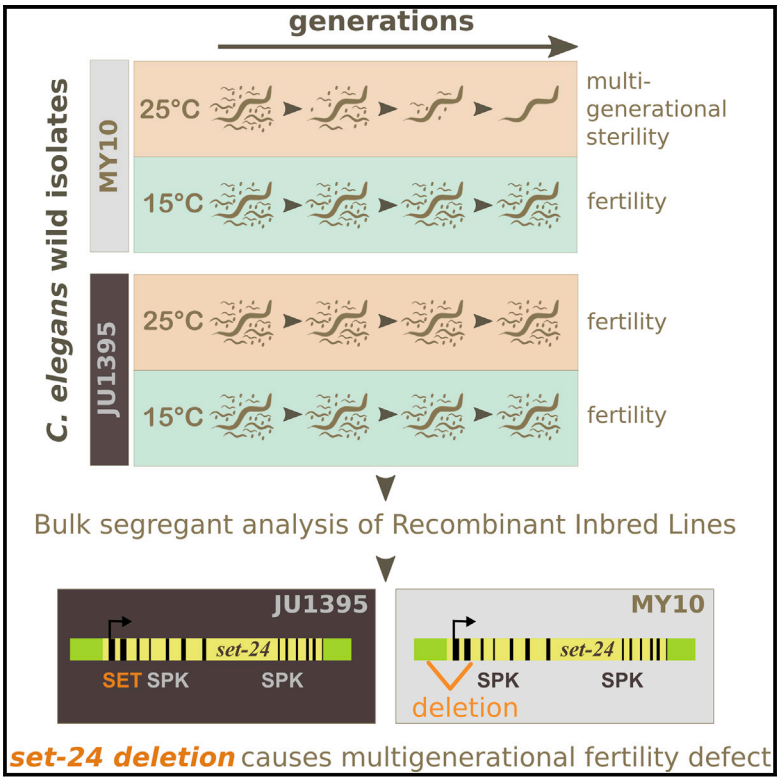
Report

Current Biology

# Natural Genetic Variation in a Multigenerational Phenotype in *C. elegans*

## Graphical Abstract




## Highlights

- *C. elegans* wild isolates show a reversible heat-sensitive mortal germline phenotype
- Pool sequencing of inbred lines was used for mapping this multigenerational trait
- We identified a polymorphism in the *set-24* gene as a major causal locus
- Natural populations may harbor genetic variation in epigenetic inheritance phenomena

## Authors

Lise Frézal, Emilie Demoinet, Christian Braendle, Eric Miska, Marie-Anne Félix

## Correspondence

eric.miska@gurdon.cam.ac.uk (E.M.), felix@biologie.ens.fr (M.-A.F.)

## In Brief

Frézal et al. discover that some *C. elegans* wild isolates become sterile after several generations at high temperature, a reversible trait that is known to correspond to alterations in non-genetic inheritance phenomena. They identify in the wild isolate MY10 a deletion in the *set-24* gene as causal for this multigenerational inheritance phenotype.

Frézal et al., 2018, Current Biology *28*, 2588–2596
August 20, 2018 
https://doi.org/10.1016/j.cub.2018.05.091

CellPress

CellPress

Current Biology
Report

# Natural Genetic Variation in a Multigenerational Phenotype in *C. elegans*

Lise Frézal,[1,2,3] Emilie Demoinet,[4] Christian Braendle,[4] Eric Miska,[2,3,5,*] and Marie-Anne Félix[1,6,*]
[1]Institut de Biologie de l'Ecole Normale Supérieure, Centre National de la Recherche Scientifique, INSERM, École Normale Supérieure, Paris Sciences et Lettres, Paris, France
[2]Wellcome Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK
[3]Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK
[4]Université Côte d'Azur, CNRS, INSERM, IBV, Nice, France
[5]Wellcome Sanger Institute, Wellcome Trust Genome Campus, Cambridge CB10 1SA, UK
[6]Lead Contact
*Correspondence: eric.miska@gurdon.cam.ac.uk (E.M.), felix@biologie.ens.fr (M.-A.F.)
https://doi.org/10.1016/j.cub.2018.05.091

## SUMMARY

Although heredity mostly relies on the transmission of DNA sequence, additional molecular and cellular features are heritable across several generations. In the nematode *Caenorhabditis elegans*, insights into such unconventional inheritance result from two lines of work. First, the mortal germline (Mrt) phenotype was defined as a multigenerational phenotype whereby a selfing lineage becomes sterile after several generations, implying multigenerational memory [1, 2]. Second, certain RNAi effects are heritable over several generations in the absence of the initial trigger [3–5]. Both lines of work converged when the subset of Mrt mutants that are heat sensitive were found to closely correspond to mutants defective in the RNAi-inheritance machinery, including histone modifiers [6–9]. Here, we report the surprising finding that several *C. elegans* wild isolates display a heat-sensitive mortal germline phenotype in laboratory conditions: upon chronic exposure to higher temperatures, such as 25°C, lines reproducibly become sterile after several generations. This phenomenon is reversible, as it can be suppressed by temperature alternations at each generation, suggesting a non-genetic basis for the sterility. We tested whether natural variation in the temperature-induced Mrt phenotype was of genetic nature by building recombinant inbred lines between the isolates MY10 (Mrt) and JU1395 (non-Mrt). Using bulk segregant analysis, we detected two quantitative trait loci. After further recombinant mapping and genome editing, we identified the major causal locus as a polymorphism in the *set-24* gene, encoding a SET- and SPK-domain protein. We conclude that *C. elegans* natural populations may harbor natural genetic variation in epigenetic inheritance phenomena.

## RESULTS

### Many *C. elegans* Wild Isolates Show a Temperature-Sensitive Mortal Germline Phenotype

*C. elegans* is routinely cultured between 15°C and 25°C [10]. At 25°C, the reference strain of *C. elegans*, N2, can be maintained for many generations (e.g., Figure 1B in [11]). While collecting new *C. elegans* wild isolates, we discovered that many display a multigenerational sterility phenotype (or mortal germline [Mrt] phenotype) when chronically exposed to temperatures such as 25°C. Figure S1 shows a multigenerational sterility assay at 25°C on 14 such wild isolates.

Focusing on three isolates with a strong Mrt phenotype and two non-Mrt isolates, we performed a multigenerational sterility assay at several temperatures ranging from 15°C to 25°C (Figures 1A and 1B). At 25°C, we observed a highly reproducible sterility phenotype for the three Mrt isolates after a few generations, with QX1211 (from California) showing the strongest phenotype, followed by MY10 (Germany) and then by JU775 (Portugal). In contrast, the isolates JU1395 (France) and JU1171 (Chile) remained fertile throughout the 20 generations of the assay (Figure 1B). The Mrt phenotype of QX1211, MY10, and JU775 was quantitatively affected by temperature (Figure 1B). For example, at 25°C, MY10 lines were fully sterile after three or four generations; at 23°C, their half-life was of four generations (range 3–6 generations), and at 21.5°C, their half-life was of six generations with a wider range between four and eleven generations. Even at 20°C, the conventional culture temperature of *C. elegans*, up to 95% of the QX1211 lines, 75% of the MY10 lines, and 40% of the JU775 lines were extinct after 20 generations (n = 20 lines for each). At 15°C, all remained fertile over 20 generations. We conclude that *C. elegans* wild isolates differ quantitatively in the temperature-sensitive Mrt phenotype and that the number of generations to sterility is highly sensitive to temperature.

We observed that the decline in fertility in the Mrt isolates was progressive, with a low brood size and a high male incidence in one or two generations prior to full sterility. In *C. elegans*, where males are X0, high male incidence is the result of meiotic errors due to non-disjunction of the X chromosome, thus of meiotic "defects." We followed the lineage of single individuals (rather than averaging over three) of the MY10 and JU775 isolates and quantified their brood size over several generations at 23°C.

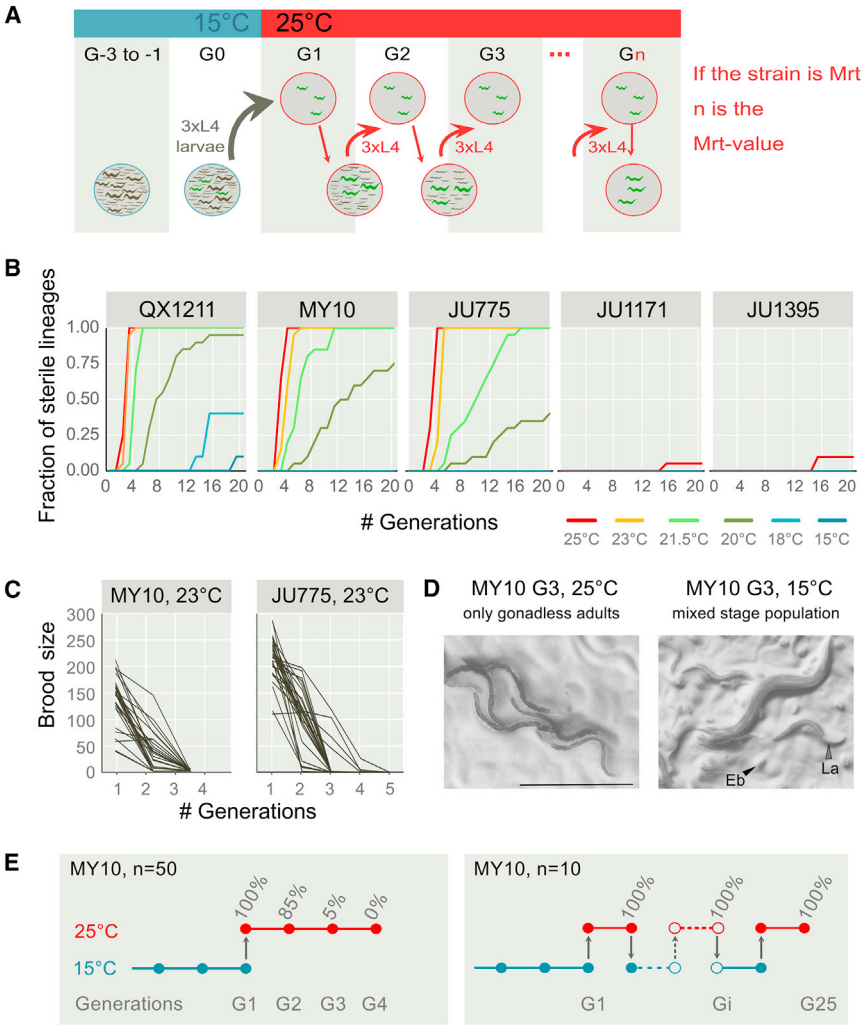



**Figure 1. A Subset of *C. elegans* Wild Isolates Displays a Strong Temperature-Sensitive Mrt Phenotype**

(A) Experimental design for mortal germline (Mrt) phenotyping. The phenotypic value is the number of generations (*n*) until sterility.

(B) The Mrt phenotype of five wild *C. elegans* isolates was followed over 25 generations at different temperatures. The proportion of sterile replicates accumulating along generations is plotted. For QX1211, MY10, and JU775, n = 20 lineages were scored at each temperature; for JU1395 and JU1171, n = 20 lineages were scored at 25°C and 23°C and 10 at 18°C and 15°C.

(C) The brood size along 30 single-individual lineages of MY10 and JU775 was manually counted from generation 1 to the generation when they became sterile at 23°C. The brood size decreases progressively in individual lineages in the generations preceding full sterility.

(D) At generation 3, all MY10 animals raised at 15°C had a normal anatomy, whereas those grown at 25°C were all sterile, with a reduced gonad and a characteristic dark intestinal color. The same scale is used for both panels. The scale bar represents 1 mm. Eb, embryo; La, larvae.

(E) Test of the reversibility of the MY10 Mrt phenotype with alternations of temperature at each generation (right). All ten replicates survived over 25 generations of such treatments, thus 13 non-consecutive generations at 25°C. With constant exposure to 25°C, all 50 lineages had a Mrt value between 2 and 4 (left).

See also Figure S1.

In this experiment, we also observed a progressive decrease in brood size and a high male incidence in the generations before full sterility (Figure 1C).

In the final generations leading to sterility in MY10 at 25°C, we observed further germline abnormalities, such as sperm and/or oocyte differentiation defects, germline atrophy, and meiotic chromosome pairing defects in oocytes (Figures 1D, 2A–2C, and S2A–S2C). The double-stranded breaks that normally occur transiently during prophase of meiosis I in the transition zone [12] were not resorbed and appeared more numerous in late pachytene of MY10 animals after three generations at 25°C (Figure 2D). These germline defects resemble previously reported temperature-sensitive Mrt phenotypes in various mutants [7, 8, 13–15].

We monitored the secondary small interfering RNA (siRNA) populations, the major effectors of RNAi pathways in *C. elegans* [16], using 5′-independent libraries, enriching for 5′ triphosphate small RNAs (sRNAs) [17, 18], in MY10 and JU1395: generation 0 was grown at 15°C and further generations were grown at 23°C until MY10 reached full sterility (Figures 2E and S3). At 15°C, the compositions of small RNA populations, and specifically the proportions of 22G small RNAs (22-nt RNAs with the first nucleotide a G, the major class of secondary siRNAs in *C. elegans*) [16], were similar in MY10 and in JU1395. In JU1395, the 22G proportion was stable over time (from the glm model; the effect of the number of generations was not significant; p = 0.967). In contrast, in MY10, the 22G small RNA pool was progressively depleted (p = 0.0034; Figures 2E and S3). This progressive change of small RNA populations in MY10 most likely corresponded to the germline atrophy described above. However, it remained unclear whether the 22G depletion was a consequence or a cause of germline atrophy, and this would be interesting to dissect further in the future.

To test whether the sterility phenotype was caused by the accumulation of irreversible damage—for example, in DNA—we submitted MY10 animals to a regime alternating between the temperatures of 15°C and 25°C at each generation, with a control at a constant temperature of 25°C (Figure 1E). Under this regime, all 10 temperature-alternation lines remained fertile after a total of 13 generations at 25°C, and all controls were sterile after four generations. This experiment rules out irreversible damage occurring at 25°C as the source of multigenerational phenotype formation.

These results overall demonstrate natural variation in the temperature-sensitive Mrt phenotype among *C. elegans* wild isolates, thus a variation in multigenerational memory of the heat environment.

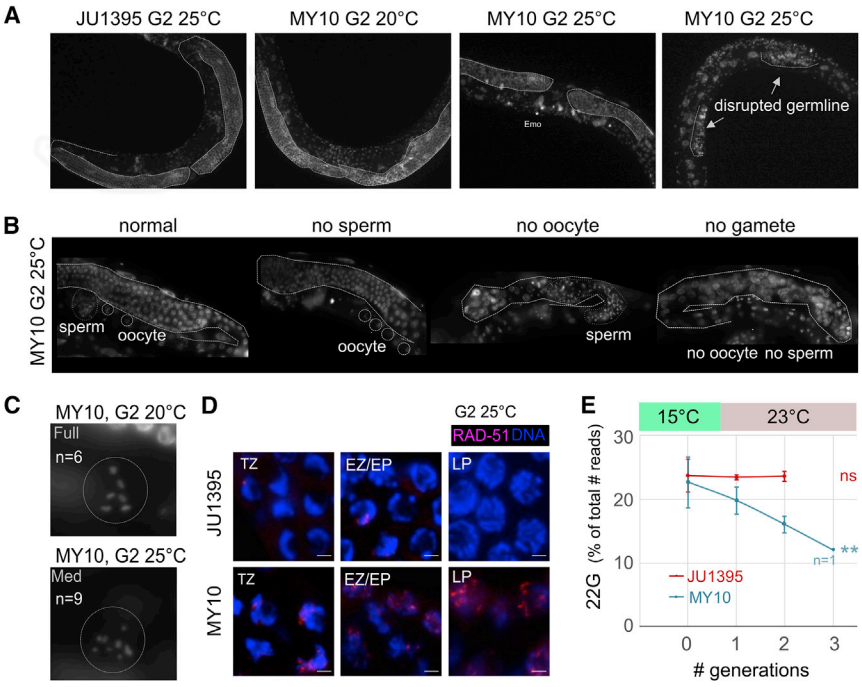



**Figure 2. Germline Integrity Defects, Chromosomal Aberrations, and 22G Small RNA Depletion in the Final Generations before or at Full Sterility**

(A) DAPI staining of JU1395 and MY10 animals at generation 2 (G2) of culture at 20°C or 25°C. Dashed lines indicate the distal germline arms. Wild-type germlines display two symmetric arms (e.g., JU1395 25°C; MY10 20°C). In contrast, at 25°C, MY10 G2 displayed a disrupted gonad with medium to small or empty arms (middle right) or displayed germlines with endomitotic oocytes that endoreplicate in the absence of sperm (left). Emo, endomitotic oocyte.

(B) At 25°C, MY10 G2 animals display various defects. Some MY10 G2 animals possess only oocytes, some only sperm, and the majority of them have neither sperm nor oocytes (rightmost panel). Dashed lines indicate the germline arms; dotted circles surround single oocytes at diakinesis or the pool of sperm in spermathecae.

(C) DAPI-stained late-G2-generation MY10 oocytes arrested in diakinesis grown at 20°C or 25°C. The dotted circle surrounds a single oocyte nucleus. The MY10 20°C oocyte displays the normal set of six paired chromosomes in diakinesis, whereas the MY10 25°C oocytes have more than six chromatin-DAPI-stained bodies, indicative of meiotic pairing defects.

(D) RAD-51 immunostaining in oocyte nuclei for MY10 and JU1395 G2 at 25°C. In JU1395, a few RAD-51 foci (red) are apparent in the transition zone (TZ) and the late zygotene to early pachytene (LZ/EP) zone and disappear in late pachytene nuclei (LP). RAD-51 foci in MY10 G2 animals persist until late pachytene and are more numerous. The scale bars represent 2 μm.

(E) Percentage of 22Gs (small RNAs of length 22 nt, starting with a guanine) in the small RNA populations in the MY10 and JU1395 strains, across generations. The dots represent the mean over three replicates per strain and per generation for generations 0, 1, and 2. The bars represent the SDs. The effect of the time (generation) on percentages of 22G was tested using a general linear model with a Poisson regression. ns, non-significant. $^{**}p < 0.005$.

See also Figures S2 and S3.

## Crosses between MY10 and JU1395 Yield Two Quantitative Trait Loci

To determine whether the natural variation in the Mrt phenotype could be mapped genetically, we crossed isolates MY10 (Mrt) and JU1395 (non-Mrt) in both directions to build recombinant inbred lines (RILs) at 15°C (Figure 3A). We then phenotyped the 120 RILs in triplicates for 25 generations at 25°C (Data S1A). Figure 3B shows the distribution of phenotypic values in the RILs: some lines exhibited a phenotypic value that was intermediate between the parental values; in addition, the distribution was biased, with more lines exhibiting a strong MY10-like Mrt phenotype than the JU1395 non-Mrt phenotype. This distribution was not consistent with segregation of a single genetic locus, indicating the involvement of at least two loci—provided the difference in the parental Mrt phenotype was indeed of genetic nature.

Using whole-genome sequencing, we next genotyped two pools of RILs corresponding to the extremes of the distribution in the Mrt phenotype (Figure 3B; Data S1), as well as the parental genome. We then assessed in each RIL pool the relative parental allele frequency across the genome. We thereby identified two chromosomal regions with a significant excess of MY10 allele in the Mrt pool compared to the non-Mrt pool, thus regions representing quantitative trait loci (QTL) for the Mrt trait. The strongest QTL on chromosome II was fully fixed for the MY10 allele in the Mrt pool and at 80% for the JU1395 allele in the non-Mrt pool. A second significant QTL was detected on chromosome V, where in both directions, about 75% of the lines had the same parental allele. For both QTLs, the MY10 variant acted expectedly in the direction of decreasing the number of generations to sterility (Figure 3C). The mtDNA showed an equal proportion of both alleles in both pools, excluding an important role of the mitochondrial genotype or of other maternal effects.

We directly tested the effect of the two QTL regions by introgressing them in both directions (i.e., into both parents) to produce near isogenic lines (NILs) (Figure 3D; Data S2). As expected, chromosome II replacements had a strong effect on the Mrt phenotype. The JU3178 line carrying the MY10 chromosome II in the non-Mrt JU1395 background became sterile after 5 or 6 generations at 25°C (Wilcoxon rank sum test with continuity correction between JU3178 versus JU1395, $p = 0.0034$, and JU3180 versus MY10, $p = 0.0036$). Conversely, the JU1395 chromosome II replacement in the MY10 background (line JU3180) partially suppressed the Mrt phenotype of MY10. Chromosome V replacement had a mild effect on the Mrt phenotype into the JU1395 background (JU3177 versus JU1395; $p = 0.0038$) and no effect on the Mrt phenotype into the MY10 background (MY10 and JU3184; $p = 0.11$). The combination of chromosomes II and V had strong effects: the introduction of MY10 chromosomes II and V in the JU1395 background aggravated the Mrt phenotype compared to chromosome II alone (JU3212 versus JU3178; $p = 0.017$); conversely, the introduction of chromosomes II and V of JU1395 in the MY10 background fully suppressed the Mrt phenotype (JU3181/JU3183 versus MY10;

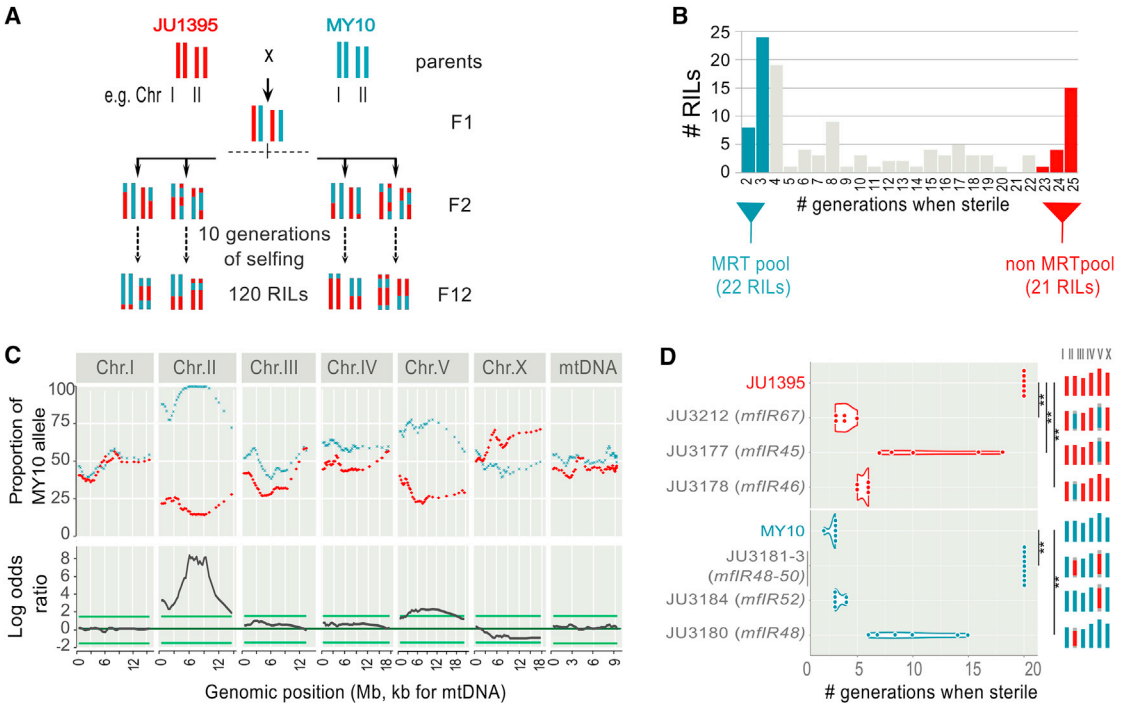



**Figure 3. Bulk Segregant Analysis of Recombinant Inbred Lines Indicates Two Major Loci**

(A) Crossing scheme for the production of the RILs. Only two chromosomes are represented.

(B) Mrt-value distribution for the 120 RILs (mean of three replicates). Full data are provided in Data S1A. The two extremes of the distribution were incorporated in the bulk segregant analysis as shown. Because few lines had a fully non-Mrt phenotype, we included in the high-trait value pool weak Mrt lines, which resisted, on average, more than 22 generations.

(C) MY10 allele proportions along the genome (six chromosomes and mtDNA) are in blue for the Mrt pool and in red for the non-Mrt pool, respectively. Full data can be found in Data S1B. Log-odd values were calculated for 1.2 Mb as explained in STAR Methods. The green lines indicate the thresholds for a significant difference between log-odd ratios at p = 0.01.

(D) Phenotyping of near isogenic lines over 20 generations confirms the QTLs detected in (C). The Mrt phenotype (5 replicates) is represented with a violin plot. The NIL genotype is represented on the right with a color code along the six chromosomes. Red, JU1395 background; blue, MY10; gray, unknown. Strain genotypes are provided in Data S2B. Wilcoxon rank sum test p values are specified as **p < 0.005.

See also Data S1 and Data S2.

p = 0.0003; Figure 3D). Altogether, the chromosome replacements validated the QTLs identified in the bulk segregant analysis. They also matched well the asymmetric distribution of RIL phenotypes (Figure 3B), which suggested that the combination of two JU1395 alleles was required for an immortal germline phenotype.

These results importantly further indicate that the natural variation in Mrt phenotype can be mapped on the genome.

## A Deletion in the Gene *set-24* Underlies the Major QTL on Chromosome II

In order to identify the molecular nature of the QTL on chromosome II, we screened for recombinants on chromosome II after crossing MY10 and the NIL JU3180 (introgression *mfIIR48*; i.e., the JU1395 chromosome V in the MY10 background; Figure 3D). This further step of recombinant mapping resulted in a 1,574,560-base pair (bp) interval (Figure 4A; Data S2B).

Analysis of the molecular variants in this interval (Data S2C) suggested as a possible candidate a deletion in the *set-24* gene in MY10, which we called *mfP23. mfP23* encompasses a 5′ upstream region and the two first exons of *set-24*, which deletes its entire predicted SET domain (Figures 4B, S4A, and S4B). SET stands for the *Drosophila melanogaster* genes *Su(var)3-9*, Enhancer-of-zeste, and Trithorax, and the SET domains are known to interact with histone tails [19, 20]. According to Interpro [21] predictions, the *C. elegans* genome codes for 34 SET-domain-containing proteins. The *set-2*, *set-25*, *set-32*, and *met-2* genes have been shown to encode methyltransferases that act on the balance between the modifications of different histone residues and thereby affect transgenerational RNAi inheritance [7–9, 22–28]. Moreover, loss of function in *set-2* and *set-32* also leads to a temperature-sensitive Mrt phenotype [7, 9, 22, 23, 26, 29]. Two SET-domain proteins, SET-5 and SET-24, in addition contain two SPK (“SET and PHD domain-containing proteins and protein kinases”) domains of unknown activity. Little is known about SET-24, which is associated with no obvious phenotype in the *C. elegans* N2 reference strain [30] (but see [31] for a possible enhancement of the fertility defects of a *spr-5* mutant). However, its putative histone-binding ability and its expression in the germline (http://wormbase.org) made the *set-24* indel polymorphism a good candidate. To test whether the *set-24(mfP23)* deletion caused the Mrt phenotype in MY10, we introduced the full-length N2 version of the *set-24* gene (YAC Y43F11A.5) into MY10 using extrachromosomal array

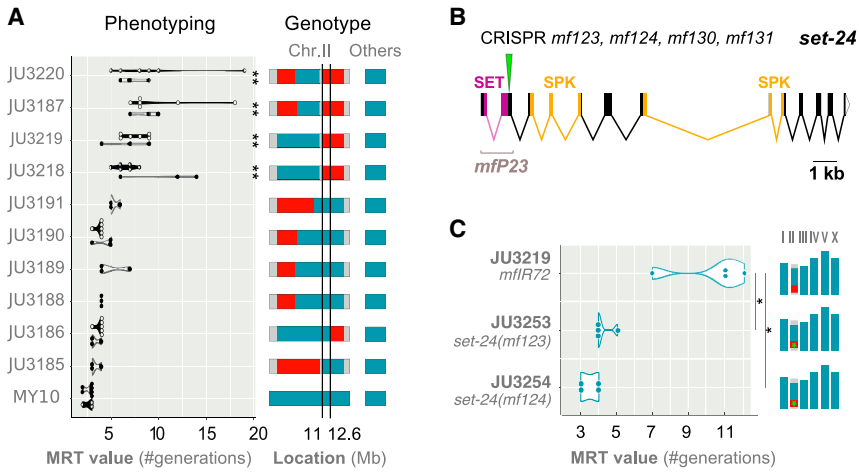



**Figure 4. The Major QTL Is Explained by a Deletion in the *set-24* Gene in MY10**

(A) Recombinant mapping of the chromosome II QTL after a cross between JU3180 (see Figure 3D) and MY10 to obtain further recombinants on chromosome II. Red areas refer to JU1395 alleles or regions, and blue areas refer to MY10 alleles or regions. Gray areas have an unknown genotype. Phenotyping of the recombinants over 20 generations is shown in the left panel. Dark and light gray represent two independent assays. Strain genotypes are provided in Data S2B. Wilcoxon rank sum test p values between MY10 and, respectively, JU3220, JU3187, JU3219, and JU3218 are specified as ** $p < 0.005$.

(B) Predicted *set-24* gene structure in the N2 reference, with the protein domains indicated by different colors. The 5′ UTR and two first exons of SET-24 are deleted in MY10 (*mfP23*). Position of the *set-24* deletion alleles obtained by genome editing in JU3219 and JU1395 are indicated by the green triangle.

(C) Phenotyping of the *set-24* deletion alleles in JU3219. In the chromosome drawings on the right, red areas refer to JU1395 regions and blue areas refer to MY10 regions. The green stars correspond to the location of *set-24* alleles *mf123* (JU3253) and *mf124* (JU3254). Wilcoxon rank sum test p values are specified as *$p < 0.05$.

See also Data S2 and Figure S4.

transgenesis. The transgene partially but significantly rescued the strong Mrt phenotype of MY10 (Figure S4C; JU3310, JU3301 compared with JU3250, JU3249; Wilcoxon rank sum test with continuity correction; $p = 0.0025$). A weak rescue was expected, as extrachromosomal arrays tend to be silenced in the *C. elegans* germline [32]. To confirm the effect of the *set-24* indel, we conversely induced CRISPR/Cas9 deletions in the intact JU1395 allele of the *set-24* gene in the NIL JU3219. These *set-24(mf123)* and *set-24(mf124)* alleles strongly enhanced the Mrt phenotype (Figure 4C). We thus conclude that the *set-24* indel underlies the major QTL on chromosome II.

To understand the evolutionary history of the *set-24(mfP23)* allele, we investigated its presence in a set of 249 wild *C. elegans* isotypes whose genomes are available on the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) website [33]. The *mfP23* allele was found to be specific to the group of isolates with a very similar genome sequence as the MY10 isotype, all sampled from the same compost heap in Roxel (Germany) in 2002 (Data S2D). Although the Roxel compost heap where MY10 was found was frequently sampled by the Schulenburg laboratory, isolates close to this isotype were not found again in Roxel or anywhere else [34, 35] (Data S2D). We thus confirmed the natural origin of the *set-24(mfP23)* allele and showed that it is a rare allele.

Many more genetic polymorphisms may modulate the Mrt phenotype of *C. elegans* wild isolates. Wild isolates, such as QX1211 and JU775, do not carry the *set-24* deletion (Figures 1 and S1); thus, their strong Mrt phenotype is likely to be explained by other genetic polymorphisms. Furthermore, our QTL mapping based on MY10 × JU1395 RILs uncovered a second QTL on chromosome V. In addition, a third QTL may be present on the left of chromosome II. Indeed, CRISPR/Cas9 deletions in the *set-24* gene did not result in a Mrt phenotype when introduced into the background of JU1395 (Figure S4E). Comparing these knockouts with the phenotype of the JU3178 chromosome II replacement line (Figure 3D), it is possible that another locus on the left of the chromosome II QTL region (Figure 3C) interacts synthetically with the *set-24* deletion to produce an intermediate value of Mrt phenotype in JU3178. That the chromosome II QTL may correspond to two molecular loci is consistent with the wide peak observed in the bulk segregant analysis. Although more replicates would be needed, Figure 4A (and Data S2) mapping results are also not inconsistent with an additional locus on the left of the original QTL peak that would have a weaker effect than *set-24* in the MY10 background. Overall, these data suggest that the temperature-sensitive Mrt phenotype in *C. elegans* is most likely influenced by many genetic polymorphisms.

## DISCUSSION

In the 20th century framework of Mendelian genetics and the modern evolutionary synthesis, genetic inheritance through DNA sequence variation was accepted as the sole mode of inheritance [36]. During the past few years, however, additional modes of inheritance have been uncovered in different organisms [27], including in *C. elegans* [3–5, 8, 9, 22, 24, 25, 27, 28, 31, 37–46]. It remains unclear whether these modes play an important role in nature and how they impact the course of evolution. In *C. elegans*, an alternative inheritance mode operates through 2° siRNAs obtained by amplification of 1° siRNAs at each generation. The inheritance of silencing through successive generations requires that 2° siRNAs are transferred into germline nuclei and impinge on histone modifications, thereby regulating gene expression across multiple generations [6, 8, 9, 22, 25, 27, 31, 39, 40]. This RNAi inheritance pathway relies on the Argonaute HRDE-1/WAGO-9 binding 2° siRNAs, MORC-1, histone-binding proteins such as HPL-2/HP1, and histone methyltransferases such as SET-2, SET-25, or SET-32 [7–9, 22–27]. Mutations in this inheritance system are specifically those resulting in a temperature-sensitive Mrt phenotype (including for null alleles). In contrast, other Mrt mutations in the reference N2

background affect other processes, for example, DNA repair or chromosome pairing mechanisms [1, 47–49], and are generally not temperature sensitive.

Here, we found natural genetic variation in the temperature-sensitive Mrt phenotype. The occurrence of this natural sterility phenotype at such relatively mild temperatures seems surprising. For example, the JU775 isolate and others with a Mrt phenotype (Figure S1) were found in or near Lisbon, Portugal in the month of July, when temperatures rise well above 20°C. We hypothesize that the multigenerational sterility is most likely suppressed by some unknown environmental factors in the wild (fluctuations in temperature may be such an environmental factor), so that the Mrt phenotype reported here only appears under particular laboratory conditions. A superficially similar phenotype was previously described at 23°C–24°C for a Bergerac strain studied by Brun [50, 51]. However, it turned out that this strain carried a temperature-sensitive allele in the *zyg-12* gene that renders the strain immediately sterile at 25°C [52], independently of the high transposition activity that arose during culture of this strain [51, 53]. When we reassayed different derivatives of the Bergerac strain [51], we did not observe the same progressive phenotype as in our newly tested *C. elegans* isolates, for which fertility at high temperature is high at the beginning and progressively declines with a visible reduction in gonad size in the last generation (Figure 1C). We instead observed a very low brood size from the first generation at 23°C.

The deletion allele in the *set-24* gene that we found as the main locus explaining the Mrt phenotype in MY10 is a rare allele, which is not surprising as we had chosen MY10 as an isolate with an extreme Mrt phenotype, a choice that is likely to yield rare alleles. This derived allele may not have been maintained for a long time maybe due to counterselection at this locus. Because of the low outcrossing rate and high linkage disequilibrium in *C. elegans* [54–57], it may also have disappeared under counterselection for a linked locus or under drift.

We distinguished here four levels of variation contributing to natural variation in the Mrt phenotype. First, the Mrt phenotype is sensitive to temperature and potentially to other environmental factors through chronic exposure. Second, the final sterility is caused by gross germline abnormalities and germline reduction in the last two generations. Whether this final sterility is caused by DNA damage, massive transposition, or non-DNA-sequence-based phenomena, such as deregulated gene expression, remains unclear. Third, we show that the multigenerational nature of the phenomenon is caused by a reversible process, thus not by DNA damage accumulation or transposition that would occur within a generation at 25°C. Examples of possible processes that may explain the multigenerational nature of the phenotype are the accumulation of histone and siRNA composition modifications. Fourth, we demonstrate that variation in the Mrt phenotype among wild isolates of *C. elegans* at a given temperature can be attributed to genetic differences, as confirmed by QTL mapping and identification of the *set-24* deletion.

In the N2 background, the temperature-sensitive Mrt phenotype corresponds to mutations in the RNAi inheritance pathway. The observed temperature-sensitive Mrt phenotype of *C. elegans* wild isolates was progressive and reversible, also suggesting an important non-genetic component in its accumulation across generations. Therefore, the diversity of the Mrt phenotype of *C. elegans* wild isolates observed under laboratory culture conditions provides an exciting model to test whether and how inheritance systems are modulated by natural genetic variation. In a now extended view of heredity in evolution, some forms of non-genetic inheritance may work in parallel to Mendelian heredity. Given this plurality of inheritance systems, how these systems themselves may vary in natural populations becomes a central question in biology. From the generally lesser stability of the variants, non-genetic inheritance may be particularly important on shorter timescales, depending on the frequency of environmental fluctuations, and may shape phenotypic evolution in this manner [58, 59].

Although more work is required to define the nature of the multigenerational memory and how it is affected by natural variation, our findings provide the first piece of evidence on genetically encoded variation for a multigenerational phenotype. The way in which SET-24 may influence histone modifications is still to be determined, but its molecular nature converges with our current understanding of multigenerational inheritance in *C. elegans*. We conclude that natural populations harbor genetic variation in phenomena of multigenerational inheritance.

## STAR★METHODS

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

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Mortal germline (Mrt) phenotyping
  - DAPI and immunostaining
  - Production of Recombinant Inbred Lines (RIL)
  - RIL phenotyping and pooling strategy
  - 5′ end independent small RNA sequencing
  - Analysis of the small RNAs
  - DNA preparation and whole genome sequencing
  - Analysis of the parental genomes
  - Quantitative trait locus (QTL) mapping
  - Construction of Near Isogenic Lines (NIL)
  - Genome editing
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Statistical analyses
  - Bulk segregant analysis
  - Small RNA analysis
- DATA AND SOFTWARE AVAILABILITY

## SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and two data files and can be found with this article online at https://doi.org/10.1016/j.cub.2018.05.091.

## ACKNOWLEDGMENTS

We are grateful to Amhed Vargas Velazquez and Fabrice Besnard for their help with genome analysis. We thank Kay Harnish from the Gurdon Institute sequencing facility for advice on genomic library preparation. We also thank Wormbase and CeNDR databases. This work was funded by a grant

from the Agence Nationale de la Recherche (ANR-14-CE10-0003-02) to C.B. and M.-A.F. This work was also supported by Cancer Research UK (C13474/A18583 and C6946/A14492) and the Wellcome Trust (104640/Z/14/Z and 092096/Z/10/Z). Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The *C. elegans* YAC Y43F11A was kindly provided by the Wellcome Sanger Institute.

## AUTHOR CONTRIBUTIONS

Conceptualization, L.F., C.B., E.M., and M.-A.F.; Methodology, L.F. and M.-A.F.; Formal Analysis, L.F. and M.-A.F.; Investigation, L.F., E.D., and M.-A.F.; Writing – Original Draft, L.F. and M.-A.F.; Writing – Review & Editing, L.F., C.B., E.M., and M.-A.F.; Visualization, L.F. and E.D.; Funding Acquisition, C.B., E.M., and M.-A.F.; Supervision, C.B., E.M., and M.-A.F.

## DECLARATION OF INTERESTS

E.M. is a founder and Director of STORM Therapeutics. This work is not related to any activity of STORM Therapeutics, and STORM Therapeutics did not contribute in any way to this work.

Received: February 15, 2018
Revised: May 16, 2018
Accepted: May 31, 2018
Published: August 2, 2018

## REFERENCES

1. Ahmed, S., and Hodgkin, J. (2000). MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*. Nature *403*, 159–164.
2. Smelick, C., and Ahmed, S. (2005). Achieving immortality in the *C. elegans* germline. Ageing Res. Rev. *4*, 67–82.
3. Grishok, A., Tabara, H., and Mello, C.C. (2000). Genetic requirements for inheritance of RNAi in *C. elegans*. Science *287*, 2494–2497.
4. Vastenhouw, N.L., Brunschwig, K., Okihara, K.L., Müller, F., Tijsterman, M., and Plasterk, R.H. (2006). Gene expression: long-term gene silencing by RNAi. Nature *442*, 882.
5. Alcazar, R.M., Lin, R., and Fire, A.Z. (2008). Transmission dynamics of heritable silencing induced by double-stranded RNA in *Caenorhabditis elegans*. Genetics *180*, 1275–1288.
6. Katz, D.J., Edwards, T.M., Reinke, V., and Kelly, W.G. (2009). A *C. elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. Cell *137*, 308–320.
7. Xiao, Y., Bedet, C., Robert, V.J., Simonet, T., Dunkelbarger, S., Rakotomalala, C., Soete, G., Korswagen, H.C., Strome, S., and Palladino, F. (2011). *Caenorhabditis elegans* chromatin-associated proteins SET-2 and ASH-2 are differentially required for histone H3 Lys 4 methylation in embryos and adult germ cells. Proc. Natl. Acad. Sci. USA *108*, 8305–8310.
8. Buckley, B.A., Burkhart, K.B., Gu, S.G., Spracklin, G., Kershner, A., Fritz, H., Kimble, J., Fire, A., and Kennedy, S. (2012). A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. Nature *489*, 447–451.
9. Spracklin, G., Fields, B., Wan, G., Becker, D., Wallig, A., Shukla, A., and Kennedy, S. (2017). The RNAi inheritance machinery of *Caenorhabditis elegans*. Genetics *206*, 1403–1416.
10. Stiernagle, T. (2006). Maintenance of C. elegans. Wormbook, 1–11.
11. Simon, M., Sarkies, P., Ikegami, K., Doebley, A.-L., Goldstein, L.D., Mitchell, J., Sakaguchi, A., Miska, E.A., and Ahmed, S. (2014). Reduced insulin/IGF-1 signaling restores germ cell immortality to *Caenorhabditis elegans* Piwi mutants. Cell Rep. *7*, 762–773.
12. Alpi, A., Pasierbek, P., Gartner, A., and Loidl, J. (2003). Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. Chromosoma *112*, 6–16.
13. Alvares, S.M., Mayberry, G.A., Joyner, E.Y., Lakowski, B., and Ahmed, S. (2014). H3K4 demethylase activities repress proliferative and postmitotic aging. Aging Cell *13*, 245–253.
14. Sakaguchi, A., Sarkies, P., Simon, M., Doebley, A.L., Goldstein, L.D., Hedges, A., Ikegami, K., Alvares, S.M., Yang, L., LaRocque, J.R., et al. (2014). *Caenorhabditis elegans* RSD-2 and RSD-6 promote germ cell immortality by maintaining small interfering RNA populations. Proc. Natl. Acad. Sci. USA *111*, E4323–E4331.
15. Herbette, M., Mercier, M.G., Michal, F., Cluet, D., Burny, C., Yvert, G., Robert, V.J., and Palladino, F. (2017). The C. elegans SET-2/SET1 histone H3 Lys4 (H3K4) methyltransferase preserves genome stability in the germline. DNA Repair (Amst.) *57*, 139–150.
16. Grishok, A. (2013). Biology and mechanisms of short RNAs in *Caenorhabditis elegans*. Adv. Genet. *83*, 1–69.
17. Pak, J., and Fire, A. (2007). Distinct populations of primary and secondary effectors during RNAi in *C. elegans*. Science *315*, 241–244.
18. Sijen, T., Steiner, F.A., Thijssen, K.L., and Plasterk, R.H. (2007). Secondary siRNAs result from unprimed RNA synthesis and form a distinct class. Science *315*, 244–247.
19. Dillon, S.C., Zhang, X., Trievel, R.C., and Cheng, X. (2005). The SET-domain protein superfamily: protein lysine methyltransferases. Genome Biol. *6*, 227.
20. Krajewski, W.A., and Reese, J.C. (2010). SET domains of histone methyltransferases recognize ISWI-remodeled nucleosomal species. Mol. Cell. Biol. *30*, 552–564.
21. Finn, R.D., Attwood, T.K., Babbitt, P.C., Bateman, A., Bork, P., Bridge, A.J., Chang, H.Y., Dosztányi, Z., El-Gebali, S., Fraser, M., et al. (2017). InterPro in 2017-beyond protein family and domain annotations. Nucleic Acids Res. *45* (D1), D190–D199.
22. Ashe, A., Sapetschnig, A., Weick, E.M., Mitchell, J., Bagijn, M.P., Cording, A.C., Doebley, A.L., Goldstein, L.D., Lehrbach, N.J., Le Pen, J., et al. (2012). piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. Cell *150*, 88–99.
23. Robert, V.J., Mercier, M.G., Bedet, C., Janczarski, S., Merlet, J., Garvis, S., Ciosk, R., and Palladino, F. (2014). The SET-2/SET1 histone H3K4 methyltransferase maintains pluripotency in the *Caenorhabditis elegans* germline. Cell Rep. *9*, 443–450.
24. Weiser, N.E., Yang, D.X., Feng, S., Kalinava, N., Brown, K.C., Khanikar, J., Freeberg, M.A., Snyder, M.J., Csankovszki, G., Chan, R.C., et al. (2017). MORC-1 integrates nuclear RNAi and transgenerational chromatin architecture to promote germline immortality. Dev. Cell *41*, 408–423.e7.
25. Lev, I., Seroussi, U., Gingold, H., Bril, R., Anava, S., and Rechavi, O. (2017). MET-2-dependent H3K9 methylation suppresses transgenerational small RNA inheritance. Curr. Biol. *27*, 1138–1147.
26. Kalinava, N., Ni, J.Z., Peterman, K., Chen, E., and Gu, S.G. (2017). Decoupling the downstream effects of germline nuclear RNAi reveals that H3K9me3 is dispensable for heritable RNAi and the maintenance of endogenous siRNA-mediated transcriptional silencing in *Caenorhabditis elegans*. Epigenetics Chromatin *10*, 6.
27. Klosin, A., Casas, E., Hidalgo-Carcedo, C., Vavouri, T., and Lehner, B. (2017). Transgenerational transmission of environmental information in *C. elegans*. Science *356*, 320–323.
28. Greer, E.L., Maures, T.J., Ucar, D., Hauswirth, A.G., Mancini, E., Lim, J.P., Benayoun, B.A., Shi, Y., and Brunet, A. (2011). Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. Nature *479*, 365–371.
29. Li, T., and Kelly, W.G. (2011). A role for Set1/MLL-related components in epigenetic regulation of the *Caenorhabditis elegans* germ line. PLoS Genet. *7*, e1001349.
30. Andersen, E.C., and Horvitz, H.R. (2007). Two *C. elegans* histone methyltransferases repress *lin-3* EGF transcription to inhibit vulval development. Development *134*, 2991–2999.
31. Greer, E.L., Beese-Sims, S.E., Brookes, E., Spadafora, R., Zhu, Y., Rothbart, S.B., Aristizábal-Corrales, D., Chen, S., Badeaux, A.I., Jin, Q.,

et al. (2014). A histone methylation network regulates transgenerational epigenetic memory in *C. elegans*. Cell Rep. *7*, 113–126.

32. Kelly, W.G., and Fire, A. (1998). Chromatin silencing and the maintenance of a functional germline in *Caenorhabditis elegans*. Development *125*, 2451–2456.
33. Cook, D.E., Zdraljevic, S., Roberts, J.P., and Andersen, E.C. (2017). CeNDR, the *Caenorhabditis elegans* natural diversity resource. Nucleic Acids Res. *45* (D1), D650–D657.
34. Haber, M., Schüngel, M., Putz, A., Müller, S., Hasert, B., and Schulenburg, H. (2005). Evolutionary history of *Caenorhabditis elegans* inferred from microsatellites: evidence for spatial and temporal genetic differentiation and the occurrence of outbreeding. Mol. Biol. Evol. *22*, 160–173.
35. Petersen, C., Dirksen, P., Prahl, S., Strathmann, E.A., and Schulenburg, H. (2014). The prevalence of *Caenorhabditis elegans* across 1.5 years in selected North German locations: the importance of substrate type, abiotic parameters, and *Caenorhabditis* competitors. BMC Ecol. *14*, 4.
36. Sapp, J. (1987). Beyond the Gene: Cytoplasmic Inheritance and the Struggle for Authority in Genetics (Oxford University Press).
37. Rechavi, O., and Lev, I. (2017). Principles of transgenerational small RNA inheritance in *Caenorhabditis elegans*. Curr. Biol. *27*, R720–R730.
38. Johnson, C.L., and Spence, A.M. (2011). Epigenetic licensing of germline gene expression by maternal RNA in *C. elegans*. Science *333*, 1311–1314.
39. Burton, N.O., Burkhart, K.B., and Kennedy, S. (2011). Nuclear RNAi maintains heritable gene silencing in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *108*, 19683–19688.
40. Gu, S.G., Pak, J., Guang, S., Maniar, J.M., Kennedy, S., and Fire, A. (2012). Amplification of siRNA in *Caenorhabditis elegans* generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint. Nat. Genet. *44*, 157–164.
41. Rechavi, O., Houri-Ze'evi, L., Anava, S., Goh, W.S.S., Kerk, S.Y., Hannon, G.J., and Hobert, O. (2014). Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. Cell *158*, 277–287.
42. Jobson, M.A., Jordan, J.M., Sandrof, M.A., Hibshman, J.D., Lennox, A.L., and Baugh, L.R. (2015). Transgenerational effects of early life starvation on growth, reproduction, and stress resistance in *Caenorhabditis elegans*. Genetics *201*, 201–212.
43. Ni, J.Z., Kalinava, N., Chen, E., Huang, A., Trinh, T., and Gu, S.G. (2016). A transgenerational role of the germline nuclear RNAi pathway in repressing heat stress-induced transcriptional activation in *C. elegans*. Epigenetics Chromatin *9*, 3.
44. Demoinet, E., Li, S., and Roy, R. (2017). AMPK blocks starvation-inducible transgenerational defects in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *114*, E2689–E2698.
45. Minkina, O., and Hunter, C.P. (2017). Stable heritable germline silencing directs somatic silencing at an endogenous locus. Mol. Cell *65*, 659–670.e5.
46. Minkina, O., and Hunter, C.P. (2018). Intergenerational transmission of gene regulatory information in *Caenorhabditis elegans*. Trends Genet. *34*, 54–64.
47. Meier, B., Clejan, I., Liu, Y., Lowden, M., Gartner, A., Hodgkin, J., and Ahmed, S. (2006). *trt-1* is the *Caenorhabditis elegans* catalytic subunit of telomerase. PLoS Genet. *2*, e18.
48. Youds, J.L., O'Neil, N.J., and Rose, A.M. (2006). Homologous recombination is required for genome stability in the absence of DOG-1 in *Caenorhabditis elegans*. Genetics *173*, 697–708.
49. Yanowitz, J.L. (2008). Genome integrity is regulated by the *Caenorhabditis elegans* Rad51D homolog *rfs-1*. Genetics *179*, 249–262.
50. Brun, J.-L. (1966). L'adaptation aux températures élevées chez un Nématode: *Caenorhabditis elegans* Maupas 1900. L'adaptation et son évolution. Ann. Biol. Anim. Biochim. Biophys. *6*, 127–158.
51. Nigon, V.M., and Félix, M.-A. (2017). History of research on C. elegans and other free-living nematodes as model organisms. Wormbook, 1–84.
52. Malone, C.J., Misner, L., Le Bot, N., Tsai, M.C., Campbell, J.M., Ahringer, J., and White, J.G. (2003). The *C. elegans* hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. Cell *115*, 825–836.
53. Moerman, D.G., and Waterston, R.H. (1984). Spontaneous unstable *unc-22 IV* mutations in *C. elegans* var. Bergerac. Genetics *108*, 859–877.
54. Barrière, A., and Félix, M.-A. (2005). High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. Curr. Biol. *15*, 1176–1184.
55. Rockman, M.V., and Kruglyak, L. (2009). Recombinational landscape and population genomics of *Caenorhabditis elegans*. PLoS Genet. *5*, e1000419.
56. Andersen, E.C., Gerke, J.P., Shapiro, J.A., Crissman, J.R., Ghosh, R., Bloom, J.S., Félix, M.-A., and Kruglyak, L. (2012). Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. Nat. Genet. *44*, 285–290.
57. Richaud, A., Zhang, G., Lee, D., Lee, J., and Félix, M.-A. (2018). The local co-existence pattern of selfing genotypes in *Caenorhabditis elegans* natural metapopulations. Genetics *208*, 807–821.
58. Bonduriansky, R. (2012). Rethinking heredity, again. Trends Ecol. Evol. *27*, 330–336.
59. Rivoire, O., and Leibler, S. (2014). A model for the generation and transmission of variations in evolution. Proc. Natl. Acad. Sci. USA *111*, E1940–E1949.
60. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics *25*, 1754–1760.
61. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal *17*, 10–12.
62. Andrews, S. (2017). FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
63. Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., et al. (2013). From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr. Protoc. Bioinformatics *43*, 11.10.1–11.10.33.
64. R Development Core Team (2015). R: a language and environment for statistical computing (R Foundation for Statistical Computing). http://www.r-project.org/
65. McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R., Thormann, A., Flicek, P., and Cunningham, F. (2016). The Ensembl variant effect predictor. Genome Biol. *17*, 122.
66. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics *25*, 2078–2079.
67. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics *29*, 15–21.
68. Milne, I., Stephen, G., Bayer, M., Cock, P.J., Pritchard, L., Cardle, L., Shaw, P.D., and Marshall, D. (2013). Using Tablet for visual exploration of second-generation sequencing data. Brief. Bioinform. *14*, 193–202.
69. Lee, R.Y.N., Howe, K.L., Harris, T.W., Arnaboldi, V., Cain, S., Chan, J., Chen, W.J., Davis, P., Gao, S., Grove, C., et al. (2018). WormBase 2017: molting into a new stage. Nucleic Acids Res. *46* (D1), D869–D874.
70. Zerbino, D.R., Achuthan, P., Akanni, W., Amode, M.R., Barrell, D., Bhai, J., Billis, K., Cummins, C., Gall, A., Girón, C.G., et al. (2018). Ensembl 2018. Nucleic Acids Res. *46* (D1), D754–D761.
71. Besnard, F., Koutsovoulos, G., Dieudonné, S., Blaxter, M., and Félix, M.-A. (2017). Toward universal forward genetics: using a draft genome sequence of the nematode *Oscheius tipulae* to identify mutations affecting vulva development. Genetics *206*, 1747–1761.
72. Ye, K., Schulz, M.H., Long, Q., Apweiler, R., and Ning, Z. (2009). Pindel: a pattern growth approach to detect break points of large deletions and

medium sized insertions from paired-end short reads. Bioinformatics *25*, 2865–2871.

73. Michelmore, R.W., Paran, I., and Kesseli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA *88*, 9828–9832.

74. Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., et al.; 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools. Bioinformatics *27*, 2156–2158.

75. Friedland, A.E., Tzur, Y.B., Esvelt, K.M., Colaiácovo, M.P., Church, G.M., and Calarco, J.A. (2013). Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. Nat. Methods *10*, 741–743.

76. Arribere, J.A., Bell, R.T., Fu, B.X., Artiles, K.L., Hartman, P.S., and Fire, A.Z. (2014). Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in *Caenorhabditis elegans*. Genetics *198*, 837–846.

## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Bacterial and Virus Strains | | |
| *Escherichia coli* strain OP50 | Caenorhabditis Genetics Center (CGC) via Paul Sternberg's laboratory | WB Strain: OP50 |
| BAC clone Y43F11A– *C. elegans* Genome consortium | Wellcome Sanger Institute https://www.sanger.ac.uk/form/Sanger_CloneRequests | WormBase ID: Y43F11A |
| Critical Commercial Assays | | |
| Nextera DNA Library Prep Kit | Illumina | FC-141-1007 |
| Site-directed mutagenesis kit | New England Biolabs | E0552S |
| PureLink Midiprep kit | Invitrogen | K210004 |
| dsDNA BR Assay Kit | ThermoFisher | Q32850 |
| TruSeq Small RNA kit | Illumina | RS-200-0012 |
| Deposited Data | | |
| Sequencing data | This paper | NCBI: PRJNA471398 and SRA: SRP145812 |
| Experimental Models: Organisms/Strains | | |
| N2, *C. elegans* | Caenorhabditis Genetics Center via Paul Sternberg's laboratory | WB Strain: N2 |
| MY10, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: MY10 |
| MY8, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: MY8 |
| MY17, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: MY17 |
| MY21, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: MY21 |
| MY22, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: MY22 |
| JU1171, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU1171 |
| JU775, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU775 |
| JU1395, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU1395 |
| QX1211, *C. elegans wild isolate* | M. Rockman | WB Strain: QX1211 |
| AB4, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: AB4 |
| CB4932, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: CB4932 |
| ED3046, *C. elegans wild isolate* | E. Dolgin | WB Strain: ED3046 |
| EG4725, *C. elegans wild isolate* | M. Ailion | WB Strain: EG4725 |
| JU363, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU363 |
| JU397, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU397 |
| JU561, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU561 |
| JU642, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU642 |
| JU774, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU774 |
| JU782, *C. elegans wild isolate* | M.-A. Félix | WB Strain: JU782 |
| KR314, *C. elegans wild isolate* | Caenorhabditis Genetics Center | WB Strain: KR314 |
| JU3004, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3005, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3006, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3007, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3008, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3009, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3010, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| JU3011, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3012, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3013, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3014, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3015, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3016, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3017, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3018, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3019, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3020, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3021, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3022, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3023, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3024, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3025, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3026, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3027, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3028, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3029, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3030, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3031, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3032, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3033, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3034, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3035, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3036, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3037, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3038, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3039, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3040, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3041, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3042, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3043, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3044, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3045, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3046, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3047, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3048, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3049, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3050, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3051, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3052, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3053, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3054, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3055, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3056, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3057, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3058, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3059, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| JU3060, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3061, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3062, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3063, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3064, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3065, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3066, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3067, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3068, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3069, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3070, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3071, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3072, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3073, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3074, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3075, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3076, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3077, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3078, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3079, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3080, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3081, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3082, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3083, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3084, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3085, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3086, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3087, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3088, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3089, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3090, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3091, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3092, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3093, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3094, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3095, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3096, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3097, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3098, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3099, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3100, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3101, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3102, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3103, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3104, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3105, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3106, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3107, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3108, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| JU3109, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3110, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3111, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3112, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3113, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3114, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3115, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3116, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3117, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3118, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3119, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3120, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3121, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3122, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3123, *C. elegans* RIL MY10 x JU1395 | This paper | Data S1A |
| JU3177, *C. elegans* RIL [*mfIR45*(V, MY10 > JU1395)] | This paper | Figure 3D, Data S2B |
| JU3178, *C. elegans* RIL [*mfIR46*(II, MY10 > JU1395)] | This paper | Figure 3D, Data S2B |
| JU3180, *C. elegans* RIL [*mfIR48*(II, JU1395 > MY10)] | This paper | Figure 3D, Data S2B |
| JU3181, *C. elegans* RIL [*mfIR49*(II&V, JU1395 > MY10)] | This paper | Figure 3D, Data S2B |
| JU3182, *C. elegans* RIL [*mfIR50*(II&V, JU1395 > MY10)] | This paper | Figure 3D, Data S2B |
| JU3183, *C. elegans* RIL [*mfIR51*(II&V, JU1395 > MY10)] | This paper | Figure 3D, Data S2B |
| JU3184, *C. elegans* RIL [*mfIR52*(V, JU1395 > MY10)] | This paper | Figure 3D, Data S2B |
| JU3212, *C. elegans* RIL [*mfIR67*(II&V, MY10 > JU1395)] | This paper | Figure 3D, Data S2B |
| JU3185, *C. elegans* NIL [*mfIR53*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3186, *C. elegans* NIL [*mfIR54*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3187, *C. elegans* NIL L [*mfIR55*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3188, *C. elegans* NIL [*mfIR56*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3189, *C. elegans* NIL [*mfIR57*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3190, *C. elegans* NIL [*mfIR58*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3191, *C. elegans* NIL [*mfIR59*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3218, *C. elegans* NIL [*mfIR71*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3219, *C. elegans* NIL [*mfIR72*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3220, *C. elegans* NIL [*mfIR73*(*recombinant* II MY10x JU1395 > MY10)] | This paper | Figure 4A, Data S2B |
| JU3253, *C. elegans* strain [*set-24*(*mf123*) in JU3219] | This paper | Figure 4C |
| JU3254, *C. elegans* strain [*set-24*(*mf124*) in JU3219] | This paper | Figure 4C |
| JU3292, *C. elegans* strain [*set-24*(*mf130*) in JU1395] | This paper | Figure S4E |
| JU3293, *C. elegans* strain [*set-24*(*mf131*) in JU1395] | This paper | Figure S4E |
| JU3249, *C. elegans* strain [*mfEx96*[*myo-2*::GFP; Y43F11A] injected in MY10] | This paper | Figure S4C |
| JU3250, *C. elegans* strain [*mfEx97*[*myo-2*::GFP; Y43F11A] injected in MY10] | This paper | Figure S4C |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| JU3301, *C. elegans* strain [*mfEx100*[*myo-2*::GFP] injected in MY10] | This paper | Figure S4C |
| JU3310, *C. elegans* strain [*mfEx101*[*myo-2*::GFP] injected in MY10] | This paper | Figure S4C |
| Oligonucleotides | | |
| Primers used to genotype RILs and other recombinants | This paper | Data S2A |
| Software and Algorithms | | |
| bwa 0.7.8-R455 | [60] | http://bio-bwa.sourceforge.net/bwa.shtml |
| cutadapt | [61] | https://github.com/marcelm/cutadapt |
| FastQC 0.11.5 | [62] | https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ |
| GATK 3.2-2 | [63] | https://software.broadinstitute.org/gatk/ |
| Picard 1.114 | Broad Institute | http://broadinstitute.github.io/picard/ |
| Pindel 0.2.5b6 | [64] | http://gmt.genome.wustl.edu/packages/pindel/ |
| Variant Effect Predictor (VEP) | [65] | https://www.ensembl.org/Tools/VEP |
| R: A Language and Environment for Statistical Computing v3.4.1 | [64] | https://www.R-project.org |
| Samtools 1.2 | [66] | http://samtools.sourceforge.net/ |
| STAR_2.4.2a | [67] | https://github.com/alexdobin/STAR |
| Tablet 1.17.08.17 | [68] | https://ics.hutton.ac.uk/tablet/ |
| vcftools v0.1.12b | [68] | http://vcftools.sourceforge.net/ |
| Other | | |
| *C. elegans* genome of reference; Wormbase release WS243 | N/A | ftp://ftp.wormbase.org/pub/wormbase/releases/WS243/species/c_elegans/PRJNA13758/ |
| Resource website Wormbase release WS262 | [69] | http://www.wormbase.org/ |
| Resource website Interpro | [21] | http://www.ebi.ac.uk/interpro/ |
| Resource website Ensembl | [70] | https://www.ensembl.org/index.html |
| Resource website CeDNR | [33] | https://elegansvariation.org/ |

## CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Marie-Anne Félix (felix@biologie.ens.fr).

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

*Caenorhabditis elegans* animals were grown on 55 mm diameter NGM plates and fed with *Escherichia coli* OP50 bacteria [10], at indicated temperatures. Hermaphrodites were used, except for crosses where hermaphrodites were mated to males. We specificy the developmental stage of the animals when relevant.

The complete list of strains used in this study and their laboratory origin can be found in the Key Resources Table. The wild origin of *C. elegans* isolates can be found in [56]. JU isolates were isolated by our laboratory, while the others were obtained through the Caenorhabditis Genetics Center.

## METHOD DETAILS

### Mortal germline (Mrt) phenotyping

Prior to starting the phenotyping assays, adult hermaphrodites were bleached and their progeny grown for at least three generations at 15°C to produce the initial populations. Phenotyping assays begun with the transfer of three L4-stage hermaphroditic larvae (generation $G_1$) onto new NGM plates that were then incubated at the chosen temperature for the assay (generally 25°C, Figure 1A).

Each generation $G_n$ was then started with three L4-stage larvae picked in the progeny of generation $G_{n-1}$. When all three individuals of generation $G_n$ were sterile, we took *n* as the value for the Mrt phenotype. Phenotyping assays were stopped after 20-25 generations and lineages that remained fertile were considered non-Mrt. Assays were replicated as indicated for each case either in methods or figure legends.

As the standard assay, three larvae were transferred at each generation, except if otherwise indicated. The original Mrt screen [1] was performed with transfer of six L1 larvae. We preferred to transfer L4 larvae when it is possible to differentiate males and hermaphrodites. We considered three as sufficient to avoid extinction due to manipulation or other hazards of life on a Petri dish such as drying on the side. Some assays focusing on specific lineages were performed by transferring a single larva, while the assays with GFP transgenes in Figure S4C were performed with ten larvae so as not to lose the extrachromosomal transgene.

### DAPI and immunostaining

DAPI staining was performed on young adult hermaphrodites. Before fixation, animals were washed in M9, then collected in cold methanol and washed 3 times with PBS-Tween 0.05% before being DAPI stained with Vectashield (Vector Laboratories) medium supplemented with DAPI.

RAD-51 immunostainings were performed on young adult hermaphrodites grown for 24 hours after the L4-stage. Animals were dissected to extrude their germline and then fixed for 10 min in paraformaldehyde, freeze-cracked and immersed in cold methanol. The purified rabbit anti-RAD-51 primary antibody (a gift from A. Gartner) was used at a 1/500 dilution. The Alexa Fluor 555 anti-rabbit antibody (Molecular Probes) was used as secondary antibody at a dilution of 1/1500.

### Production of Recombinant Inbred Lines (RIL)

Recombinant Inbred Lines (RILs) were obtained starting from crosses between MY10 and JU1395 in both directions of crossing: MY10 males with JU1395 hermaphrodites, and MY10 hermaphrodites with JU1395 males. We genotyped the F1 progeny using *niDf250*del-F/R primers to identify heterozygote individuals. For each cross direction, one heterozygote hermaphrodite F1 was randomly chosen. 120 recombinant inbred lineages (60 in each direction of the cross) were then built by transferring one hermaphrodite per generation for 11 generations (Figure 3A). To prevent allele segregation biases which could emerge from temperature associated traits, animals were grown at 15°C throughout RIL production.

### RIL phenotyping and pooling strategy

We measured the Mrt phenotype of each RIL at 25°C in triplicates for 25 generations. We decided to use a pooling strategy for the genotyping, focusing on the two extremes of the phenotypic distribution: a pool of 21 RILs with a non-Mrt phenotype and another pool of 22 RILs with a MY10-like phenotype. The 120 RILs, their Mrt-values and the RILs included in the QTL mapping are listed in Data S1A.

### 5′ end independent small RNA sequencing

Our aim was to monitor the small RNA populations along the progressive onset of fertility defects. We characterized the small RNA composition in JU1395 and in MY10, across generations and for three independent replicates each. Following the Mrt phenotyping design explained above, each generation was started with three L4 larvae from the previous generation, generation 0 (G0) was grown at 15°C and subsequent generations were grown at 23°C. As MY10 was sterile at generation 3 for two replicates and at generation 4 for one replicate, the siRNA monitoring was performed until generation 2 in triplicates for MY10 and JU1395. We added the siRNA data for the remaining replicate of MY10 that was fertile at generation 3 and sterile at generation 4. At each generation, adults were harvested more than 32 hr after the L4-to-adult molt. Each sample was immediately homogenized in Trizol (Invitrogen) and stored at −80°C. RNA from each sample was extracted with isopropanol and chloroform (2.5:1). In order to obtain all small RNAs in a 5′-end independent manner (with and without triphosphate ends), samples were treated with 5′ polyphosphatase (Epicenter/Illumina) for 30 min. Small RNA libraries were generated from 1 μg polyphosphatase-treated total RNA using the TruSeq Small RNA kit (Illumina), following the manufacturer's instructions. Libraries were sequenced using the Illumina HiSeq 1500 to generate 36-nucleotide single-end reads.

### Analysis of the small RNAs

Adaptors were removed from the Fastq files using the program Cutadapt v1. Fastq sequences were then trimmed to leave only reads of length between 16 and 33 nucleotides and were aligned using STAR (version STAR_2.4.2a) to the N2 reference genome (WS235 genome version ftp://ftp.wormbase.org/pub/wormbase/releases/WS235/species/c_elegans/c_elegans.WS235.genomic.fa.gz), reporting in a bam file only the best single alignment with up to one mismatch allowed. For each generated bam files, we extracted the counts of reads grouped according to their length and the identity of their first nucleotide. The reads with secondary alignment were not taken into account in our analysis. Data were normalized to the total number of reads with a length between 16 to 33 nucleotides. Plots of the proportions of siRNA grouped according to their length and their first nucleotide were generated to illustrate the small RNA population differences between generations.

### DNA preparation and whole genome sequencing

Genomic DNA was extracted from mixed-stage growing populations of each RIL as described in [71]. DNA concentrations were quantified using a Qubit fluorometer with the dsDNA BR Assay Kit (ThermoFisher) and adjusted to 1 μg/μL. For pool sequencing, DNAs of RILs of a given pool (as specified in Data S1A) were mixed in equimolar proportions. We also prepared DNA from the parents, MY10 and JU1395. Four paired-end libraries were built using the Nextera DNA Library preparation kit (Illumina), following manufacturer's instructions and sequenced on an Illumina HiSeq1500 (100 bp paired reads).

### Analysis of the parental genomes

Sequence quality was checked using FastQc. Reads were filtered for quality, mapped to the genome of the *C. elegans* reference N2 strain (WS243 genome version ftp://ftp.wormbase.org/pub/wormbase/releases/WS243/species/c_elegans/PRJNA13758/c_elegans.PRJNA13758.WS243.genomic.fa.gz) and variants called, following the first 7 steps of the mapping-by-sequencing pipeline (andalousian-map_Portable.sh) described in [71].

The Pindel software [72] was used to detect in homozygous indels in MY10 and JU1395 genomes. We used the bam format outputs from the above mapping pipeline as input files, specifying 250 as the expected average insert size. Parent-specific deletions were identified and those in the mapping interval manually checked using Tablet [68].

The Ensembl Variant Effect Predictor [65] and the WBcel235 annotation of the genome of reference were used to annotate impacts of the parent-specific SNPs and deletions (Data S2C).

### Quantitative trait locus (QTL) mapping

As first described in [73], the purpose of bulk segregant analysis is to detect genomic regions where parental allele proportions deviate between groups of contrasted phenotypes, here between the Mrt and non-Mrt pools.

To do so, we first defined a set of SNPs (markers) between the two parents, MY10 and JU1395, along the genome. Using the *compare* function of the vcftools software [74], we listed the positions where SNP were homozygous and different between the two parental genomes. As a second step, we selected these positions in the genomic sequences of the Mrt and non-Mrt pools. For each pool and each marker, we extracted the total number of reads and the number of reads corresponding to the MY10 allele. Except for the mitochondrial DNA, we excluded positions where the total number of reads was below 20, above 250 (putative repeats) or where quality was not maximal. Proportions of MY10 alleles were calculated for each marker as the ratio of read counts with the MY10 allele divided by the total number of reads at this SNP. Data are provided in Data S1B. To minimize the effect of heterogeneous distribution of SNP along the chromosomes, we first calculated the mean allele frequencies on a 300kb window size with no overlap. We then displayed the distribution of MY10 allele proportions along chromosomes in a sliding window manner with a window size of 1.2Mb and step of 300kb. See below in 'Statistical analysis' for the log-odds ratio calculation.

### Construction of Near Isogenic Lines (NIL)

Near Isogenic Lines were produced in order to confirm the QTL identified by bulk segregant analysis. We crossed MY10 and JU1395 to introduce chromosome II, chromosome V or both from one parent into the genetic background of the other parent through repeated backcrosses. All crosses were made at 18°C. Chromosomes were followed by PCR-based genotyping using the primers listed in Data S2A. These primers were designed to amplify regions neighboring MY10- or JU1395-specific deletions (ranging from 100 to 2520 bp). PCR products were separated on a 1.5% agarose gel to discriminate between alleles. We cannot rule out that the tips of the chromosomes outside the genotyped markers were from the other parent. NILs were genotyped over 20 generations.

To fine map the QTL, we crossed JU3180 (Figure 3D) and MY10 to obtain further recombinants of chromosome II in the MY10 background. The resulting recombinant and homozygous F3 progeny were phenotyped over 20 generations using the primers in Data S2A.

The genotypes of NILs and chromosome II recombinants are provided in Data S2B.

### Genome editing

We used the CRISPR/Cas9 target design and reagents described in [75]. We targeted the second exon of the *set-24* gene, with the guide sequence 5′-gtaacgcggcaagaactctaCGG −3′ with the final 'CGG' representing the PAM motif for the Cas9. We replaced the *dpy-10* target site with the *set-24* target site in the pjA58 plasmid from [76] using the site-directed mutagenesis kit (New England Biolabs) and the primers 5′- aagaactctaGTTTTAGAGCTAGAAATAGC-3′ and 5′- gccgcgttacAAGACATCTCGCAATAGG-3′. DH5-alpha bacteria were transformed with the modified pJA58 plasmid, pU6-*set24*-sgRNA2. Bacteria were then grown overnight at 37°C in liquid LB medium and plasmids were extracted using a PureLink Midiprep kit (Invitrogen). The *set-24* target site in pU6-*set24*-sgRNA2 was confirmed by Sanger sequencing.

We then injected the following mix into gonads of JU3219 and JU1395 hermaphrodites: 100 ng/μL of the pU6-*set24*-sgRNA2 plasmid, together with 50 ng/μL of the Cas9 coding plasmid (*Peft-3*::*Cas9*-SV40 NLS::*tbb-2* 3′UTR [75];) and 40 ng/μL of the pjA58 plasmid as co-injection marker. We singled the F1 progeny from plates with the highest number of animals displaying the Dpy phenotype. F1 were screened for *set-24* editing by screening for deletions in a 107 bp region around the PAM site. The primers 5′-GAAACTCCACTGCATTGT-3′ and 5′-TTTTCCTCGGCAATACG-3′ were used to generated PCR products which were loaded onto a 3%-agarose gel to identify samples with a smaller PCR product length. Broods from independent P0 animals were found positive and rendered homozygous. Frameshifting deletions introduced in the exon 2 of *set-24* were confirmed by Sanger sequencing. The

resulting lines, JU3253, JU3254, JU3292, JU3293, were given the allele names *mf123*, *mf124, mf130* and *mf131*, respectively and were phenotyped over 25 generations. Alleles sequences are specified in Figure S4B.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Statistical analyses

Statistical analyses were performed using R v3.3.2 [64]. The details on the number of replicates (animals or lines) and the tests can be found in the Results section and the Figure legends.

### Bulk segregant analysis

In our design, the limiting factor for QTL mapping was the low number of pooled RILs pooled and not the read coverage. Indeed, we recovered a minimum of 82 reads per 300kb window, thus had a large excess of reads compared to the number of RILs in the pool (21 and 22). To test whether differences in MY10 allele proportions between the two pools were significantly different from expectations under a random distribution, we first calculated for all previously defined windows the log-odds ratio as: $\log(m_1/(21\text{-}m_1))/(m_2/(22\text{-}m_2)))$, $m_1$ being the MY10 allele proportion multiplied by the number of RIls (22) in the Mrt pool and $m_2$ the MY10 allele proportion multiplied by the number of RILs (21) in the non-Mrt pool. We calculated the threshold of significance ($p = 0.01$) for log-odds ratios in a 2-tailed manner. Using the binomial law, we simulated log-odds ratios for 1 million randomized draws of the two pools.

### Small RNA analysis

In order to test, in each genotype, the effect of the time (i.e., generation) on the percentage of 22G in small RNA population, we used the following general linear model with a Poisson regression: %22G = replicates + #generations/replicates + $\varepsilon$.

## DATA AND SOFTWARE AVAILABILITY

The accession number for the genomic and small RNA sequence data reported in this paper is NCBI: PRJNA471398 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA471398). Code is available from sources indicated and on request.