Natural variations in reproductive aging phenotypes reveal the importance of early reproductive period in *Caenorhabditis elegans*

Jiseon Lim[*,†,§], Jun Kim[*,‡,§], and Junho Lee[*,†,‡,1]

[*]Department of Biological Sciences, Seoul National University, Seoul, Korea 08826

[†]Institute of Molecular Biology and Genetics, Seoul National University, Seoul, Korea 08826

[‡]Research Institute of Basic Sciences, Seoul National University, Seoul, Korea 08826

[§]Co-first authors

Natural variations in reproductive aging

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[1]Corresponding author: Junho Lee, Ph.D.

Department of Biological Sciences, Seoul National University, Gwank-ro 1, Gwanak-gu, Seoul, Korea 08826. E-mail: elegans@snu.ac.kr

Abstract

Although reproductive capacity is a major factor in individual fitness, aging of the reproductive system precedes somatic aging and may reduce the total brood size. Genetic studies have led to the development of a body of evolutionary theory in the nematode *Caenorhabditis elegans,* but these studies did not take into account current knowledge about the natural history of *C. elegans*. To enhance our understanding of reproductive aging in *C. elegans*, we measured and compared two reproductive aging-related traits—the number of progeny and the X-chromosome nondisjunction rate—of 96 wild strains during early, late and total reproductive periods. We found that the two traits exhibited natural phenotypic variation, with few outliers, and that the brood size and the X-chromosome nondisjunction rate were not genetically correlated. Contrary to a previous hypothesis, that reproductive aging contributes to the generation of an optimal total number of offspring, we found that the total brood size did not converge to an optimal value, and early brood size was more constant than total brood size among wild strains. We speculate that reproductive aging is a by-product of a rapid increase in the initial population size, which might be related to the boom-and-bust lifestyle of *C. elegans*. We also identified loci and candidate genetic variants significantly associated with X-chromosome nondisjunction rate in the late and total reproductive periods. Our results provide an insight into reproductive aging in wild *C. elegans* strains.

Introduction

Reproductive aging involves age-related dysfunction in the reproductive system, and generally becomes apparent before somatic aging. In *Caenorhabditis elegans*, the regulation of the reproductive schedule by reproductive aging has been thought to have evolved to optimize total brood size and increase individual fitness, and it has been found that some wild strains exhibit little variation in their brood size (HODGKIN AND BARNES 1991; HUGHES *et al.* 2007). Oocyte quality begins to decrease after the first two to three days of adulthood, whereas adult worms mostly die at day 15–20 (COLLINS *et al.* 2007). The oocyte-production rate of unmated *C. elegans* hermaphrodites is highest on the second day of adulthood and dramatically declines thereafter, so these individuals produce around 90% of their progeny during the first three days of adulthood (KLASS 1977; HUANG *et al.* 2004). The X-chromosome nondisjunction rate also increases in this period, increasing the number of male progeny in aged hermaphrodites (ROSE AND BAILLIE 1979; LUO *et al.* 2010). These reproductive aging phenotypes are known to be associated with several genetic pathways, such as TGF-β signaling, insulin/IGF-1 signaling, and dietary restriction pathways (HUANG *et al.* 2004; HUGHES *et al.* 2007; LUO *et al.* 2009; LUO *et al.* 2010).

These studies, however, have several limitations, as they were mostly conducted before the natural history of *C. elegans* was thoroughly investigated. The work was based on a lab-adapted reference strain or a few wild strains, so the reproductive aging phenotypes have not yet been measured in hundreds of recently collected wild strains (HODGKIN AND BARNES 1991; HUGHES *et al.* 2007; LUO *et al.* 2009; LUO *et al.* 2010; COOK *et al.* 2017; KOCSISOVA *et al.* 2019). Most of the previous work used mated hermaphrodites, but hermaphrodites of

natural populations rarely mate with males (BARRIÈRE AND FÉLIX 2005). One of the theoretical works suggested that *C. elegans* should have an optimal brood size to reduce oscillations in population size, but recent studies on the ecology of *C. elegans* showed that it has a highly fluctuating boom-and-bust lifestyle (HUGHES *et al.* 2007; CUTTER 2015; FRÉZAL AND FÉLIX 2015). Recent extensive collection of wild *C. elegans* have provided a deeper understanding of its natural history, and larger wild strain sets and their whole-genome sequencing data, allow us to thoroughly study the reproductive aging phenotypes in wild strains of *C. elegans* (FÉLIX AND BRAENDLE 2010; FRÉZAL AND FÉLIX 2015; COOK *et al.* 2017; CROMBIE *et al.* 2019).

In this study, we used unmated hermaphrodites of 96 wild *C. elegans* strains, and measured their natural variations in oocyte-production and X-chromosome nondisjunction rates during reproductive periods. The majority of the wild strains exhibited reproductive aging phenotypes that began near the early reproductive period, and the two traits were not correlated, implying that different genetic mechanisms may underlie these traits. We found that early brood size was more likely than total brood size to have a constant value. We also conducted genome-wide association mapping to identify candidate genetic variants for these phenotypic variations. Our study provides further insights into the natural history of *C. elegans*.

Materials and Methods

C. elegans culture

All wild strains were maintained at 20 °C on NGM lite plates seeded with OP50. All strains and their phenotypic values are listed in Table S1.

Measuring brood size and male-production rate

Four virgin hermaphrodites (L4 stage) of each wild strain were transferred separately to new plates, then transferred again every 12–24 h. Three plates were used on the same day, and each experiment was replicated five times. After about 2 d, the offspring could be distinguished by secondary sex characteristics, such as the shape of the tail tip in males. Time constraints prevented examination of all 96 strains at once, so about 15 wild strains were measured in blocks, with strain CB4856 as an internal control. The 96 wild strains were chosen by considering their genetic diversity (COOK *et al.* 2017). The brood size was calculated as the ratio of the number of progeny to the number of parents, and the male-production rate was calculated as the ratio of the number of males to the number of progeny. These phenotypic values were measured for each period (early, late, total) and each replicate. Phenotypic change was calculated as (late-early)/(late+early), with the average of all replicates for each strain in the late and early positions. Since NIC260 was an outlier for the male-production rate and ECA36 was an outlier for both the male-production rate and the brood size, these strains were excluded from the following procedures.

Comparing standard deviations and estimating heritability

To compare the standard deviations of the phenotypic values for each trait and period, tests of equality of standard deviations were conducted using Minitab 19 (https://www.minitab.com).

First, the Anderson-Darling test was implemented to test the normality of the distributions of the phenotypic values of brood size and male-production rate. Normality was not rejected for brood size, but was rejected for male-production rate. Therefore, an F-test was performed to compare the standard deviations of brood size, but Bonett's test and Levene's test were performed for the standard deviations of the male-production rate. Broad-sense heritability was calculated as described in Gimond et al. (2019) using the lmer function in the lme4 package (BATES *et al.* 2014).

Calculating phenotypic differences between wild strains collected in the same environment

Wild strains collected at the same location on the same day were grouped by referring to strain data provided by *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) (COOK *et al.* 2017). The difference between the maximum and minimum values of each phenotype in each group was calculated, and these values were compared with the mean of the phenotypic difference of 30 pairs randomly selected from 94 wild strains.

Correlation analysis and genome-wide association mapping

Correlations were quantified using Pearson’s correlation using DatFrame.corr() from the pandas package in Python (MCKINNEY 2010). Genome-wide association mapping was conducted with the web-based Genetic Mapping in CeNDR (COOK *et al.* 2017) using the mean phenotypic values of five replicates of 94 wild strains. For phenotypic changes in the early and late periods, the mapping was conducted with the cegwas package, as in the CeNDR webpage (https://github.com/AndersenLab/cegwas2). The variance explained by each QTL and the environmental conditions of the wild strains that we used were obtained

from the mapping in CeNDR. JU311 was excluded from the male-production rate change because the total male-production rate of the strain was zero.

Filtering and categorizing interval variants in associated loci

The interval variants of the male-production rates in the late and total reproductive periods were analyzed only for protein-coding genes in all associated loci. Lists of interval variants were acquired by Genetic Mapping in CeNDR (COOK *et al.* 2017). These data included the variants' *p*-values for the phenotypes using Spearman's correlation tests and their putative impact on the gene, as estimated by SnpEff (CINGOLANI *et al.* 2012). The variants of each trait were filtered by p-value < 0.05, and then among the filtered variants, only those common to both traits were chosen. The common variants were categorized according to their putative impact, and whether they were variants of a gene that is associated with a high incidence of male progeny phenotype. The same process was carried out for genes that had physical, genetic, or regulatory interactions with TGF-β Sma/Mab ligand DLB-1 or insulin/IGF-1 receptor DAF-2 (HARRIS *et al.* 2020).

Calculating linkage disequilibrium between QTL peaks

We measured the linkage disequilibrium between pairs of QTL peaks by calculating the Pearson's correlation coefficient (HILL AND ROBERTSON 1968).

Data availability statement

All genotypes, marker information, and mapping process are available in the Caenorhabditis elegans Natural Diversity Resource (https://www.elegansvariation.org/). All phenotypes are listed in Table S1 and the whole procedure was detailed in Materials and Methods.

Results

To understand natural variation in reproductive aging phenotypes, we measured the brood size and the male-production rate, parameters that reflect oocyte-production and X-chromosome nondisjunction rates, respectively. We counted all progeny of 96 wild strains, and checked their sexes in two different reproductive periods: 0–36 (early) and 36–72 (late) hours after adulthood, as *C. elegans* worms produce around 90% of their progeny during these periods (Figure S1, Table S1). Almost all wild strains exhibited continuous phenotypic distributions for all the traits, but we identified a few outliers (Figure S2). ECA36 had the smallest total brood size, of less than 50, which was one-third of the second lowest brood size. ECA36 and NIC260 were outliers in the rate of male production over the total reproductive period. The male-production rates of ECA36 and NIC260 were around 6% and 0.6%, respectively, which were 30 and 3 times higher than the third highest rate. We excluded these two outliers from further analysis.

We then compared the phenotypes in the early and late reproductive periods, to test whether reproductive aging begins at similar times in wild strains. We found that the average brood size was 148 in the early period and 96 in the late periods (Figure 1A). The average male-production rate was 0.05% in the early period and 0.12% in the late periods (Figure 1B). Of the 94 wild strains, 87 (92.5%) laid more eggs, and 82 (87.2%) produced fewer male progeny in the early period than in the late period (Figure 1C, D), suggesting that the reproductive aging patterns in wild strains may be similar to that of the reference strain (KLASS 1977; ROSE AND BAILLIE 1979; HUANG *et al.* 2004). The variance in brood size in the early reproductive period was lower than those in the late or total reproductive periods (Figure 1A),

a finding that does not support the previous prediction that the total brood size has an optimal value (HUGHES *et al.* 2007). The male-production rate also had lower variance in the early reproductive period than that in the late period, but the variance was similar to that over the total reproductive period (Figure 1B).

To further examine whether there are optimal phenotypic values, we compared strains that shared the same environmental conditions, and were collected in the same location on the same day. The wild strains that we used may have specific optimal values in their different natural environments, but their phenotypes might be disturbed by culturing under the same laboratory conditions, resulting in a loss of the optimal values. We hypothesized that if strains from the same environment have their own optimal values, their phenotypic differences should be smaller than those of randomly chosen strain pairs. Among the 94 strains, we could classify 22 strains into nine groups based on their collection metadata. We found that the phenotypic differences in the nine groups were not markedly lower than those of random pairs (Figure 2), supporting the contention that optimal phenotypic values may not exist.

We analyzed correlations by genotype for the following eight traits: brood size and male-production rate in early, late and total reproductive periods, and changes in these values between the early and late periods, to test whether these traits share an underlying genetic architecture. The brood size and male-production rate were not significantly correlated (Figure 3A), implying that different genetic mechanisms may mediate the two traits. In contrast, the total brood size was weakly positively correlated with the early brood size, but strongly positively correlated with the late brood size, as the phenotypic values of the early

brood size were similar among all wild strains (Figure 1A). Phenotypic variations in the late brood size may therefore have primarily affected the total brood size. The total male-production rate was highly positively correlated with the early and late male-production rates, but the early and late male-production rates were weakly correlated to each other, suggesting that they might also have different genetic architectures.

We conducted genome-wide association mapping to investigate the underlying genetic architecture of these traits. We found that several loci were significantly associated with the male-production rates of late and total periods (Figure 3B), but could not find any significant loci for the other six traits (Figure S3). The significant loci for male-production rates in the late and total periods were located on chromosomes IV and V, and largely overlapped, as they were highly correlated (Figure 3A,B), suggesting that they might share significant components of the genetic architecture. We investigated further, using correlations between phenotypic variations and genetic variants at the loci, and found that 3,050 variants were significantly correlated with phenotypes, and were shared between the two traits (Figure 4A).

We classified these shared variants according to the known phenotypes that appeared when the functions of the genes containing the variants were impaired by mutation or RNA interference. Among these genes, 11 were known to be associated with the high incidence of male progeny (Him) phenotype (Figure 4B) (HARRIS *et al.* 2020). We categorized the shared variants by their putative impacts using SnpEff, and found that *zim-2* contains a high-impact variant and that *coh-3*, *him-17*, *him-5*, *him-8*, *lex-1*, *rad-51*, *sao-1*, *srgp-1*, *zim-1*, *zim-2* and *zim-3* contain moderate-impact, low-impact or modifier variants (Table S2) (CINGOLANI *et al.*

2012; COOK *et al.* 2017).

In addition to genes with the Him phenotype, we also analyzed genes that exhibited physical, genetic or regulatory interactions with major components of the TGF-β or insulin/IGF-1 signaling pathways, which are known to be associated with changes in the male-production rate during reproductive aging (LUO *et al.* 2010; HARRIS *et al.* 2020). We found some genes that interact with DAF-2, the insulin/IGF-1 receptor (Figure 4B), but could not find any genes for DBL-1, the Sma/Mab TGF-β-related ligand. Among the DAF-2 interacting genes, *daf-10*, *rpn-7*, *hsp-12.6*, *elo-1*, *fat-4*, *fat-3*, *gst-4*, *daf-14*, *epi-1*, *mep-1*, *let-60*, *par-5*, *let-653*, *fkb-4*, *mes-4*, *mtl-2* and *cyp-42A1* have moderate-impact, low-impact or modifier variants (Table S3) (CINGOLANI *et al.* 2012; COOK *et al.* 2017).

We divided wild strains according to their alleles, and calculated the phenotypic variances that could be explained by each locus (Figure 5). We found that for every locus, only five to eight wild strains contained high-male-production rate alleles, and the other approximately 90 strains had low-male-production alleles (Figure 5A, B). The variances explained by each locus ranged from 11%–17% for late male-production rates, and from 15% to 19% for total male-production rates, values which are similar (Figure 5A, B), and are also similar to the heritability values for those traits (12% and 18%, respectively; Figure 1B). These loci exhibited strong linkage disequilibrium, even between loci on two different chromosomes (Figure 5C, D).

Discussion

Reproductive schedules may be modulated to increase individual fitness by optimizing the proportion of resources devoted to the progeny. We showed that reproductive aging for brood size and male-production rate had similar times of onset among wild *C. elegans* strains. Their reproductive aging probably begins within 1.5 days after adulthood, which results in decreasing oocyte-production rate and increasing X-chromosome nondisjunction rates, as previously reported in the laboratory-adapted reference strain. We also analyzed the patterns of each trait during the first three days of adulthood, and found that variances increased as wild strains aged. Earlier studies predicted that reproductive aging may lead to the optimal total brood size, to increase individual fitness in *C. elegans*, but our results demonstrated that early brood size has much smaller variance than the total brood size does, suggesting that total brood size may not be more optimized than early brood size.

We hypothesize that the life history of *C. elegans* may affect this phenomenon. The majority of *C. elegans* wild strains may increase the number of hermaphrodites in the early reproductive period by accelerating oocyte production and suppressing the production of male progeny, which may contribute to the achievement of rapid population growth in the early period. As *C. elegans* emigrants disperse to seek new ephemeral resources, this rapid population growth may be beneficial for their boom-and-bust lifestyle, and reproductive aging would be a by-product that occurs as population growth becomes less important in the late reproductive period.

Different genetic variations may underlie phenotypic variations in oocyte production and X-chromosome nondisjunction rates, as these traits were only weakly correlated to each other. TGF-β signaling, insulin/IGF-1 signaling, dietary restriction and other pathways modulate reproductive aging in *C. elegans*. TGF-β signaling and insulin/IGF-1 signaling pathways have been reported to be associated with oocyte quality controls mechanisms, such as chromosome segregation. Notch signaling pathway may affect the oocyte-production rate, as it controls the fates of germline stem cells (LUO *et al.* 2009; LUO *et al.* 2010; KOCSISOVA *et al.* 2019). These signaling pathways have genetic variations in wild strains, and they may affect phenotypic variations in reproductive aging phenotypes, but we could not find any loci significantly associated to any of the traits except late and total male-production rates. These loci contain some components of the insulin/IGF-1 pathway and genes known to be related to the X-chromosome nondisjunction rate. The genes and variants which mediate the phenotypic differences are still poorly understood.

Our genome-wide association mapping experiments identified only a few associated loci for two out of eight traits. If these associated loci contain more than 10,000 variants, only low resolution could be achieved using around 100 wild strains, so our negative results could arise because the number of wild strains that we used was not large enough for fine mapping. It is also possible that the wild strains that we used do not have enough genetic diversity, as *C. elegans* has experienced a chromosome-scale selective sweep, and its hermaphroditism prevents the mixture of different genetic backgrounds (BARRIÈRE AND FÉLIX 2005; DOLGIN *et al.* 2007; ANDERSEN *et al.* 2012; GIMOND *et al.* 2013). These characteristics of *C. elegans* might result in low genetic diversity in wild strains. Our data showed that more than 90% of

the wild strains that we used contained the same allele at each peak of the associated locus (Figure 5A, B). A recent collection of *C. elegans* from Hawaii exhibits much higher genetic diversity than the set we used, which could possibly be used to resolve other loci associated with the remaining traits (CROMBIE *et al.* 2019).

Our results do not support the previous prediction of optimal total brood size. The phenotypic variation in total brood size we observed was slightly higher than that previously reported (235–353 vs. 154–330) (HODGKIN AND BARNES 1991), and wild strains that shared the same environment did not have smaller phenotypic differences than those of randomly selected wild strain pairs (Figure 2). Brood size in the early reproductive period had significantly lower variance than those in the late and total reproductive periods. Variance of the early male-production rate was also lower than that of the late male-production rate. We propose that these changes in variances may result from the importance of the early reproductive period in *C. elegans*.

The increases in phenotypic variance throughout the aging process have several possible explanations. Individuals with the same genetic background can become different because they randomly accumulate different experiences, resulting in increased phenotypic variation over time. However, increased phenotypic variation was observed among the average phenotypic values of each wild strain rather than those of each individual, and averaging individual phenotypic values probably smoothed out the random differences between individuals. The increased variance could also result from unknown laboratory conditions that changed the gene-environment interactions of our wild strains from those in their natural

environments. The optimal brood size, therefore, might be hidden in our experiment, although this explanation may not fully account for the way in which phenotypic variation increased in the late reproductive period. Lastly, the phenomenon may be better explained by the importance of the early reproductive period compared to the total period. Previous theoretical predictions assumed that *C. elegans* has an optimal total brood size to distribute finite resources efficiently, thus avoiding fluctuations in population size, but *C. elegans* populations in nature exhibit a boom-and-bust lifestyle that repeats cycles of colonization, proliferation, depletion of resources and dispersal (FÉLIX AND BRAENDLE 2010; CUTTER 2015; FRÉZAL AND FÉLIX 2015). Thus, rapid population growth in the early reproductive period could be more important than growth over the total period. Our results showed that almost all wild strains appear to be more fecund in the early reproductive period, consistent with previous reports (KLASS 1977; HODGKIN AND BARNES 1991; HUANG *et al.* 2004). The early brood size may be maximized by allocating the minimum resources required just for development (PEREZ *et al.* 2017). The male-production rate remained low throughout not only the early, but also the total period, implying that spontaneous male production and chromosome segregation were restricted, in order to reduce the high cost of males and aneuploidy (SMITH AND MAYNARD-SMITH 1978; CUTTER *et al.* 2019).

Rapid population growth in the early reproductive period might thus be important, but after this the importance of population growth decreases in the late reproductive period, resulting in increased variance (FISHER 1958). When founders lay eggs in their late period, the early offspring become sexually mature and ready to produce progeny, the grandchildren of the founders. This pattern increases the possibility of competition between late progeny and their

nieces rather than sisters, and therefore leads to tens to hundreds of times lower reproductive values of later progeny. Male production, on the other hand, is maintained at a low level over the total reproductive period, implying a tight inhibition of outcrossing and its associated cost. There are outliers with several or tens of times higher male-production rates in *C. elegans* wild strains. These outliers may be selected under outcrossing-favoring environments, such those involving co-evolving parasites (MORRAN *et al.* 2009). Our results seem to support the last of these explanations: that is, that reproductive aging phenotypes may be a by-product of the boom-and-bust lifestyle in *C. elegans*, which intensifies the importance of rapid population growth in the early reproductive period.

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Literature Cited

Andersen, E. C., J. P. Gerke, J. A. Shapiro, J. R. Crissman, R. Ghosh *et al.*, 2012 Chromosome-scale selective sweeps shape Caenorhabditis elegans genomic diversity. Nature genetics 44**:** 285.

Barrière, A., and M.-A. Félix, 2005 High local genetic diversity and low outcrossing rate in Caenorhabditis elegans natural populations. Current Biology 15**:** 1176-1184.

Bates, D., M. Mächler, B. Bolker and S. Walker, 2014 Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.

Cingolani, P., A. Platts, L. L. Wang, M. Coon, T. Nguyen *et al.*, 2012 A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 6**:** 80-92.

Collins, J. J., C. Huang, S. Hughes and K. Kornfeld, 2007 The measurement and analysis of age-related changes in Caenorhabditis elegans.

Cook, D. E., S. Zdraljevic, J. P. Roberts and E. C. Andersen, 2017 CeNDR, the Caenorhabditis elegans natural diversity resource. Nucleic acids research 45**:** D650-D657.

Crombie, T. A., S. Zdraljevic, D. E. Cook, R. E. Tanny, S. C. Brady *et al.*, 2019 Deep sampling of Hawaiian Caenorhabditis elegans reveals high genetic diversity and admixture with global populations. Elife 8**:** e50465.

Cutter, A. D., 2015 Caenorhabditis evolution in the wild. Bioessays 37**:** 983-995.

Cutter, A. D., L. T. Morran and P. C. Phillips, 2019 Males, Outcrossing, and sexual selection in Caenorhabditis Nematodes. Genetics 213**:** 27-57.

Dolgin, E. S., B. Charlesworth, S. E. Baird and A. D. Cutter, 2007 Inbreeding and outbreeding depression in Caenorhabditis nematodes. Evolution: International Journal

of Organic Evolution 61: 1339-1352.

Félix, M.-A., and C. Braendle, 2010 The natural history of Caenorhabditis elegans. Current biology 20: R965-R969.

Fisher, R. A., 1958 *The genetical theory of natural selection*. Рипол Классик.

Frézal, L., and M.-A. Félix, 2015 The natural history of model organisms: C. elegans outside the Petri dish. Elife 4: e05849.

Gimond, C., R. Jovelin, S. Han, C. Ferrari, A. D. Cutter *et al.*, 2013 Outbreeding depression with low genetic variation in selfing Caenorhabditis nematodes. Evolution 67: 3087-3101.

Harris, T. W., V. Arnaboldi, S. Cain, J. Chan, W. J. Chen *et al.*, 2020 WormBase: a modern model organism information resource. Nucleic acids research 48: D762-D767.

Hill, W., and A. Robertson, 1968 Linkage disequilibrium in finite populations. Theoretical and applied genetics 38: 226-231.

Hodgkin, J., and T. M. Barnes, 1991 More is not better: brood size and population growth in a self-fertilizing nematode. Proceedings of the Royal Society of London. Series B: Biological Sciences 246: 19-24.

Huang, C., C. Xiong and K. Kornfeld, 2004 Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. Proceedings of the National Academy of Sciences 101: 8084-8089.

Hughes, S. E., K. Evason, C. Xiong and K. Kornfeld, 2007 Genetic and pharmacological factors that influence reproductive aging in nematodes. PLoS genetics 3: e25.

Klass, M. R., 1977 Aging in the nematode Caenorhabditis elegans: major biological and environmental factors influencing life span. Mechanisms of ageing and development 6: 413-429.

Kocsisova, Z., K. Kornfeld and T. Schedl, 2019 Rapid population-wide declines in stem cell number and activity during reproductive aging in C. elegans. Development 146.

Luo, S., G. A. Kleemann, J. M. Ashraf, W. M. Shaw and C. T. Murphy, 2010 TGF-β and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. Cell 143**:** 299-312.

Luo, S., W. M. Shaw, J. Ashraf and C. T. Murphy, 2009 TGF-ß Sma/Mab signaling mutations uncouple reproductive aging from somatic aging. PLoS Genet 5**:** e1000789.

McKinney, W., 2010 Data structures for statistical computing in python, pp. 51-56 in *Proceedings of the 9th Python in Science Conference*. Austin, TX.

Morran, L. T., M. D. Parmenter and P. C. Phillips, 2009 Mutation load and rapid adaptation favour outcrossing over self-fertilization. Nature 462**:** 350-352.

Perez, M. F., M. Francesconi, C. Hidalgo-Carcedo and B. Lehner, 2017 Maternal age generates phenotypic variation in Caenorhabditis elegans. Nature 552**:** 106-109.

Rose, A., and D. Baillie, 1979 The effect of temperature and parental age on recombination and nondisjunction in Caenorhabditis elegans. Genetics 92**:** 409-418.

Smith, J. M., and J. Maynard-Smith, 1978 *The evolution of sex*. Cambridge University Press Cambridge.

Figures

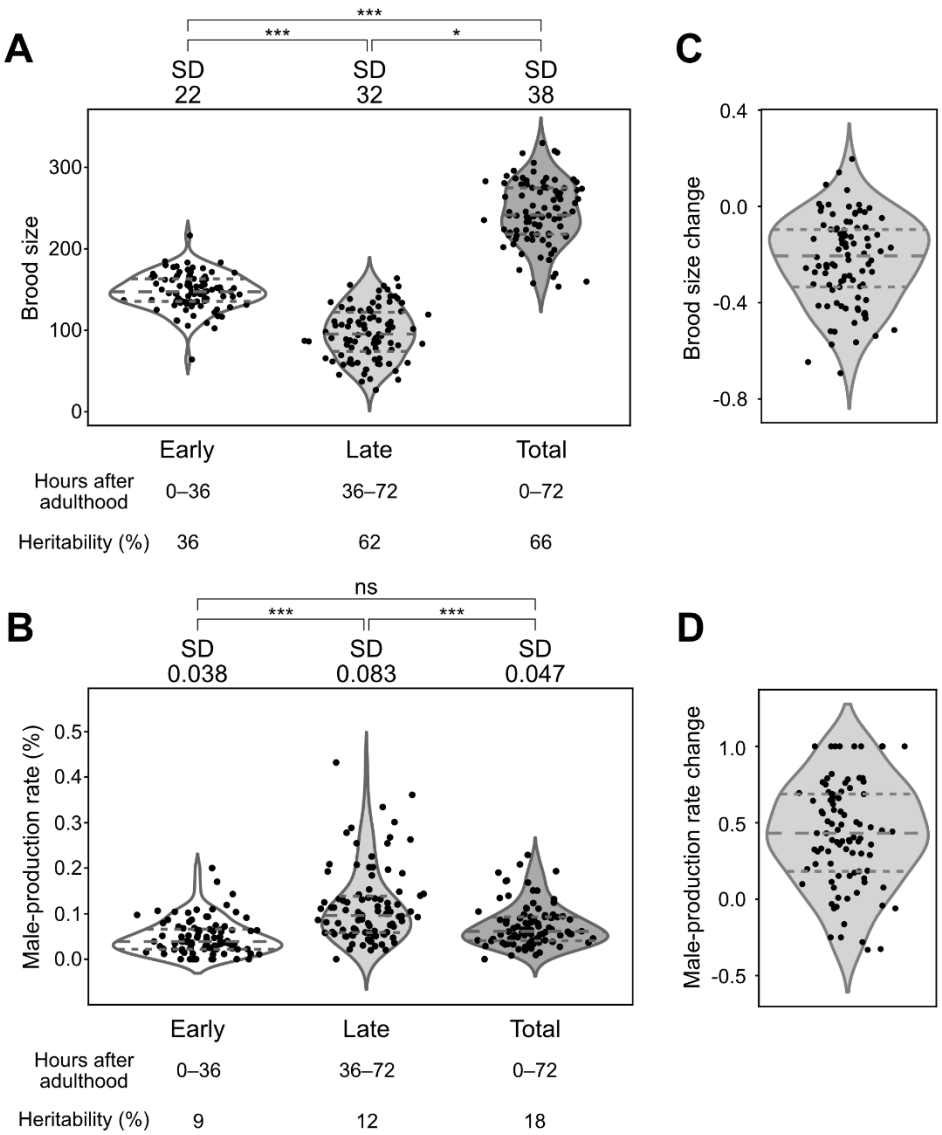

Figure 1. Phenotypic distributions of brood size and male-production rate in each period, and their changes over time. A, B: Distributions of (A) brood size and (B) male-production rate in the early, late and total periods in the 94 wild strains. Each dot represents the average phenotypic value of a wild strain. The *p*-value was used to compare standard deviations using F-tests for brood size, and Bonett's and Levene's tests for male-production rate (*: $p < 0.05$; ***: $p < 0.001$; ns: nonsignificant). Horizontal dashed lines represent (bottom to top) 1st, 2nd and 3rd quartiles. SD, standard deviation C, D: Distributions of change in (C) brood size and (D) male-production rate between two reproductive periods in the 94 wild strains. Phenotypic change was calculated as (late-early)/(late+early).

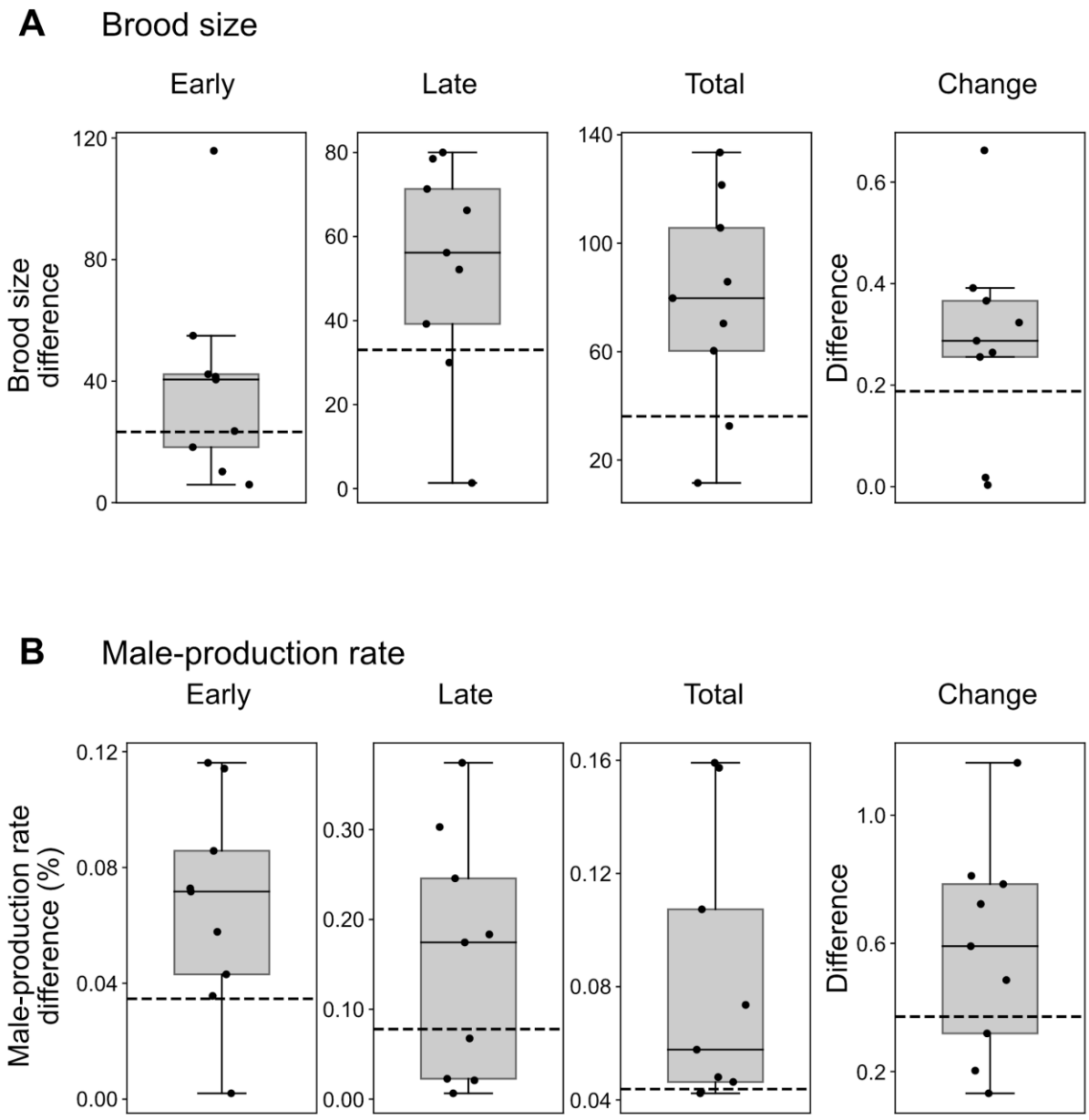

Figure 2. Phenotypic differences between wild strains collected in the same environment. Each dot represents a difference between the phenotypic values within the groups of strains collected in the same environment. (A) brood size and (B) male-production rate. Black solid lines in boxes denote medians, and the bottom and top of the boxes represent 25% and 75% quartiles, respectively. Whiskers extend to 1.5X interquartile range from the boxes. Horizontal dashed lines indicate the average of phenotypic differences of 30 pairs randomly selected from 94 strains. Phenotypic differences were calculated by subtracting the minimum value from the maximum value within the group.

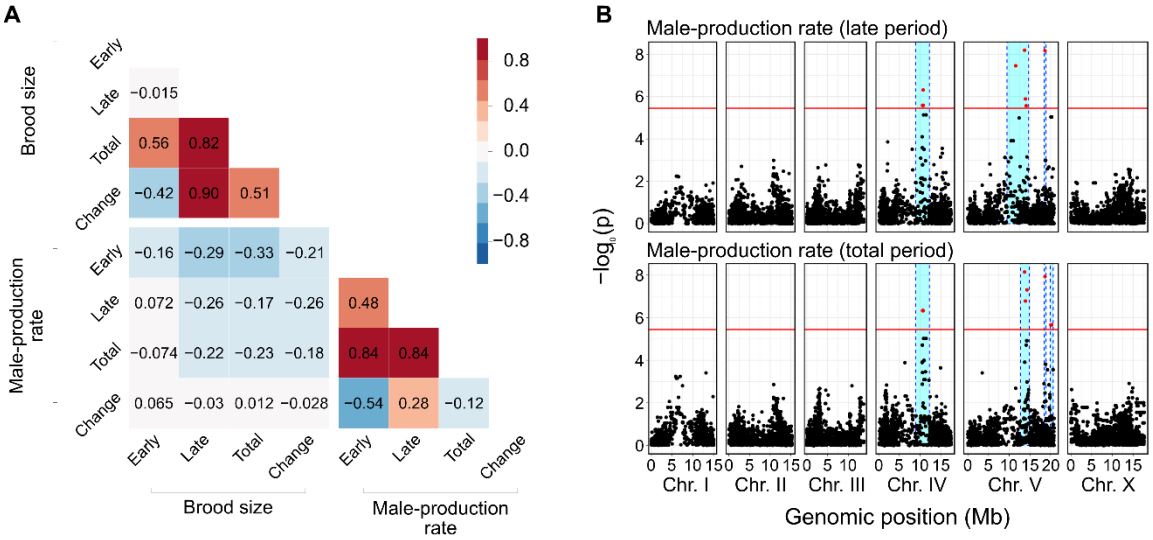

Figure 3. Correlation matrix and genome-wide association mapping for brood size and male-production rate. (A) Heatmap of Pearson correlations between brood size and male-production rate of early, late and total reproductive periods and their changes over time. (B) Manhattan plots for genome-wide association mapping of male-production rates in the late and total periods. Red horizontal lines represent the adjusted Bonferroni cut-off for 5% (-logP= 5.44) and red dots indicate SNVs beyond that threshold. Cyan bars show 95% confidence intervals.

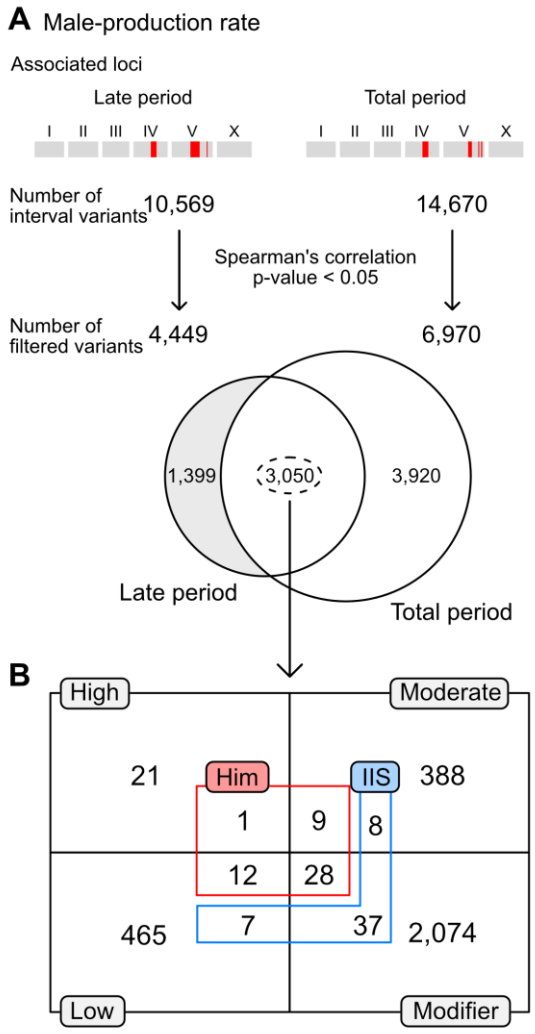

Figure 4. Filtering candidate variants in loci significantly associated with male-production rates in the late and total reproductive periods. (A) Filtering scheme. All interval variants in the loci were filtered according to their correlation with the traits. The loci for the two traits shared thousands of common variants. (B) The number of overlapped and filtered variants were categorized by their putative impacts on genes, as estimated by SnpEff (gray boxes and black lines) (CINGOLANI *et al.* 2012). These variants were further categorized by gene functions, including: genes known to interact with the IIS receptor, DAF-2 (blue), or to exhibit Him phenotypes (red), as annotated in the WormBase. Him: high incidence male progeny.

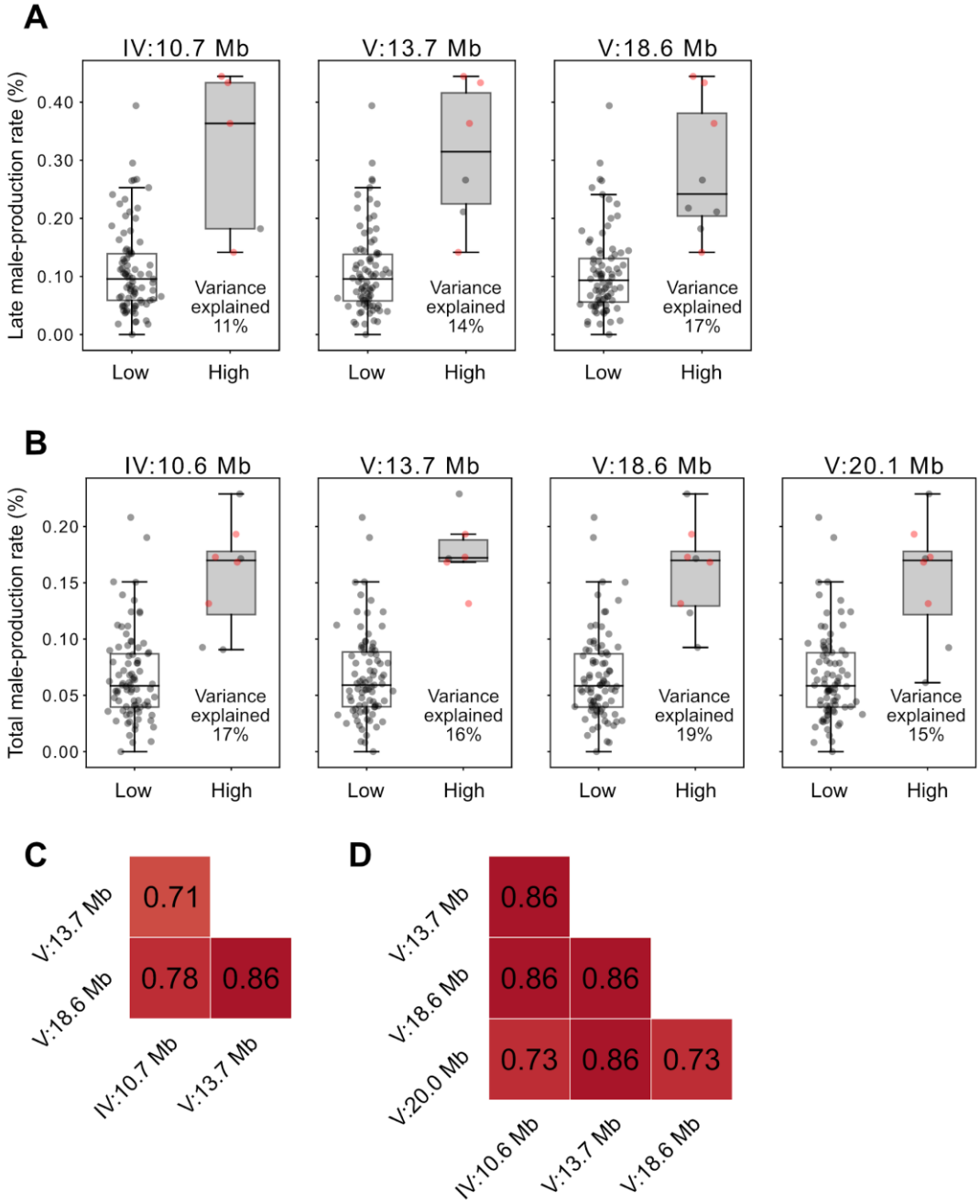

Figure 5. Phenotypic effects of each allele and linkage disequilibrium between associated loci. A, B: Phenotypic differences by peak genotypes of each associated locus for male-production rate in the late (A) and total (B) reproductive periods. Positions of each peak are on the top. Each dot represents the phenotypic value of a strain. The four strains that are marked with red dots had high alleles at all peaks for both the late and total reproductive periods. Variance explained by each locus is represented at the bottom right corner of each box. C, D: Heatmaps of linkage disequilibrium (Pearson's correlation coefficient, r) between peaks for male-production rate in the late (C) and total (D) reproductive periods.

Supplemental Materials

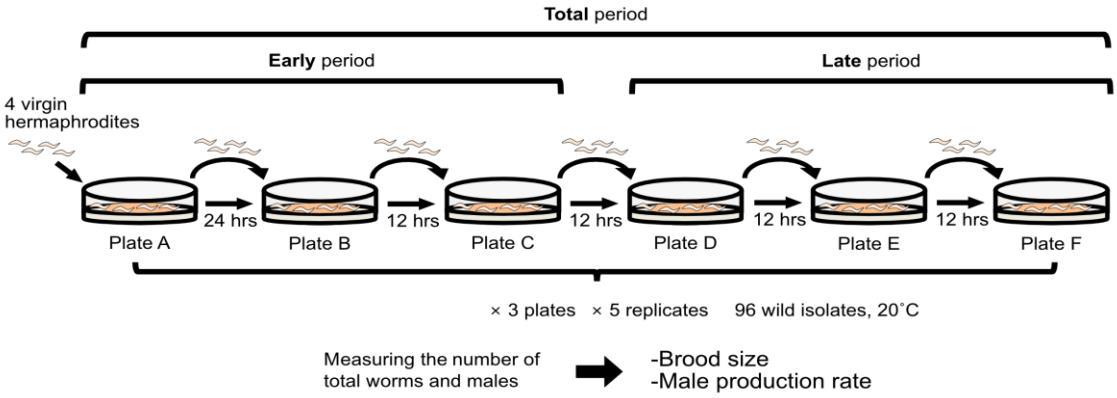

Figure S1. Experimental scheme for measuring the brood size and male-production rate over time. The oocyte-production rates and X-chromosome nondisjunction rates were measured in two different reproductive periods. The earlier period was 48 hours after the last larval stage and the later period was the following 36 hours. Four unmated L4 hermaphrodites for each wild strain were raised on the first medium for 24 hours and transferred to fresh media every 12 hours for a total of 84 hours. As the last larval stage of C. *elegans* takes around 12 hours to develop into the adult stage, we used the first 48 hours to 36 hours after entering adulthood. We used three plates for each replicate and counted five replicates in total.

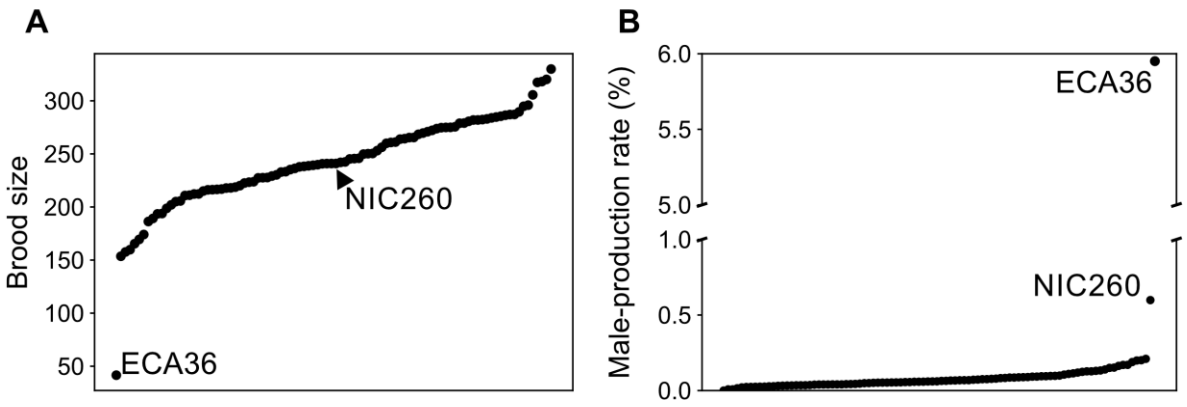

Figure S2. Phenotypic distributions of brood sizes and male-production rates, including outliers. Phenotypic variations for (A) brood size and (B) male-production rate in the total reproductive period including two outliers, ECA36 and NIC260 (arrowheads).

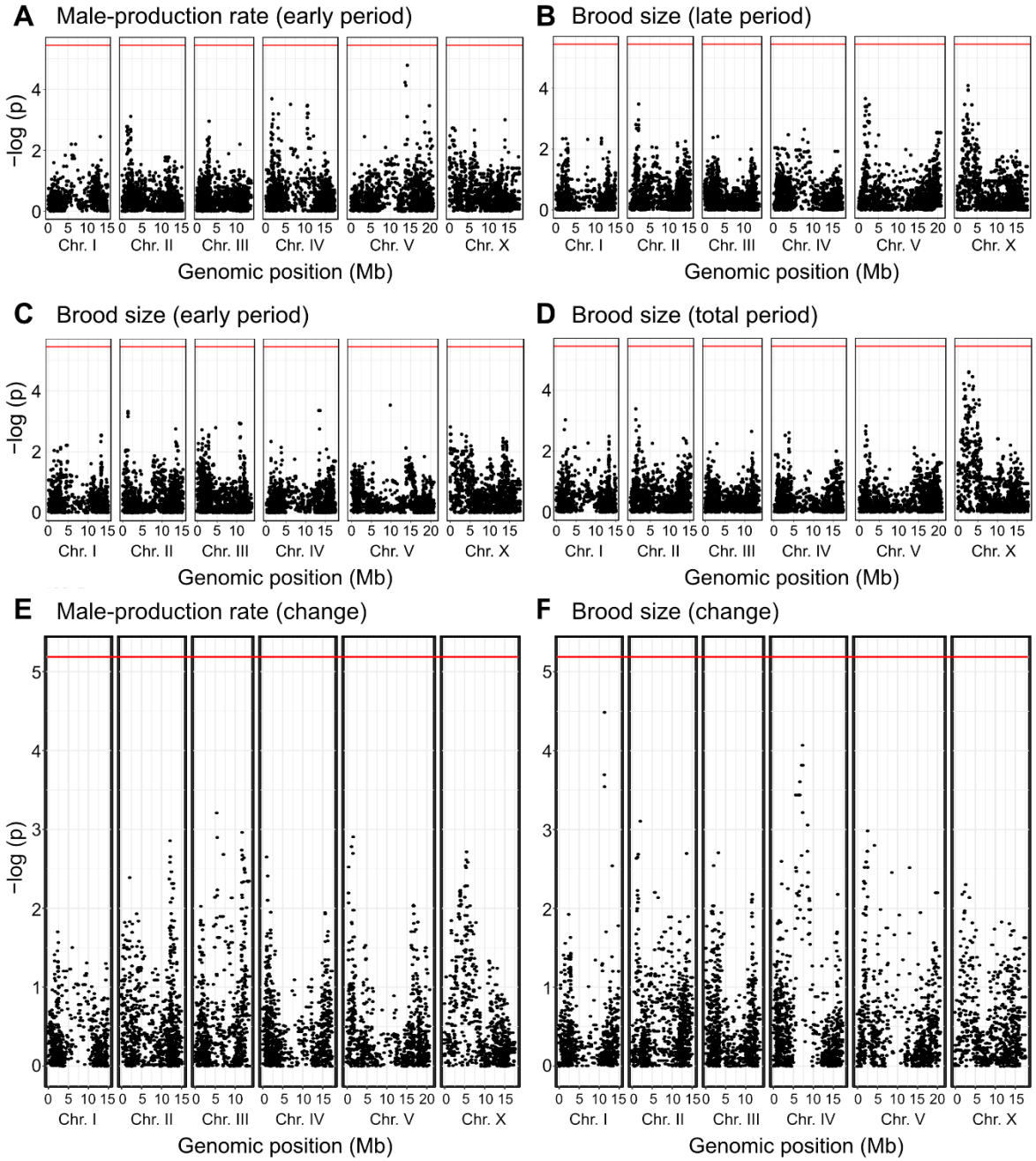

Figure S3. Manhattan plots representing genome-wide association mapping results. (A) male-production rate of the early reproductive period. (B-D) Brood size of late (B), early (C), and total (D) reproductive periods. (E-F) Phenotypic changes between early and late reproductive periods of male-production rate (E) and brood size (F).

(Separately provided)

Table S1. Average values of brood size and male-production rate (%) over time for all wild isolates used in the project

Table S2. Candidate variants within genes which exhibit the Him phenotypes

Table S3. Candidate variants within genes which interact with the IIS receptor, DAF-2

	early_broo	early_male	late_brood	late_male_	total_broo	total_male	brood_size	male_production_rate
AB1	147.55	0.00032	81.6	0.00241	229.15	0.00095	-0.2878	0.765453
BRC20067	132.4333	0.000199	83.88333	0.000603	216.3167	0.000353	-0.22444	0.504163
CB4854	149.8667	0.000298	124.85	0.000589	274.7167	0.000409	-0.09106	0.327369
CB4856	135.6417	0.000407	82.36111	0.00076	218.0028	0.000541	-0.24681	0.373583
CB4932	147.55	0	126.25	0.001041	273.8	0.000441	-0.07779	1
CX11271	146.1167	0.000255	114.6833	0.00059	260.8	0.000402	-0.12053	0.397288
CX11276	144.4	0.000286	106	0.000986	250.4	0.000514	-0.15335	0.550746
CX11285	125.2	0.000809	40.21667	0.002042	165.4167	0.001139	-0.51375	0.432307
CX11314	147.3833	8.98E-05	90.41667	0.000486	237.8	0.000261	-0.23956	0.688069
DL200	153.7167	0.000183	129.25	0.000986	282.9667	0.00056	-0.08646	0.68747
DL226	145.4667	0.000361	99.76667	0.000451	245.2333	0.000409	-0.18635	0.110838
DL238	152.0167	0.001083	58.78333	0.001259	210.8	0.001102	-0.44228	0.075146
ECA248	176.1833	0.00035	88.16667	0.000972	264.35	0.000545	-0.33296	0.470456
ECA36	34.43333	0.061222	7.116667	0.051437	41.55	0.059511	-0.65744	-0.08685
ED3005	134.3167	0.00061	102.0333	0.001377	236.35	0.000885	-0.13659	0.386008
ED3017	141.2667	0	86.15	0.000386	227.4167	0.000152	-0.24236	1
ED3040	174.45	0.000154	111.0167	0.001133	285.4667	0.000584	-0.22221	0.76014
ED3046	145.5167	0.000103	60.15	0.001044	205.6667	0.000355	-0.41507	0.819642
ED3048	178.45	0.000728	141.7333	0.000523	320.1833	0.000664	-0.11467	-0.16355
ED3049	137	0	61.7	0.00075	198.7	0.000241	-0.37896	1
ED3052	121.95	0.00082	116.3167	0.000835	238.2667	0.000836	-0.02364	0.008928
ED3073	184.7	0.000421	94.18333	0.002952	278.8833	0.001305	-0.32457	0.750518
ED3077	161.6667	0.000142	155.7167	0.000657	317.3833	0.000366	-0.01875	0.644069
EG4349	155.4	0.000211	150.2167	0.001748	305.6167	0.000967	-0.01696	0.78438
EG4725	165.8	0	98.1	0.000637	263.9	0.000211	-0.25654	1
JT11398	168.8667	0.000314	58.71667	0.001786	227.5833	0.000606	-0.484	0.700757
JU1088	166.05	0	46.18333	0.000671	212.2333	0.00008	-0.56479	1
JU1172	111.75	0.000622	111.6	0.001189	223.35	0.000877	-0.00067	0.313396
JU1200	153.8667	0.000227	107.2833	0.000808	261.15	0.000425	-0.17838	0.560664
JU1212	166.0667	0.000426	163.8667	0.000214	329.9333	0.000314	-0.00667	-0.33133
JU1213	125.5333	0.001004	124.6667	0.002672	250.2	0.001887	-0.00346	0.453607
JU1242	135.6167	0.000376	76.66667	0.000409	212.2833	0.000401	-0.27769	0.041794
JU1246	147.05	0.000718	139.3833	0.001037	286.4333	0.000921	-0.02677	0.181716
JU1409	144.4	0.000348	73.41667	0.001632	217.8167	0.000611	-0.32589	0.648675
JU1440	117.3333	0.000175	110.4	0.000369	227.7333	0.00025	-0.03044	0.357276
JU1530	169.3333	0.000495	148.8167	0.00112	318.15	0.000789	-0.06449	0.387179
JU1568	143.9833	0	135.05	0.001472	279.0333	0.000668	-0.03202	1
JU1580	136.45	0.000586	138.55	0.001297	275	0.000924	0.007636	0.377699
JU1581	108.9833	0.000125	84.51667	0.000534	193.5	0.000339	-0.12644	0.621215
JU2007	157.3	0.000127	83.4	0.001089	240.7	0.000391	-0.30702	0.791178
JU2464	138.1	0.001092	78.76667	0.001592	216.8667	0.001273	-0.27359	0.186156
JU2466	105.5333	0.000642	140.3333	0.000957	245.8667	0.000824	0.14154	0.197155
JU2513	162.1	0.00073	133.8333	0.001035	295.9333	0.000862	-0.09552	0.172739
JU2519	145.0167	0.00096	60.05	0.002529	205.0667	0.001503	-0.41434	0.449712
JU2522	139.0833	0.000103	126.3	0.000784	265.3833	0.000429	-0.04817	0.768654
JU258	141.5833	0.000244	60.43333	0.000479	202.0167	0.000325	-0.4017	0.324975

JU310	128.3667	0.000351	153.9667	0.000655	282.3333	0.000526	0.090673	0.302578
JU311	166.5833	0	50	0	216.5833	0	-0.53828	
JU323	183.2667	0.001988	39.3	0.002001	222.5667	0.0021	-0.64685	0.003284
JU346	134.95	0.000259	154.6167	0.000238	289.5667	0.000236	0.067918	-0.04219
JU360	154.45	0.000881	64.23333	0.000495	218.6833	0.000752	-0.41254	-0.28079
JU367	164.6833	0.000901	65.56667	0.002328	230.25	0.00133	-0.43047	0.441976
JU393	177.5833	0.000255	90.86667	0.001195	268.45	0.0006	-0.32303	0.648055
JU406	130.7667	0.000903	93.01667	0.001451	223.7833	0.001191	-0.16869	0.232643
JU440	150.0167	0.000282	109.9667	0.000627	259.9833	0.000409	-0.15405	0.379231
JU774	160.85	0.000477	77.93333	0.002648	238.7833	0.000973	-0.34725	0.694635
JU778	177.1167	0.00062	106.8167	0.001233	283.9333	0.000895	-0.24759	0.331126
JU792	131.3167	0.000564	134.15	0.0005	265.4667	0.000563	0.010673	-0.06012
JU830	102.4	0	83.91667	0.000181	186.3167	8.21E-05	-0.0992	1
JU847	152.7167	0.000502	58.38333	0.00091	211.1	0.000588	-0.44687	0.289153
KR314	216.3667	0.000369	58.56667	0.000222	274.9333	0.000352	-0.57396	-0.24981
LKC34	170.7333	0.000456	104.7833	0.003939	275.5167	0.00152	-0.23937	0.792342
MY1	163.8333	0	85.98333	0.000892	249.8167	0.000333	-0.31163	1
MY16	171.6	0.000985	69.36667	0.003635	240.9667	0.001634	-0.42426	0.573532
MY18	171.5667	0.000267	115.5	0.00059	287.0667	0.0004	-0.19531	0.377554
MY23	116.6167	0.001408	36.96667	0.004334	153.5833	0.001991	-0.51861	0.509538
NIC1	157.25	0.000208	123.4333	0.001313	280.6833	0.000716	-0.12048	0.726842
NIC166	133.5167	0.000223	101.7167	0.000414	235.2333	0.000281	-0.13518	0.298631
NIC195	156.1167	0.000716	128.5833	0.000364	284.7	0.000537	-0.09671	-0.32634
NIC199	134.7333	0.000893	107.3	0.002249	242.0333	0.001396	-0.11335	0.431539
NIC2	148.25	0.000372	123.9333	0.000709	272.1833	0.000494	-0.08934	0.311342
NIC207	162.7167	0.000594	119.25	0.000782	281.9667	0.000687	-0.15416	0.13627
NIC236	135.5167	0.000196	106.9333	0.000957	242.45	0.000489	-0.11789	0.660412
NIC242	151.7333	0.000272	87.56667	0.001391	239.3	0.000645	-0.26814	0.673096
NIC251	183.1833	0.000627	57.75	0.001821	240.9333	0.000946	-0.52061	0.487792
NIC252	129.8	0.000381	86.35	0.00112	216.15	0.000682	-0.20102	0.492418
NIC255	152.6167	0	134.55	0.00055	287.1667	0.000289	-0.06291	1
NIC256	124.9	0.00091	95.2	0.002659	220.1	0.001698	-0.13494	0.489983
NIC260	156.9167	0.004355	83.96667	0.008844	240.8833	0.005988	-0.30284	0.340111
NIC261	64.15	0.001128	95.63333	0.001415	159.7833	0.001238	0.197038	0.113167
NIC265	179.95	0.000771	65.63333	0.004444	245.5833	0.001702	-0.46549	0.704286
NIC266	135.9333	0.000572	103.9	0.002177	239.8333	0.001273	-0.13356	0.584013
NIC271	117.6833	0.001733	51.75	0.002112	169.4333	0.002009	-0.38914	0.098638
NIC272	138.7667	0.000627	76.25	0.000853	215.0167	0.000686	-0.29075	0.152632
NIC274	137.3333	0.000196	51.85	0.000178	189.1833	0.000257	-0.45185	-0.05013
NIC275	171.6667	0.000385	123.1833	0.000523	294.85	0.000459	-0.16443	0.152259
NIC276	129.4	0.000526	64.43333	0.000494	193.8333	0.000522	-0.33517	-0.03155
NIC277	146.15	0.000684	87.11667	0.001444	233.2667	0.000992	-0.25307	0.357293
NIC3	142.0167	0.000461	111.0667	0.000736	253.0833	0.000582	-0.12229	0.229669
PB303	167.3333	0.000381	88.91667	0.001407	256.25	0.000727	-0.30602	0.573541
PX179	158.65	0.000186	112.25	0.000972	270.9	0.000528	-0.17128	0.679391
QG2075	112.2	0.001196	45.41667	0.00106	157.6167	0.001057	-0.42371	-0.06051
QG557	148.0833	0.000215	84.81667	0.00187	232.9	0.000862	-0.27165	0.793664

QW947	163.2167	0.000661	106.4	0.000397	269.6167	0.00064	-0.21073	-0.25008
QX1212	139.9833	0.000426	141.95	0.001005	281.9333	0.000756	0.006976	0.404311
QX1794	147.3333	0.000691	26.71667	0.000805	174.05	0.000776	-0.693	0.076065

_change

CHROM	POS	gene_nam	gene_id	impact	late_male_	total_male_spearman_cor_p
IV	10282516	rad-51	WBGene0C	LOW	0.035242	0.033362
IV	10282788	rad-51	WBGene0C	MODIFIER	0.03163	0.003114
IV	10282840	rad-51	WBGene0C	MODIFIER	0.027912	0.018979
IV	10548060	zim-1	WBGene0C	MODIFIER	0.025741	0.022901
IV	10551413	zim-1	WBGene0C	MODIFIER	0.023693	0.001629
IV	10552467	zim-1	WBGene0C	MODIFIER	0.023693	0.001629
IV	10552593	zim-1	WBGene0C	MODIFIER	0.00196	0.000104
IV	10553225	zim-1	WBGene0C	MODIFIER	0.034119	0.002835
IV	10553948	zim-1	WBGene0C	MODIFIER	0.035242	0.033362
IV	10555007	zim-1	WBGene0C	LOW	0.035242	0.033362
IV	10555321	zim-2	WBGene0C	MODIFIER	0.001887	0.000103
IV	10555524	zim-2	WBGene0C	MODIFIER	0.035242	0.033362
IV	10556789	zim-2	WBGene0C	MODIFIER	0.001887	0.000103
IV	10557100	zim-2	WBGene0C	MODERATE	0.029574	0.002269
IV	10557114	zim-2	WBGene0C	MODERATE	0.029574	0.002269
IV	10557189	zim-2	WBGene0C	MODIFIER	0.001887	0.000103
IV	10557209	zim-2	WBGene0C	MODIFIER	0.029574	0.002269
IV	10557342	zim-2	WBGene0C	MODERATE	0.001887	0.000103
IV	10558022	zim-2	WBGene0C	HIGH	0.001887	0.000103
IV	10559496	zim-3	WBGene0C	MODERATE	0.029574	0.002269
IV	10559575	zim-3	WBGene0C	LOW	0.001887	0.000103
IV	10559590	zim-3	WBGene0C	LOW	0.001887	0.000103
IV	10559793	zim-3	WBGene0C	MODERATE	0.029574	0.002269
IV	10560163	zim-3	WBGene0C	LOW	0.029574	0.002269
IV	10560369	zim-3	WBGene0C	LOW	0.001887	0.000103
IV	10560434	zim-3	WBGene0C	LOW	0.029574	0.002269
IV	10560744	zim-3	WBGene0C	LOW	0.029574	0.002269
IV	10562249	him-8	WBGene0C	MODERATE	0.001887	0.000103
IV	10562422	him-8	WBGene0C	MODIFIER	0.029574	0.002269
IV	10562643	him-8	WBGene0C	MODIFIER	0.001887	0.000103
IV	10562769	him-8	WBGene0C	MODIFIER	0.001887	0.000103
IV	10563322	him-8	WBGene0C	MODERATE	0.005182	0.000349
IV	10563493	him-8	WBGene0C	MODERATE	0.001887	0.000103
IV	10563612	him-8	WBGene0C	MODIFIER	0.029574	0.002269
IV	10563897	him-8	WBGene0C	MODIFIER	0.001887	0.000103
IV	11573540	srgp-1	WBGene0C	MODIFIER	0.035242	0.033362
IV	11578665	srgp-1	WBGene0C	MODIFIER	0.030273	0.025391
IV	12083201	lex-1	WBGene0C	LOW	0.035242	0.033362
IV	12083232	lex-1	WBGene0C	MODERATE	0.035242	0.033362
IV	12083827	lex-1	WBGene0C	MODIFIER	0.035242	0.033362
IV	12084139	lex-1	WBGene0C	MODIFIER	0.044728	0.004121
V	13176908	him-17	WBGene0C	MODIFIER	0.035214	0.0163
V	13178557	him-17	WBGene0C	LOW	0.035214	0.0163
V	13964693	sao-1	WBGene0C	MODIFIER	0.023952	0.004139
V	14090774	him-5	WBGene0C	MODIFIER	0.003718	0.002139
V	14090957	him-5	WBGene0C	MODIFIER	0.012777	0.001285

V	14091464	him-5	WBGene0(	LOW	0.003718	0.002139
V	14092357	him-5	WBGene0(	MODIFIER	0.00692	0.000549
V	14457568	coh-3	WBGene0(	MODIFIER	0.032259	0.004623
V	14458395	coh-3	WBGene0(	LOW	0.032259	0.004623

CHROM	POS	gene_nam	gene_id	impact	late_male_	total_male_spearman_cor_p
IV	9139368	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9139704	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9140529	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9140854	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9142990	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9143168	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9144386	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9144938	daf-10	WBGene0C	MODIFIER	0.036121	0.03418
IV	9143367	daf-10	WBGene0C	MODERATE	0.036121	0.03418
IV	9316845	rpn-7	WBGene0C	MODERATE	0.035242	0.033362
IV	9446650	hsp-12.6	WBGene0C	MODIFIER	0.035242	0.033362
IV	9527139	elo-1	WBGene0C	LOW	0.031797	0.029975
IV	9799632	fat-4	WBGene0C	MODIFIER	0.035242	0.033362
IV	9804049	fat-3	WBGene0C	MODIFIER	0.035242	0.033362
IV	9804677	fat-3	WBGene0C	MODERATE	0.035242	0.033362
IV	9805637	fat-3	WBGene0C	MODIFIER	0.036121	0.03418
IV	10143034	gst-4	WBGene0C	MODIFIER	0.020358	0.003684
IV	10255550	daf-14	WBGene0C	LOW	0.03163	0.003114
IV	10251733	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10252780	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10253202	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10253308	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10253455	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10254045	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10255494	daf-14	WBGene0C	MODIFIER	0.048758	0.01026
IV	10254220	daf-14	WBGene0C	MODERATE	0.048758	0.01026
IV	10254950	daf-14	WBGene0C	LOW	0.048758	0.01026
IV	10673908	epi-1	WBGene0C	LOW	0.00012	4.29E-05
IV	10676230	epi-1	WBGene0C	LOW	0.003495	0.001145
IV	10667075	epi-1	WBGene0C	MODIFIER	0.004113	0.000143
IV	10678435	epi-1	WBGene0C	MODERATE	0.035242	0.033362
IV	11526816	mep-1	WBGene0C	MODERATE	0.029254	0.027576
IV	11689030	let-60	WBGene0C	MODIFIER	0.006452	0.006141
IV	11820643	par-5	WBGene0C	MODIFIER	0.035242	0.033362
IV	11891050	let-653	WBGene0C	MODIFIER	0.035242	0.033362
IV	11887317	let-653	WBGene0C	MODIFIER	0.035242	0.033362
IV	11887539	let-653	WBGene0C	MODIFIER	0.035242	0.033362
IV	11889901	let-653	WBGene0C	MODIFIER	0.035242	0.033362
IV	11889902	let-653	WBGene0C	MODIFIER	0.035242	0.033362
IV	11889903	let-653	WBGene0C	MODIFIER	0.035242	0.033362
IV	11886524	let-653	WBGene0C	MODERATE	0.035242	0.033362
IV	11889585	let-653	WBGene0C	MODERATE	0.035242	0.033362
IV	11890237	let-653	WBGene0C	LOW	0.035242	0.033362
IV	11890727	let-653	WBGene0C	LOW	0.035242	0.033362
V	12785819	fkb-4	WBGene0C	MODIFIER	0.041814	0.026451
V	12786048	fkb-4	WBGene0C	MODIFIER	0.041814	0.026451

V	13433220	mes-4	WBGene0C	MODIFIER	0.024489	0.006257
V	13433895	mes-4	WBGene0C	MODIFIER	0.024489	0.006257
V	14018657	mtl-2	WBGene0C	MODIFIER	0.00153	9.46E-05
V	18912750	cyp-42A1	WBGene0C	MODIFIER	0.009795	0.008587
V	18912506	cyp-42A1	WBGene0C	MODIFIER	0.017892	0.026172
V	18912770	cyp-42A1	WBGene0C	MODIFIER	0.01896	0.02664