 20 DGRP lines with high mating success of DGRP females with O/S males and 20 lines with low mating success of DGRP females with O/S males. We also chose 20 DGRP lines with high and 20 DGRP lines with low mating success of males with O/S females. We then paired the 20 high female mating success DGRP lines with 20 high DGRP male and 20 low DGRP male mating success lines. We also paired the 20 low female mating success lines with 20 high DGRP male and 20 low DGRP male mating success lines, for a total of 80 pairs of lines. The mating success between females and males from different DGRP strains can be predicted with high accuracy using mating

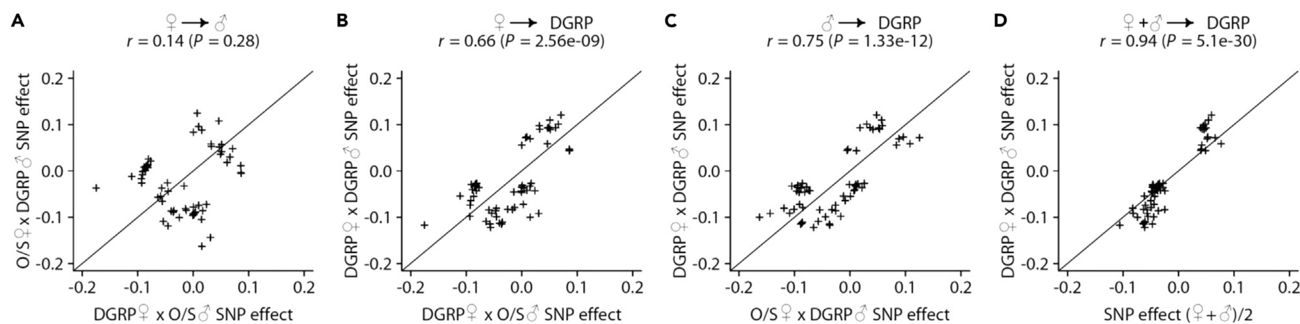

Figure 2. Prediction of the additive effects of variants associated with DGRP × DGRP mating success from the additive effects of variants associated with male and female mating success with an unrelated genotype

(A) Scatterplot of SNP effects estimated from GWA analysis of mating success between DGRP females and O/S males and that between O/S females and DGRP males.

(B) Scatterplot of SNP effects estimated from GWA analysis of mating success between DGRP females and O/S males and that between DGRP females and males of the same genotype.

(C) Scatterplot of SNP effects estimated from GWA analysis of mating success between O/S females and DGRP males and that between DGRP females and males of the same genotype.

(D) Scatterplot of average SNP effects estimated from GWA analysis of mating success between O/S females and DGRP males and that between DGRP females and O/S males, and that between DGRP females and males of the same genotype. Above each plot, the mating types and prediction model, correlation, and p value of the correlation are indicated.

behaviors measured against the tester strain (Figures 1G and 1H; $r = 0.78$ for an additive model and $r = 0.87$ for an multiplicative model). These observations suggest that males and females contribute independently to the mating success.

Quantitative traits correlated with male and female mating behavior

Drosophila mating behavior relies on pheromonal, visual, acoustic, and other cues[11,17]; and may be genetically correlated with additional traits related to fitness. Several of these traits have been quantified previously in the DGRP under the same rearing conditions used in this study. The independence between female and male contribution to mating success allows us to separate this complex trait into the female and male components. Therefore, we assessed the genetic correlations of female and male mating success with O/S F_1 individuals with the primary female (7,11-heptacosadiene and 5,9-heptacosadiene) and male (7-tricosene and the relative proportion of 7-tricosene) sex pheromones[18] and with gene expression of *Desat1*,[19] which is involved in both the emission and perception of sex pheromones[20,21] (Table S3). We also assessed the genetic correlations of female and male mating success with O/S F_1 individuals with male aggressive behavior,[22] startle response,[15] starvation resistance,[15] chill coma recovery time,[15] phototaxis,[23] sleep and activity traits,[24] body size and metabolic traits,[19] food consumption,[25] and lifespan.[26] All of these quantitative traits are genetically variable in the DGRP. We used the correlations of line means to estimate genetic correlations between these traits since these means estimate the mean genotypic values of each line. Female mating success is significantly negatively correlated with day sleep, day sleep bout length, waking activity, phototaxis, food consumption, and lifespan; and male mating success is positively correlated with phototaxis and free glycerol, and negatively correlated with night sleep (Table S3).

GWA analyses of mating success

We performed three GWA analyses for mating success: for DGRP females and DGRP males, for DGRP females and O/S males, and for O/S females and DGRP males. At a nominal significance level of $p < 10^{-5}$, we identified 64 single nucleotide polymorphisms (SNPs) and insertion-deletion (indel) polymorphisms associated with mating success. These variants are in or near 47 genes; 11 SNPs or indels were 1 kb from the nearest gene and were considered intergenic (Table S4A). Two adjacent genes, *CalpB* and *Taf2*, had four polymorphisms associated with mating success of DGRP females and DGRP males and DGRP females and O/S males; all other polymorphisms were unique to one of the GWA analyses (Table S4A). The correlation between SNP effects estimated for the female and male mating behavior was not significant (Figure 2A, $r = 0.14$, $p = 0.28$), which is consistent with our observation that mating success of DGRP females and O/S males (female component) is not correlated with that of O/S females and DGRP males (male component). However, both the female (Figure 2B, $r = 0.66$, $p = 2.56^{-9}$) and male (Figure 2C, $r = 0.75$, $p = 1.33^{-12}$) components individually can predict variant effects well, and we can nearly perfectly predict the effects of variants associated with mating success of DGRP females and DGRP males from the sum of the additive effects of variants associated with mating success of DGRP females and O/S males and O/S females and DGRP males (Figure 2D, $r = 0.94$, $p = 5.10^{-30}$; Table S4B). This is consistent with the high correlation between the mating behavior of the DGRP female × DGRP male pairs and that predicted from the DGRP female × O/S male and O/S female by DGRP male pairs. Many of the candidate genes have plausible functions given the focal trait (mating success) and genetically correlated traits (Table S4C).

RNAi of candidate genes

We chose 11 candidate genes from the three GWA analyses for which *UAS*-RNAi constructs in a common genetic background were publicly available (*CG33144*, *CG43102*, *CR32773*, *DmsR-2*, *Dpr6*, *HPS4*, *MESK2*, *Ote*, *SKIP*, *Svil*, and *trol*). We crossed the *UAS*-RNAi stocks to each of three ubiquitously expressed *GAL4* drivers with different strengths (*Act-GAL4* > *Ubi-GAL4* > *Ubi-GAL4[156]*).[27] We also crossed the progenitor control strain to the three *GAL4* drivers. We evaluated the mating success of F_1 RNAi and F_1 control females and males when paired with O/S males and females, respectively. RNAi targeting of nine candidate genes affected mating success in at least one sex and for at least one *GAL4* driver (Table S5). RNAi-mediated reduction in expression of *MESK2* and *Svil* did not have any significant effects. In general, the effects of RNAi were much greater for males than females. Mating success was significantly decreased for male *GAL4* > *UAS*-RNAi of *CG43102*, *CR32733*, *DmsR-2*, *dpr6*, *HPS4*, *Ote*, and *SKIP*. Mating success of male *Ubi-GAL4* > *UAS-trol* RNAi was increased, but deceased for male *Act-GAL4* > *UAS-trol* RNAi. Female mating success was increased for *GAL4* > *UAS*-RNAi of *CG33144*, *DmsR-2*, *dpr6*, *Ote*, and *SKIP*; and decreased for *GAL4* > *UAS*-RNAi of *CG43102*, *CR32773*, and *trol* females. Therefore, the mating behaviors of RNAi female × O/S male and O/S female × RNAi male are also not correlated.

DISCUSSION

There is significant naturally occurring genetic variation for mating success in *D. melanogaster*. The mating success of females and males is not genetically correlated when DGRP individuals are paired with males and females of a common, unrelated genotype; and the genetic correlations between mating success and other fitness-related phenotypes are different between males and females. However, the mating behavior of DGRP × DGRP pairs between same and different genotypes can be accurately predicted from that of the males and females with an unrelated genotype, and the effects of variants associated with male and female mating success with an unrelated genotype predict the effects of variants associated with DGRP × DGRP mating success nearly perfectly. RNAi against 11 candidate genes affecting mating success confirms that nine of these genes indeed affect mating success and that the effects are not the same for females and males. These data are not consistent with maintenance of genetic variation for pre-copulatory mating success by antagonistic sexual pleiotropy (where alleles for mating success have opposite effects in females and males). Indeed, a genetic correlation of zero implies that female and male pre-copulatory mating behaviors can evolve independently.

None of the nine candidate genes that affect mating success of *GAL4* > *UAS*-RNAi flies have been implicated previously in mating behavior. *CR32773* is a long noncoding RNAi with no prior functional annotation that we can now infer affects mating behavior. *trol* encodes a secreted heparan sulfate proteoglycan that regulates cell-signaling by multiple growth factors and affects multiple developmental processes.[28] The remaining seven candidate genes have all been implicated in olfaction or other sensory processes and could therefore plausibly affect mating behavior. *CG33144* is involved in protein ubiquitination and *CG43102* is predicted to have guanyl-nucleotide exchange factor activity and positively regulates the Rho protein signal transduction pathway.[28] *CG33144* is associated with variation in the DGRP for olfactory responses to 2-phenyl ethyl alcohol, 2-heptanone and ethyl butyrate; and *CG43102* is associated with variation in the DGRP for olfactory responses to d-carvone.[29] *DmsR-2* encodes a G-protein coupled receptor; *dpr6* is involved in synapse organization; *HPS4* regulates gene silencing by small RNAs in adult flies; and *Ote* encodes a protein that mediates transcriptional silencing in conjunction with BMP/Dpp signaling.[28] Adult flies in which *DmsR-2*, *dpr6*, *HPS4*, and *Ote* expression has been knocked down using RNAi fail to avoid noxious high temperatures,[30] indicating a role in thermosensation. Finally, *SKIP* is involved in olfactory perception.[31]

Limitations of the study

The GWAS was limited to detecting variants associated with pre-copulatary mating success with moderately large effects and intermediate allele frequencies due to the small size of the DGRP. More loci with smaller effects or with lower minor allele frequencies would likely be identified in larger mapping populations. The functional validations using RNAi show that perturbations of expression of genes in closest proximity to the associated SNPs do affect mating behavior. However, RNAi can have off-target effects. Although the effects of *GAL4* drivers with different strengths have different effects on mating success, their actual gene expression levels were not quantified. Finally, the effects of RNAi do not necessarily re-capitulate the effects of causal SNPs; evaluation of SNP effects requires germ-line base editing in the same genetic background and subsequent functional analyses.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- QUANTIFICATION AND STATISTICAL ANALYSIS
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  - Genome-wide association analyses

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109837.

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AUTHOR CONTRIBUTIONS

T.F.C.M. and R.R.H.A. supervised and funded the research; A.Y. conducted the experiments; A.Y., W.H. and T.F.C.M. analyzed the data; and A.Y., W.H. and T.F.C.M. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw and analyzed phenotype data	This paper	Tables S1, S2, S3, S4, and S5; https://github.com/qgg-lab/dgrp-mating
DGRP Sequences	Huang et al.[16]	NCBI SRA (Accessions in Table S1;[16])
Experimental models: Organisms/strains		
DGRP lines	Bloomington *Drosophila* Stock Center (BDSC)	Table S1; https://bdsc.indiana.edu/stocks/wt/dgrp.html
Oregon	Laboratory of Trudy Mackay	N/A
Samarkand	Laboratory of Trudy Mackay	N/A
RNAi of *CG33144* (*P{KK101512}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 108583
RNAi of *CG43102* (*P{KK102017}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 110150
RNAi of *CR32773* (*P{KK106738}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: no longer available
RNAi of *DmsR-2* (*P{KK110024}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 109513
RNAi of *dpr6* (*P{KK112634}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 103521
RNAi of *HPS4* (*P{KK102510}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 103762
RNAi of *MESK2* (*P{KK107898}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 105492
RNAi of *Ote* (*P{KK109866}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 105308
RNAi of *SKIP* (*P{KK107352}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 109892
RNAi of *Svil* (*P{KK103279}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 108962
RNAI of *trol* (*P{KK105502}VIE-260B*)	Vienna *Drosophila* Resource Center	VDRC: 110494
KK RNAi Control (*y,w^{1118};P{attP,y^{+},w^{3}}*)	Vienna *Drosophila* Resource Center	VDRC: 60100
Act-GAL4 (*P{Act5C-GAL4}25FO1*)	Bloomington *Drosophila* Stock Center (BDSC)	BDSC: 4414
Ubi-GAL4 (*P{Ubi-GAL4.U}2*)	Bloomington *Drosophila* Stock Center (BDSC)	BDSC: 32551
Ubi-GAL4[156]	Laboratory of Trudy Mackay	N/A
Software and algorithms		
DGRP web server	https://flydgrp.org	N/A
R	https://www.r-project.org	Version 4.2.0
Custom Code	This paper	https://doi.org/10.5281/zenodo.10730022

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contract, Trudy Mackay (tmackay@clemson.edu).

Materials availability

All DGRP lines and *Act-GAL4* (*P{Act5C-GAL4}25FO1*) and *Ubi-GAL4* (*P{Ubi-GAL4}2*) are available from the Bloomington Drosophila Stock Center (BDSC) (https://bdsc.indiana.edu/). KK *UAS*-RNAi lines and their control (including the transgene landing site) are available from the Vienna *Drosophila* Resource Center (https://www.viennabiocenter.org/vbcf/vienna-drosophila-resource-center/). *Ubi-GAL4[156]* and the Oregon and Samarkand strains used in this study are freely available on request from the lead contact. This study did not generate any unique reagents.

Data and code availability

- Raw DGRP DNA sequence have been deposited in the NCBI Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra) under accession numbers listed in Table S1 of Huang et al.[16]All primary phenotype data are provided in the Supplementary Tables and in

a GitHub repository: https://github.com/qgg-lab/dgrp-mating, including an archived release with https://doi.org/10.5281/zenodo.10730022.

- All original code has been deposited in a GitHub repository: https://github.com/qgg-lab/dgrp-mating, including an archived release with https://doi.org/10.5281/zenodo.10730022.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Drosophila stocks

The 205 inbred, sequenced DGRP lines were derived from inseminated females collected in Raleigh, NC USA,[15,16] and inbred by 20 generations of full sib mating. Oregon and Samarkand are common wild type stocks, unrelated to the DGRP lines. They have been inbred by 20 generations of full sib mating in our laboratory. The *GAL4* driver lines *Act-GAL4* (*P{Act5C-GAL4}25FO1*) and *Ubi-GAL4* (*P{Ubi-GAL4.U}2*) were obtained from the BDSC and their major chromosomes that do not contain the drivers were replaced with *Canton-S-B* chromosomes (*CSB, w^{1118}*)[32] to minimize background genotype effects. The *Ubi-GAL4[156]* stock was created by introducing the original *Ubi-GAL4* transgene onto the third chromosome of *CSB* by *Δ2-3* transposase-mediated hopping.[25] All stocks were maintained in small mass matings in narrow polystyrene vials (25 mm diameter x 95 mm height, Genessee Scientific) with 10 mL cornmeal-agar-molasses medium (2.76 L H_20, 33 g yeast, 163 g cornmeal, 16 g agar, 200 mL molasses, 30 mL tegosept (diluted in 10% ethanol from stock solution with 50 g tegosept in 500 mL 95% ethanol), 14.4 mL propionic acid) at 25°C, 70% humidity, and a 12 h light:dark cycle (lights on at 6:30 a.m.).

METHOD DETAILS

Drosophila Genetic Reference Panel mating assays and associations with other quantitative traits

We assessed mating behavior using no-choice assays. For each assay, five virgin females and 10 males were placed without anesthesia in a vial with 1 mL of cornmeal-agar-molasses medium. There were 5–10 replicate vials for each pair of genotypes. Mating was observed directly and the time to copulation was recorded. Each copulating couple was immediately removed using a mouth aspirator through a slit window in a sponge plug.[33] The total number of matings was recorded after 1 h for matings involving DGRP lines, and after 15 min, 30 min and 1 h for matings involving RNAi and control lines. All assays were conducted between 8 a.m. and 11 a.m. (starting 1 h 30 min after lights on) under full lighting at 25°C. We used an Oregon/Samarkand (O/S) cross as a healthy reference strain. We assessed mating behavior for DRGP females and Oregon/Samarkand (O/S) F_1 hybrid males, O/S F_1 hybrid females and DGRP males, and DGRP females and DGRP males from the same DGRP line, and DGRP females to DGRP males from different DGRP lines. In addition, we determined correlations of the male and female component of mating behavior (as determined by their mating behaviors with (O/S) F_1 hybrid females and males, respectively) with other relevant quantitative traits in males and females measured previously for these DGRP lines, under the same environmental conditions.

RNAi functional analyses

We used three ubiquitously expressed *GAL4* drivers with different strengths[27] to knock down expression of candidate genes: *Act-GAL4*, *Ubi-GAL4*, and *Ubi-GAL4[156]*. We selected 11 candidate genes to evaluate whether RNAi knockdown of gene expression affected mating performance with O/S males or females. We crossed females of each driver line to the Vienna KK collection RNAi line and the co-isogenic control line (*y,w^{1118}*;*P*{*attP,y^+,w^3*}) and assessed mating of the F_1 females or males from these crosses with O/S males or females at 15 min, 30 min and 1 h. There were 10 replicate vials each with 10 males and 5 virgin females of the appropriate genotype (O/S or *GAL4*/*UAS*-RNAi F_1) and 20 replicate vials each with 10 males and five virgin females for the control genotypes (O/S or *GAL4*/control). These mating data were analyzed using Fisher Exact tests of mating data for each RNAi line and appropriate control.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantitative genetics of mating behavior in the Drosophila Genetic Reference Panel

We partitioned variation in mating behavior among and within DGRP lines for each of the three pairs of genotypes tested (DGRP females x O/S males, O/S females x DGRP males, and DGRP females x DGRP males) using single classification, random effects ANOVA models ($Y = \mu + L + \varepsilon$, where Y is the mating success phenotype, μ is the overall mean, L is DGRP line and ε is the residual). We estimated the broad sense heritability (H^2) of mating as $H^2 = \frac{\sigma_L^2}{\sigma_L^2+\sigma_\varepsilon^2}$, where σ_L^2 is the among-line variance component and σ_ε^2 is the within-line variance component. To estimate the genetic correlation of mating behavior between females and males, we performed a two-way mixed model ANOVA that fitted the main fixed effect of sex (S), the main random effect of DGRP line (L), and the random sex by line interaction ($S \times L$) ($Y = \mu + S + L + S \times L + \varepsilon$) for the data of DGRP females x O/S males and O/S females x DGRP males. We estimated the cross-sex genetic correlation of mating behavior (r_{GFM}) as $r_{GFM} = \frac{\sigma_{SxL}^2}{\sigma_{SxL}^2+\sigma_L^2}$, where σ_{SxL}^2 and σ_L^2 are the variance components due to the sex by line interaction and line, respectively.[3]

We predicted the mating behavior of the DGRP female x DGRP male pairs from that of the DGRP female x O/S male and O/S female x DGRP male pairs for the same DGRP lines using additive and multiplicative models. The additive expectation was the average of the DGRP male and female mating proportions with the O/S genotype and the multiplicative model was the product of the DGRP male and female mating proportions with the O/S genotype.

Genome-wide association analyses

We performed three GWA analyses for mating behavior: DGRP females x DGRP males, DGRP females x O/S males and O/S females x DGRP males. Each analysis tested the associations of 1,920,276 single nucleotide/multiple nucleotide polymorphisms (SNPs, MNPs) and insertion/deletion polymorphisms with minor allele frequencies $\geq$0.05 with mating behavior, and was performed using the DGRP web server, https://flydgrp.org.[16] This analysis accounts for effects of *Wolbachia* infection, cryptic relatedness due to major inversions, and residual polygenic relatedness.